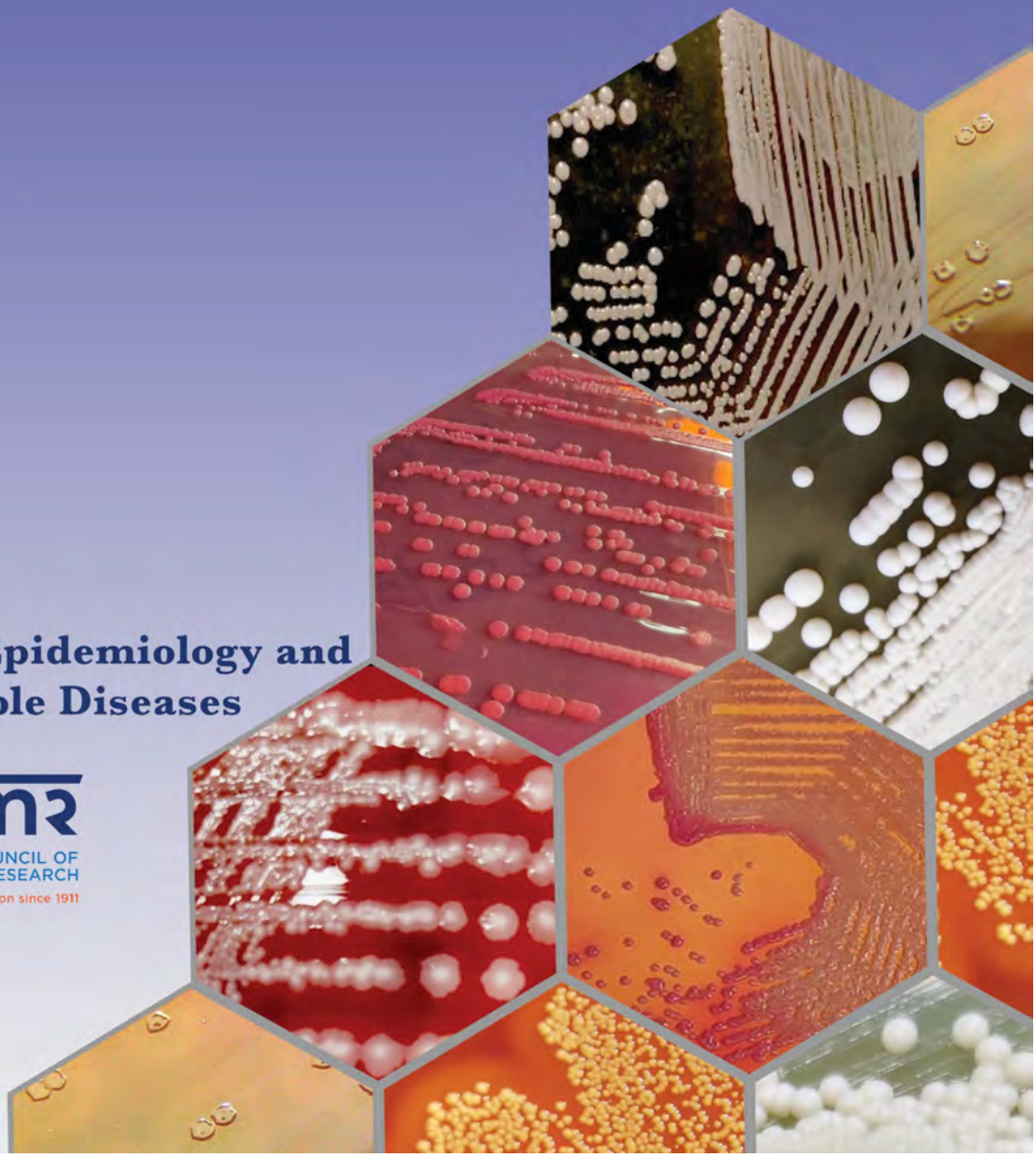
Annual Report

January 2021 to December 2021

Antimicrobial Resistance Research and Surveillance Network



Division of Epidemiology and Communicable Diseases

INDIAN COUNCIL OF MEDICAL RESEARCH

Serving the nation since 1911

CONTENTS

List of acronyms

AMRSN	Antimicrobial Resistance Research & Surveillance Network
AMS	Antimicrobial Susceptibility
BAL	Bronchoalveolar lavage
BSI	Blood stream infections
CARD	Comprehensive Antibiotic Resistance Database
CAUTI	Catheter associated urinary tract infections
CDS	Coding sequence regions
CGPS	Center for Genomic Pathogen Surveillance
CLABSI	Catheter associated blood stream infections
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase-negative Staphylococci
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
CRE	Carbapenem resistant Enterobacterales
CSF	Cerebrospinal fluid
DI	Deep infections
DEC	Diarrheagenic *E coli*
ESBLs	Extended spectrum beta lactamases
HAI	Hospital acquired Infections
HCAI	Health Care Associated infections
HCWs	Health care workers
ICU	Intensive care unit
IPC	Infection prevention and Control
OPD	Out-patient department
LOS	Length of stay
LRT	Lower Respiratory tract
MBL	Metallo-beta-lactamase
MFS	Major Facilitator superfamily
MIC	Minimum inhibitory concertation
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
NFGNB	Non fermenting Gram-negative bacilli
OXA	Oxacillinases
PBP2a	Penicillin binding protein 2a
PMQR	Plasmid mediated quinolone resistance
QUAST	Quality assessment tool
RC	Regional centers
RGI	Resistance gene identifier
SCC *mec*	Staphylococcal cassette chromosome mec
SI	Superficial infections
SD	Standard deviation
SS	Sterile body fluids
ST	Sequence types
TMP-SMX	Trimethoprim sulfamethoxazole
UTI	Urinary Tract infections
VRE	Vancomycin-resistant enterococci
WGS	Whole-genome sequencing

Executive summary

ICMR- Antimicrobial Resistance Surveillance network

The Indian Council of Medical Research (ICMR) has been supporting research on antimicrobial resistance through the Antimicrobial Resistance Research & Surveillance Network (AMRSN) since 2013. The data collected from the network has enabled compilation of drug resistance data on six pathogenic groups on antimicrobial resistance from the country. Data collected from the network is used to track resistance trends and to better understand mechanisms of resistance in the key priority pathogens using genomics and whole genome sequencing (WGS). This is the fifth detailed report on AMR trends and patterns from the country, published by ICMR. Since the network collects data from tertiary care hospitals, the data presented in this report is not reflective of the community levels of AMR in the country and should not be extrapolated to community settings. This report also includes the trends of resistance of key pathogens to the critically important antimicrobials which should guide the prevention and treatment interventions for AMR in the country.

Structure and framework for the ICMR-AMRSN

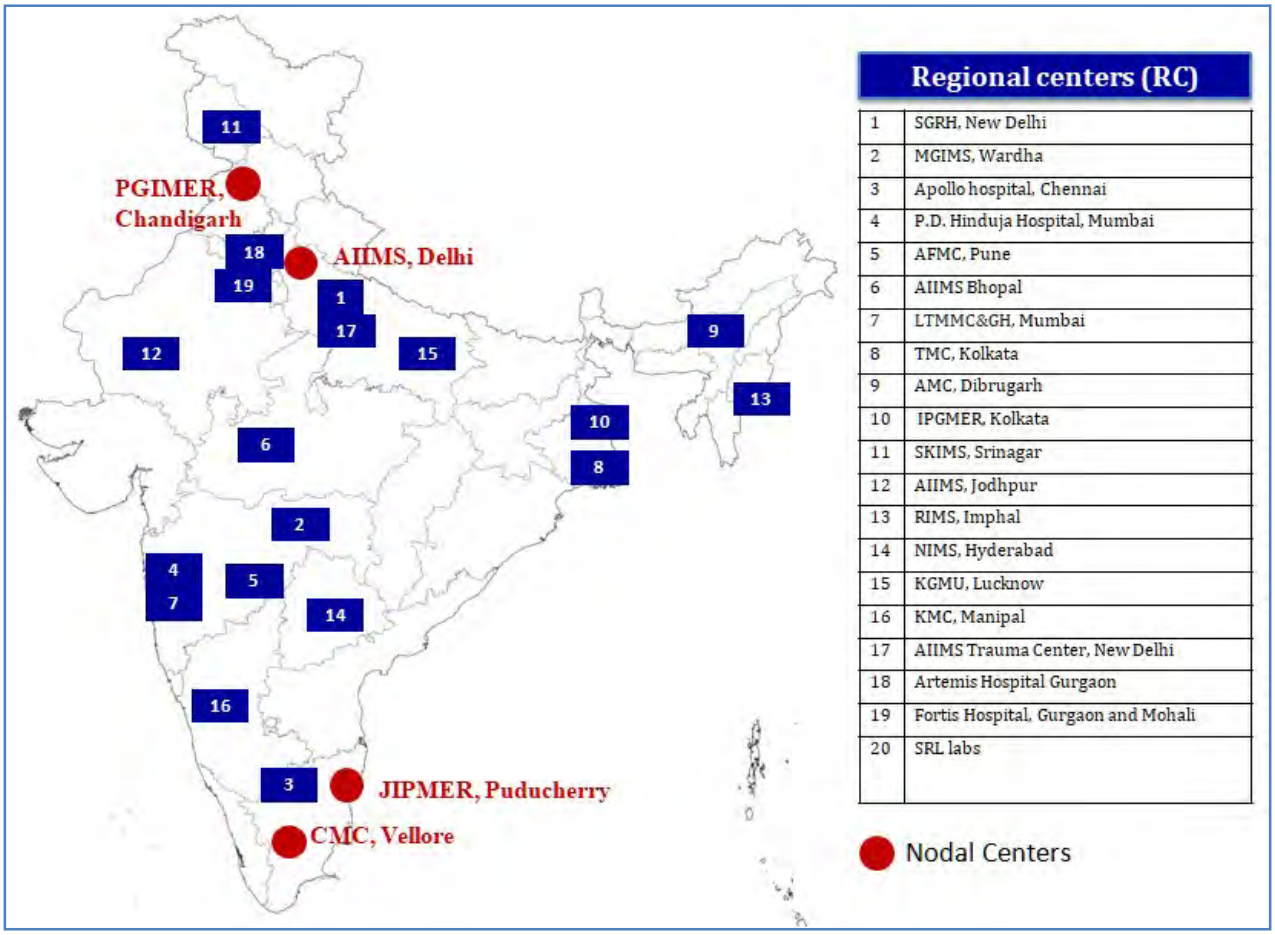
Under AMRSN, there are seven nodal centres (NCs) for each pathogenic group in four tertiary care hospitals.

(i) *Enterobacterales* causing sepsis (PGIMER, Chandigarh)
(ii) Gram-negative non-fermenters (CMC Vellore)
(iii) Gram-positives: staphylococci and *enterococci,* (JIPMER, Puducherry)
(iv) Typhoidal *Salmonella* (AIIMS New Delhi)
(v) Diarrhoeagenic bacterial organisms (CMC Vellore),
(vi) Fungal pathogens (PGIMER, Chandigarh).
(vii) *Streptococcus pneumoniae* (CMC Vellore)

There are twenty regional laboratories (regional centres, RCs) from tertiary care hospitals to provide data and fixed number of isolates for each pathogenic group [Figure (i)]. The RCs carry out only AMST; however, the NCs also focus on the identified resistant organisms and carry out detailed molecular studies on the respective group of pathogens.

One of the main objectives of establishing this network was to bring about harmonization and uniformity in the AMS testing procedures being followed for bacteriology and mycology. This was accomplished by formulating standard operating procedures (SOPs) on bacteriology and mycology, based on the Clinical Laboratory Standards Institute (CLSI)

guidelines. The SOPs are revised periodically to include the changes proposed by CLSI and are used for training of all the participating hospitals. All the network laboratories perform microbiological investigations; using standard biochemical identification (up to species level) and carry out antimicrobial susceptibility on all clinical isolates received using SOPs. The network captures quantitative data, *i.e.* minimum inhibitory concentration (MICs) or zone diameters in disc diffusion tests which are more significant than the qualitative data (interpretations as susceptible, intermediate or resistant), that indicate only broad trends for many drug-organism combinations. Phenotypic assays for the detection of mechanisms of resistance are performed for isolates at each centre. Each NC and RC determines the antibiogram of the isolates against panel (available antimicrobials of choice) with breakpoints recommended by ICMR SOPs. The records are auto validated using a set criteria decided by set of experts and ICMR data management team. This auto-validation module automatically checks for any unacceptable patterns and highlights the records that require manual interventions.



Regional centers (RC)	
1	SGRH, New Delhi
2	MGIMS, Wardha
3	Apollo hospital, Chennai
4	P.D. Hinduja Hospital, Mumbai
5	AFMC, Pune
6	AIIMS Bhopal
7	LTMMC&GH, Mumbai
8	TMC, Kolkata
9	AMC, Dibrugarh
10	IPGMER, Kolkata
11	SKIMS, Srinagar
12	AIIMS, Jodhpur
13	RIMS, Imphal
14	NIMS, Hyderabad
15	KGMU, Lucknow
16	KMC, Manipal
17	AIIMS Trauma Center, New Delhi
18	Artemis Hospital Gurgaon
19	Fortis Hospital, Gurgaon and Mohali
20	SRL labs

Figure (i): Antimicrobial Resistance Research & Surveillance Network (AMRSN): Nodal and Regional Centers

Genotypic characterisation

Molecular characterization of the resistance mechanisms is performed by corresponding NCs for pathogens [Figure (ii)]. Sixty resistant isolates per species, per year, are shared by RCs for molecular characterization with NCs. Each NC tests the isolates receives from RCs and other NCs for AMR genes. Molecular data is shared with the respective RCs and entered in online AMR portal

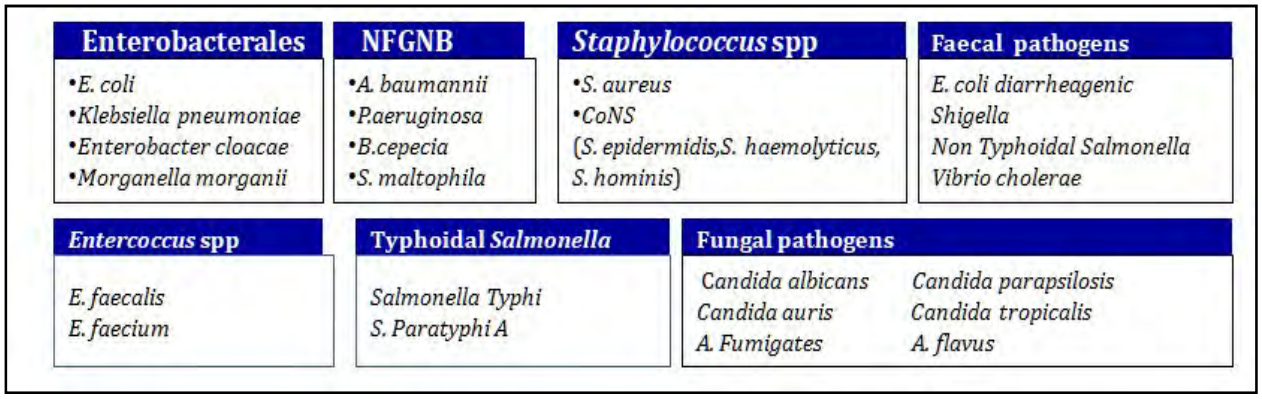


Figure (ii): List of species for genotypic characterisation

Highlights of data 2021:

- This report presents data from January 1st, 2021 to December 31st, 2021. Total number of culture positive isolates studied during the year 2021 was 95,728.
- *Escherichia coli* was the most commonly isolated pathogen followed by the *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.
- Imipenem susceptibility of *E. coli* has dropped steadily from 86% in 2016 to 64% in 2021 and that of *Klebsiella pneumoniae* dropped steadily from 65% in 2016 to 45% in 2020 and was at 43% for the year 2021.
- Resistance to carbapenems in *Acinetobacter baumannii* was recorded as 87.5% in the year 2021, limiting the availability of available treatment options. In *A. baumannii*, there is no significant change in the susceptibility trends to all the tested antibiotics compared to last year. Susceptibility to minocycline was close to 50% (45% to 65.6%) making it most susceptibile antibiotic after colistin for *Acinetobacter baumannii.*

- In *Pseudomonas aeruginosa*, more than 60% susceptibility was observed for various aminoglycosides and fluoroquinolones in 2021. There is a consistent increase in susceptibility to all the major antipseudomonal drugs in the last few years.
- *In Staphylococcus aureus*, susceptibility to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA. MRSA rates are increasing each year from 2016 to 2021 (28.4% to 42.6%). The anti MRSA antibiotics such as vancomycin and daptomycin showed excellent in vitro activity (100% against MRSA isolates). Linezolid resistance was encountered in both MRSA and CoNS isolates albeit at very low rates of 0.1%.
- Vancomycin resistance in enterococci (*E. faecalis* and *E. faecium*) was 14.9%, however, the rate was 6 times higher in *E. faecium* compared *to E. faecalis* (25.4% vs 3.8%). 37.5% of *Enterococcus faecium* causing blood stream infections (BSIs) were vancomycin resistant.
- In fungal pathogens, antifungal susceptibility profiling revealed more than 90% fluconazole susceptibility in *C. tropicalis*, *C. albicans* and *C. utilis* (~94%), but declining susceptibility rates (78%-80%) were reported in *C. parapsilosis* and *C. glabrata* thus requiring close monitoring in next few years.
- *C. auris* and *C. krusei* were predominantly resistant to fluconazole with extremely low susceptibility percentages of 2.6% and 2.9%, respectively.
- *C. auris and C. parapsilosis* isolates showed an increased trend from 2016 to 2021. *C. auris* and *C. parapsilosis* were found in 0.04% and 0.23% of isolates in 2017 respectively, which rose to 0.2% and 0.3% in 2021.
- *Aspergillus flavus* was the most common aspergillus species identified among *Aspergillus* followed by *A. fumigatous.*
- *Rhizopus arrhizus*, the most common mucorales, was predominantly susceptible to amphotericin B.
- The data suggest a high terbinafine resistance rate (11.4%) and therapeutic failure in *Trichophyton mentagrophytes-Trichophyton interdigitale* complex. Itraconazole is suggested as the drug of choice for dermatophytoses.
- There has been no significant change in the overall antimicrobial susceptibility pattern of *Salmonella* Typhi or *S*. Paratyphi A from India and the pattern remaining uniform across all the participating centers in the AMR network. *S.* Typhi is 100 % susceptible to cephalosporins and azithromycin. Other drugs which retained good susceptility for *Salmonella* Typhi or *S*. Paratyphi A were ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.

- Among diarrheal pathogens (Diarrheagenic *E. coli*, *Shigell*a spp. and *Salmonella*) norfloxacin susceptibility was poor, except for *Aeromonas and Vibrio*. Empirical use of norfloxacin for treatment of bacterial diarhhoea is strongly discouraged.
- Among meningeal isolates of *S. pneumoniae,* resistance to penicillin and cefotaxime was 77% and 23% respectively. Hence, monotherapy with either of these antibiotics is not recommended in the meningeal infections. Current ICMR guidelines of combination therapy (cephalosporins with vancomycin) are recommended.

Health Care Associated Infections

Health-care-associated bloodstream infections and urinary tract infections are common in Indian hospitals and the pathogens causing HAIs are highly drug resistant. This year's report includes a section on health care associated infections (HAI) surveillance* which is being undertaken in a network of 39 tertiary-level hospitals. This network provides valuable data on Hospital acquired Infections (HAI) burden, and is helpful in identifying and monitoring HAI levels in a hospital for appropriate intervention. The regional distribution of the participating centers is shown in Figure (iii). This surveillance focused on BSIs (Primary and secondary BSIs) and UTIs (Catheter associated and non-catheter associated). A total of 1, 50,744 Central line days and 2, 64,344 urinary catheter days were reported during this period.

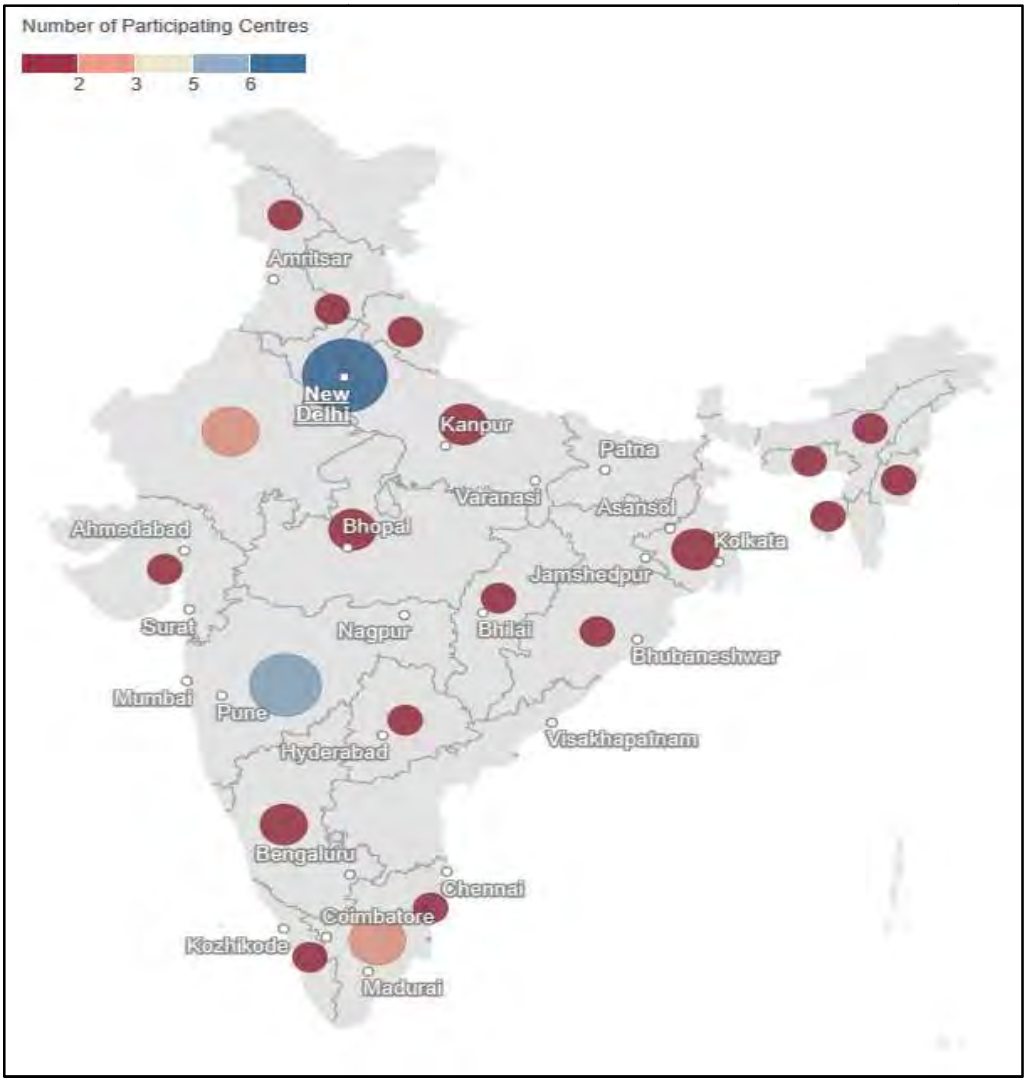


Figure (iii): Participating Centers in the HAI/ IPC network

**This HAI surveillance is being technically coordinated by the ICMR and AIIMS and funded in part by the CDC as part of a cooperative agreement (No 1U2GGH001869).*

Chapter 1 Summary of surveillance data

Total number of culture positive isolates studied during the year 2021 was 95,728. Of these, **18,988** were from blood, **19,319** from urine, **16,746** Lower Respiratory tract (LRT), **19,592** Superficial infections, **8,125** Deep infections, 995 CSF, **2,787** Sterile spaces (SS), 651 Faeces and **8525** others. Majority of the isolates were from Enterobacterales except *Salmonella and Shigella* (49.5%) followed by Non fermenting Gram-negative bacilli (NFGNB) (27.4%), staphylococci (12%), enterococci (5.9%), *fungi* (3.6%), Typhoidal *Salmonella* (0.5%), and streptococci (0.4%) (Table 1.1). In the distribution of major group of organisms in different specimens, member of the Enterobacterales group were the commonest isolates in urine (76.5%), sterile body fluids (SS) (58.6%), deep infections (DI) (49.7%), others (48.8%), superficial infections (SI) (44.9%), blood (38.5%), LRT (37.9%) and CSF (34%). Non fermenting Gram-negative bacilli (NFGNB) group were the predominant isolates in the lower respiratory tract (54.7%), CSF (43.9%), superficial infections (SI) (27.3%), blood (24.9%), deep infection (DI) (24.5%), sterile sites (SS) (23.3%),others (22.7%), and urine (10.1%). *Staphylococci* constituted 20.7% of the superficial infections (SI) followed by deep infection (DI) (19.9%), blood infection (19.3%), and CSF (7.9%). Enterococci group constituted 10.2% of the isolates from CSF followed by urine (10%), sterile body fluid (9.2%), blood (7%), superficial infections (5.5%), and deep infections (3.8%), and Typhoidal *Salmonella* group constituted 2.3% of the isolates from blood. *Yeast* group were significant isolates in the blood infection (7.8%) (Table 1.1 and Figure 1.1).

The distribution of top 10 isolates from different specimens is presented in Table 1.2 and Figure 1.2. *Escherichia coli* was most commonly isolated (24.7%) followed by the *Klebsiella pneumoniae* (18%), *Acinetobacter baumannii* (12.9%), *Pseudomonas aeruginosa* (12.1%), and *Staphylococcus aureus* (9.2%). Among these isolates, *Escherichia coli* was the most predominant isolate from the urine (52.3%), *K. pneumoniae* from the LRT (25.3%), *Acinetobacter baumannii* from LRT (30.4%), *S. aureus* from DI (19%), *Enterococcus faecalis* and *Enterococcus faecium* from Urine (4.5%), and (4.2%) respectively. The relative distribution of the various species isolated from patients in the out-patient department (OPD), admitted to the wards and intensive care unit (ICUs) are presented in Table 1.3 and Figures 1.3a &1.3b. Top 5 isolates in descending order in OPD specimen were *E. coli, K. pneumoniae, S. aureus , P. aeruginosa* and *Enterococcus faecalis*; in Wards *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter baumannii* and *S. aureus*; and in ICU *Acinetobacter baumannii*, *K. pneumoniae, E. coli, P. aeruginosa* and *S. aureus. Enterococcus faecium* was common isolate from the ICU (3.1%) followed by ward and OPD; whereas, *E. faecalis* was common isolate from the OPD (2.8%) followed by the wards and the ICU. (Table 1.3, Figure 1.3).

Table 1.1: Specimen wise distribution of major groups of organisms

Isolate	Culture positive																			
	Total n=95728		Blood n=18988		Urine n=19319		LRT n=16746		Superficial Infection n=19592		Deep Infection n=8125		CSF n=995		SS n=2787		Faeces n=651		Others n=8525	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enterobacterales except Salmonella and Shigella	47399 (49.5)	100	7307 (38.5)	15.4	14778 (76.5)	31.2	6353 (37.9)	13.4	8792 (44.9)	18.5	4039 (49.7)	8.5	338 (34)	0.7	1634 (58.6)	3.4	0 (0)	0	4158 (48.8)	8.8
NFGNB	26185 (27.4)	100	4720 (24.9)	18	1955 (10.1)	7.5	9161 (54.7)	35	5339 (27.3)	20.4	1987 (24.5)	7.6	437 (43.9)	1.7	650 (23.3)	2.5	0 (0)	0	1936 (22.7)	7.4
Staphylococci	11482 (12)	100	3658 (19.3)	31.9	304 (1.6)	2.6	738 (4.4)	6.4	4058 (20.7)	35.3	1615 (19.9)	14.1	79 (7.9)	0.7	124 (4.4)	1.1	0 (0)	0	906 (10.6)	7.9
Enterococci	5647 (5.9)	100	1332 (7)	23.6	1939 (10)	34.3	65 (0.4)	1.2	1072 (5.5)	19	309 (3.8)	5.5	101 (10.2)	1.8	257 (9.2)	4.6	0 (0)	0	572 (6.7)	10.1
Fungi	3452 (3.6)	100	1485 (7.8)	43	264 (1.4)	7.6	383 (2.3)	11.1	175 (0.9)	5.1	107 (1.3)	3.1	39 (3.9)	1.1	90 (3.2)	2.6	0 (0)	0	909 (10.6)	26.3
Diarrhoeal bacterial pathogens	714 (0.7)	100	10 (0.1)	1.4	6 (0)	0.8	4 (0)	0.6	7 (0)	1	6 (0.1)	0.8	0 (0)	0	23 (0.8)	3.2	651 (100)	91.2	7 (0.1)	1
Typhoidal Salmonella	472 (0.5)	100	435 (2.3)	92.2	5 (0)	1.1	2 (0)	0.4	12 (0.1)	2.5	4 (0)	0.8	1 (0.1)	0.2	8 (0.3)	1.7	0 (0)	0	5 (0.1)	1.1
Streptococci	377 (0.4)	100	41 (0.2)	10.9	68 (0.4)	18	40 (0.2)	10.6	137 (0.7)	36.3	58 (0.7)	15.4	0 (-)	0	1 (0)	0.2	0 (-)	0	32 (0.4)	8.5

Note:

1. **Blood** includes: Blood-central catheter, Blood-peripheral and Peripheral catheter-blood.
2. **LRT** (Lower Respiratory Tract) includes: BAL, Sputum, Lung aspirate, Endotracheal aspirate (ETA) and Lobectomy tissue (Lung tissue).
3. **SSI: Superficial Infection** includes SST (Skin & Soft Tissue), Pus/exudate, Wound swab, Superficial Biopsy and Superficial Tissue.
4. **Deep Infection** includes: Abscess aspirate, Pus aspirate, Deep Biopsy and Deep Tissue.
5. **SS** (Sterile sites) includes: Fluid from sterile spaces, abdominal fluid, Intracostal tube fluid, Pancreatic drain fluid, Pericardial fluid, Peritoneal fluid and Pleural fluid.

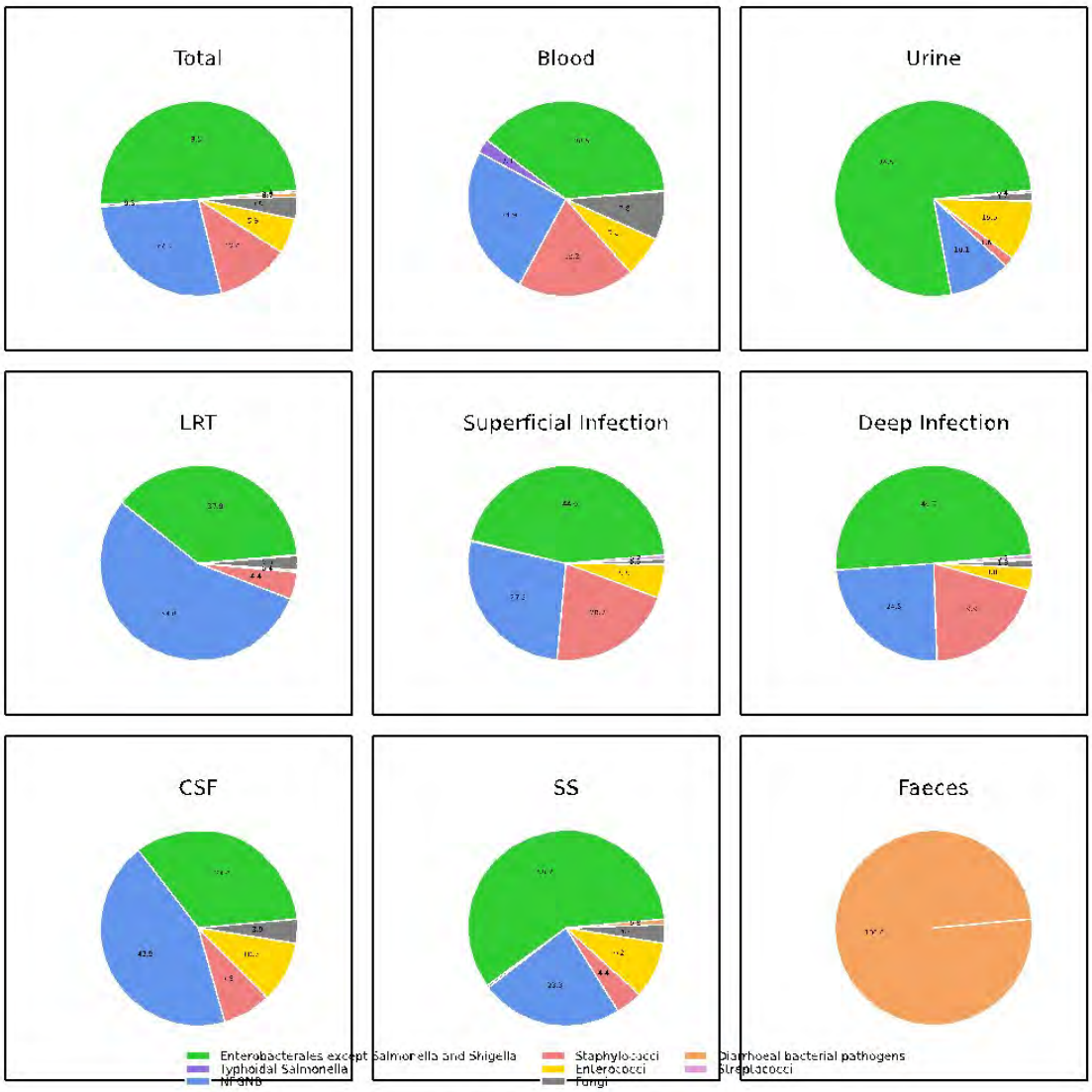


Figure 1.1: Specimen wise distribution of major groups of organisms

Table 1.2: Isolation distribution of top 10 isolates from different specimens

Organism	Total	Blood	LRT	Superficial Infection	Deep Infection	SS	Faeces	Urine
Escherichia coli	23629/95728 (24.7)	3096/18988 (16.3)	1338/16746 (8)	3980/19587 (20.3)	1911/8125 (23.5)	874/2787 (31.4)	0/651 (0)	10096/19319 (52.3)
Klebsiella pneumoniae	17216/95728 (18)	3270/18988 (17.2)	4238/16746 (25.3)	2952/19587 (15.1)	1158/8125 (14.3)	520/2787 (18.7)	0/651 (0)	3583/19319 (18.5)
Acinetobacter baumannii	12393/95728 (12.9)	2508/18988 (13.2)	5088/16746 (30.4)	1845/19587 (9.4)	752/8125 (9.3)	312/2787 (11.2)	0/651 (0)	415/19319 (2.1)
Pseudomonas aeruginosa	11622/95728 (12.1)	1336/18988 (7)	3291/16746 (19.7)	3066/19587 (15.6)	1085/8125 (13.4)	244/2787 (8.8)	0/651 (0)	1398/19319 (7.2)
Staphylococcus aureus	8827/95728 (9.2)	1663/18988 (8.8)	701/16746 (4.2)	3719/19587 (19)	1563/8125 (19.2)	109/2787 (3.9)	0/651 (0)	230/19319 (1.2)
Enterococcus faecium	2422/95728 (2.5)	700/18988 (3.7)	20/16746 (0.1)	402/19587 (2.1)	109/8125 (1.3)	124/2787 (4.4)	0/651 (0)	810/19319 (4.2)
Enterococcus faecalis	2373/95728 (2.5)	472/18988 (2.5)	14/16746 (0.1)	546/19587 (2.8)	129/8125 (1.6)	66/2787 (2.4)	0/651 (0)	871/19319 (4.5)
Enterobacter cloacae	1644/95728 (1.7)	356/18988 (1.9)	182/16746 (1.1)	462/19587 (2.4)	217/8125 (2.7)	40/2787 (1.4)	0/651 (0)	206/19319 (1.1)
Proteus mirabilis	1611/95728 (1.7)	71/18988 (0.4)	91/16746 (0.5)	607/19587 (3.1)	350/8125 (4.3)	35/2787 (1.3)	0/651 (0)	286/19319 (1.5)
Enterococus spp.	852/95728 (0.9)	160/18988 (0.8)	31/16746 (0.2)	124/19587 (0.6)	71/8125 (0.9)	67/2787 (2.4)	0/651 (0)	257/19319 (1.3)

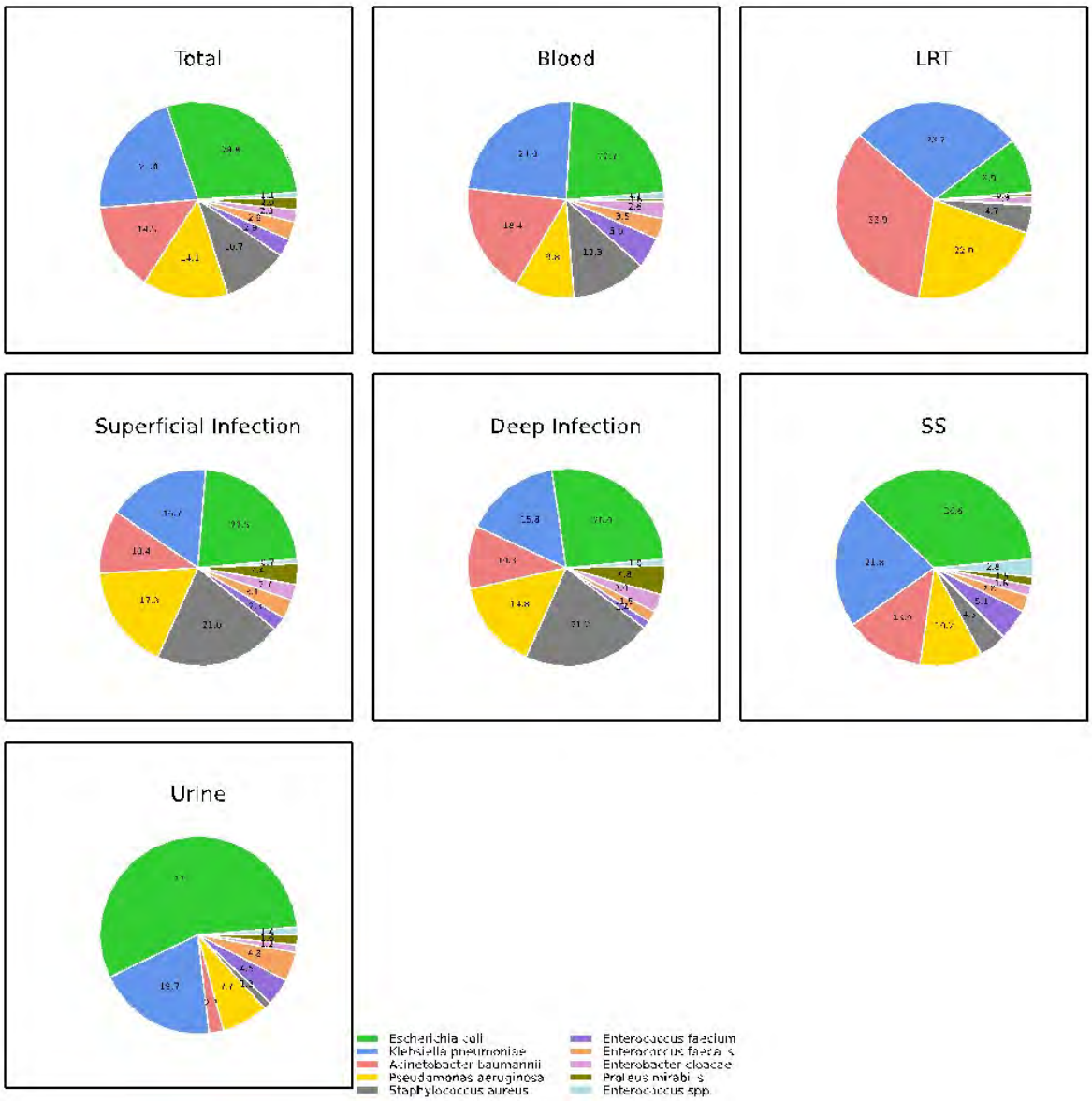


Figure 1.2: Isolation distribution of top 10 isolates from different specimens

Table 1.3: Distribution of top 10 isolates from all specimens across OPD, ward and ICU

Organism	Total n(%)	OPD n(%)	Ward n(%)	ICU n(%)
Escherichia coli	23629/95728 (24.7)	7630/23643 (32.3)	13328/51633 (25.8)	2671/20452 (13.1)
Klebsiella pneumoniae	17216/95728 (18)	3446/23643 (14.6)	9397/51633 (18.2)	4373/20452 (21.4)
Acinetobacter baumannii	12393/95728 (12.9)	1287/23643 (5.4)	5611/51633 (10.9)	4948/20452 (24.2)
Pseudomonas aeruginosa	11622/95728 (12.1)	3098/23643 (13.1)	6099/51633 (11.8)	2425/20452 (11.9)
Staphylococcus aureus	8827/95728 (9.2)	3132/23643 (13.2)	4573/51633 (8.9)	1122/20452 (5.5)
Enterococcus faecium	2422/95728 (2.5)	311/23643 (1.3)	1482/51633 (2.9)	629/20452 (3.1)
Enterococcus faecalis	2373/95728 (2.5)	671/23643 (2.8)	1339/51633 (2.6)	363/20452 (1.8)
Enterobacter cloacae	1644/95728 (1.7)	477/23643 (2)	880/51633 (1.7)	287/20452 (1.4)
Proteus mirabilis	1611/95728 (1.7)	519/23643 (2.2)	886/51633 (1.7)	206/20452 (1)
Enterococcus spp	852/95728 (0.9)	179/23643 (0.8)	532/51633 (1)	141/20452 (0.7)
Others	13686/95728 (14.3)	2893/23643 (12.2)	7506/51633 (14.5)	3287/20452 (16.1)

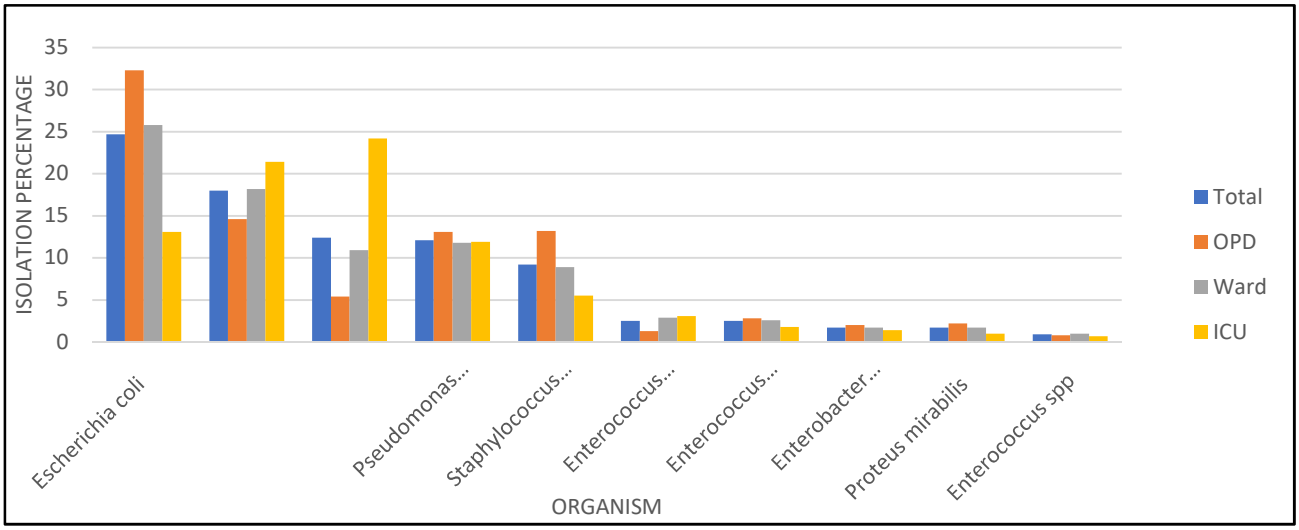


Figure 1.3a: Distribution of top 10 isolates from all specimens across OPD, ward and ICU

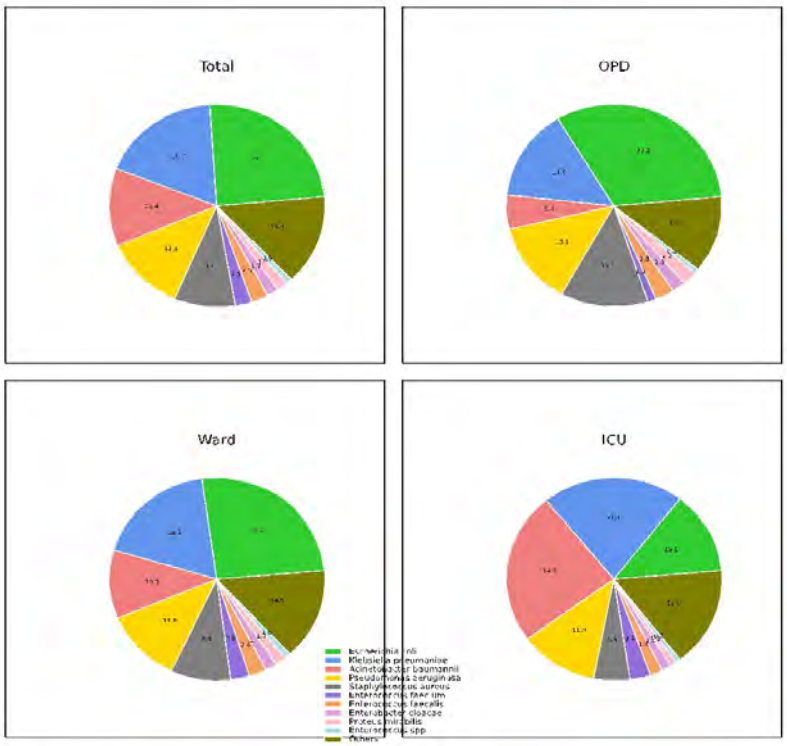


Figure 1.3b: Distribution of species of organisms in isolates from OPD, ward and ICU

Table 1.4 Yearly isolation trends of top 10 isolates from all samples

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)	Year-2021 (%)
Escherichia coli	2143/11604 (18.5)	10413/45521 (22.9)	19317/74295 (26)	30652/108465 (28.3)	16483/65561 (25.1)	23629/95728 (24.7)
Klebsiella pneumoniae	1354/11604 (11.7)	6735/45521 (14.8)	11062/74295 (14.9)	18456/108465 (17)	11810/65561 (18)	17216/95728 (18)
Acinetobacter baumannii	396/11604 (5.5)	3361/45521 (7.4)	4550/74295 (6.1)	8533/108465 (7.9)	6851/65561 (10.4)	12393/95728 (12.9)
Pseudomonas aeruginosa	556/11604 (4.8)	5689/45521 (12.5)	8883/74295 (12)	12638/108465 (11.7)	7843/65561 (12)	11622/95728 (12.1)
Staphylococcus aureus	1978/11604 (17)	5708/45521 (12.5)	8782/74295 (11.6)	12320/108465 (11.4)	6281/65561 (9.6)	8827/95728 (9.2)
Enterococcus faecium	288/11604 (2.5)	937/45521 (2.1)	1476/74295 (2)	2700/108465 (2.5)	1994/65561 (3)	2422/95728 (2.5)
Enterococcus faecalis	229/11604 (2)	1034/45521 (2.3)	2014/74295 (2.7)	2895/108465 (2.7)	2101/65561 (3.2)	2373/95728 (2.5)
Enterobacter cloacae	69/11604 (0.6)	619/45521 (1.4)	1097/74295 (1.5)	1495/108465 (1.4)	1057/65561 (1.6)	1644/95728 (1.7)
Proteus mirabilis	193/11604 (1.7)	882/45521 (1.9)	1285/74295 (1.7)	1958/108465 (1.8)	1236/65561 (1.9)	1611/95728 (1.7)
Enterococcus spp.	153/11604 (1.3)	421/45521 (0.9)	711/74295 (1)	1079/108465 (1)	703/65561 (1.1)	852/95728 (0.9)

Yearly isolation rates of top ten isolates from all samples showed a steady increase of *Klebsiella pneumoniae* from 11.7% in 2016 to 18% in 2021 (Table 1.4, Figure 1.4) and *A. baumannii* from 6.1% in 2018 to 12.9% in 2021 without much change in the isolation rates of the other species. There was a marginal decline in isolation rates of *Staphylococcus aureus*.

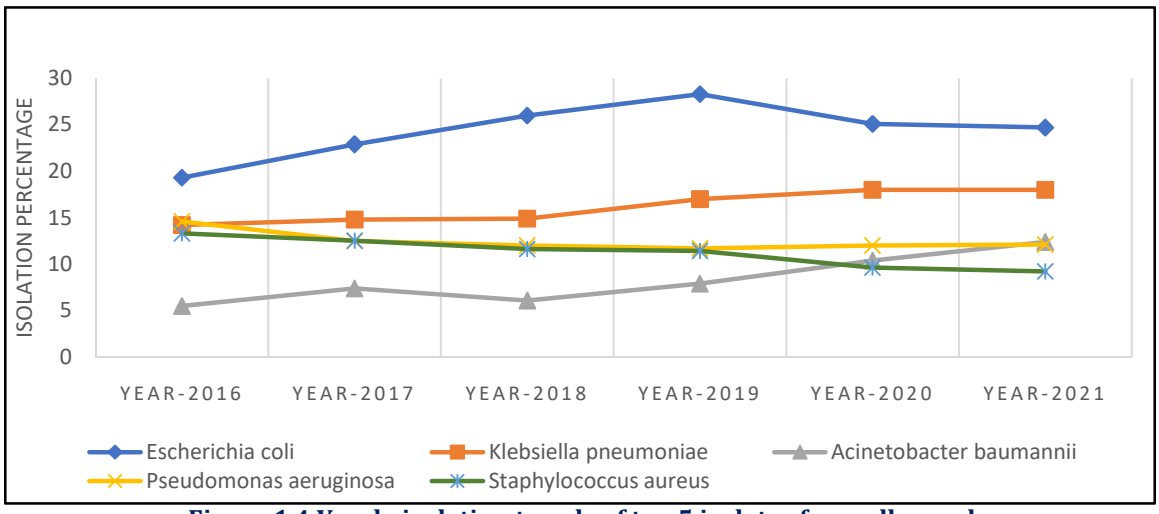


Figure 1.4 Yearly isolation trends of top 5 isolates from all samples

Enterobacterales

Of the overall isolates Enterobacterales (except *Salmonella and Shigella*) constituted a major group (49.5%) (Table 1.1). Out of a total of 95,728 culture positive isolates, specimen percentage wise distribution of major species within family Enterobacterales is shown in the Table 1.5 and Figures 1.5a and 1.5b. Overall, *Escherichia coli* was the commonest species (24.7%) followed by *Klebsiella pneumoniae* (18%), *Enterobacter cloacae* and *Proteus mirabilis* (1.7%) (Table 1.5). *Escherichia coli* was the most predominant isolate from the urine (52.3%), sterile site (31.4%), others (24%), Deep infections (23.5%), superficial infection (20.3%), blood (16.3%) and CSF (13.3%). *Klebsiella pneumoniae* was the most predominant isolate in the lower respiratory tract (25.3%), sterile sites (SS) (18.7), urine (18.5%), blood (17.2%), and superficial infection (15.1%), deep infection (DI) (14.3%) and CSF (13.2). *Enterobacter cloacae* constituted 2.7 % of deep infections and 2.4% of superficial infections and CSF. *Proteus mirabilis* was common in 4.3 % of deep and 3.1% of superficial infections and other specimens (1.7%). *Klebsiella species* constituted 1.3% of sterile site infections (SS). Isolates from the regional centers (RC 4) had higher percentage isolate rate of *E. coli*, *Proteus mirabilis and Enterobacter cloacae* than the rest of RCs (Table 1.6). Centre wise distribution showed that regional centres (RC) 2 and 4 had highest number of blood isolates than rest of RCs.

Table 1.5: Specimen wise distributions of major species of Family Enterobacterales except *Salmonella and Shigella*

Isolate	Culture positive																			
	Total n=95728		Blood n=18988		Urine n=19319		LRT n=16746		Superficial Infection n=19592		Deep Infection n=8125		CSF n=995		SS n=2787		Faeces n=*0		Others n=9176	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Escherichia coli	23629 (24.7)	100	3096 (16.3)	13.1	10096 (52.3)	42.7	1338 (8)	5.7	3980 (20.3)	16.8	1911 (23.5)	8.1	132 (13.3)	0.6	874 (31.4)	3.7	*0 (-)	0	2202 (24)	9.3
Klebsiella pneumoniae	17216 (18)	100	3270 (17.2)	19	3583 (18.5)	20.8	4238 (25.3)	24.6	2954 (15.1)	17.2	1158 (14.3)	6.7	131 (13.2)	0.8	520 (18.7)	3	*0 (-)	0	1362 (14.8)	7.9
Enterobacter cloacae	1644 (1.7)	100	356 (1.9)	21.7	206 (1.1)	12.5	182 (1.1)	11.1	462 (2.4)	28.1	217 (2.7)	13.2	24 (2.4)	1.5	40 (1.4)	2.4	*0 (-)	0	157 (1.7)	9.5
Proteus mirabilis	1611 (1.7)	100	71 (0.4)	4.4	286 (1.5)	17.8	91 (0.5)	5.6	607 (3.1)	37.7	350 (4.3)	21.7	13 (1.3)	0.8	35 (1.3)	2.2	*0 (-)	0	158 (1.7)	9.8
Citrobacter koseri	477 (0.5)	100	26 (0.1)	5.5	196 (1)	41.1	30 (0.2)	6.3	131 (0.7)	27.5	48 (0.6)	10.1	5 (0.5)	1	5 (0.2)	1	*0 (-)	0	36 (0.4)	7.5
Morganella morganii	416 (0.4)	100	40 (0.2)	9.6	89 (0.5)	21.4	13 (0.1)	3.1	154 (0.8)	37	69 (0.8)	16.6	1 (0.1)	0.2	17 (0.6)	4.1	*0 (-)	0	33 (0.4)	7.9
Serratia marcescens	387 (0.4)	100	95 (0.5)	24.5	46 (0.2)	11.9	112 (0.7)	28.9	56 (0.3)	14.5	48 (0.6)	12.4	9 (0.9)	2.3	6 (0.2)	1.6	*0 (-)	0	15 (0.2)	3.9
Klebsiella spp.	311 (0.3)	100	54 (0.3)	17.4	31 (0.2)	10	135 (0.8)	43.4	22 (0.1)	7.1	7 (0.1)	2.3	4 (0.4)	1.3	37 (1.3)	11.9	*0 (-)	0	21 (0.2)	6.8
Providencia rettgeri	144 (0.2)	100	17 (0.1)	11.8	39 (0.2)	27.1	28 (0.2)	19.4	17 (0.1)	11.8	19 (0.2)	13.2	1 (0.1)	0.7	12 (0.4)	8.3	*0 (-)	0	11 (0.1)	7.6

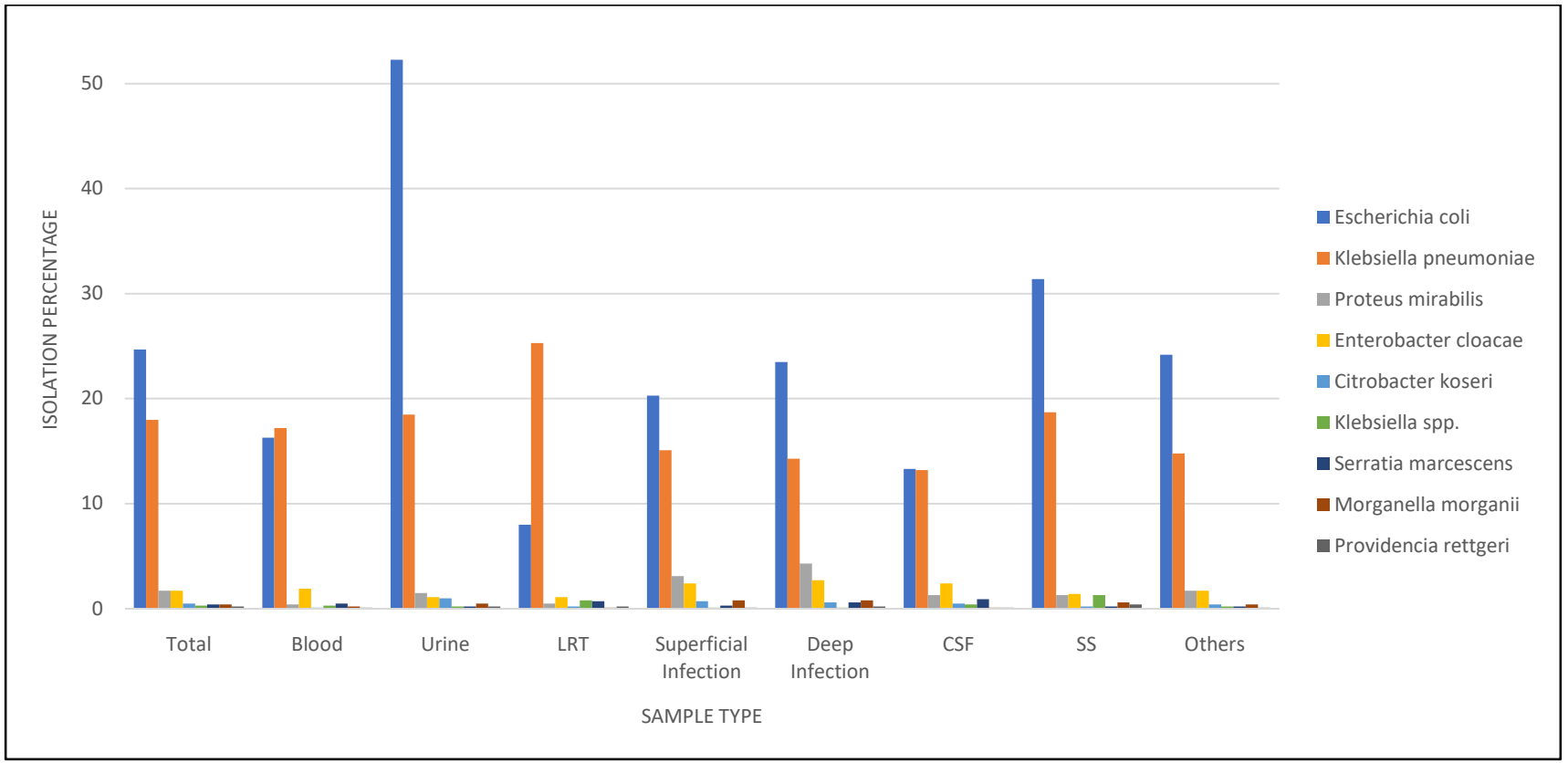


Figure 1.5a: Specimen wise distribution of major species of Family Enterobacterales except Salmonella and Shigella

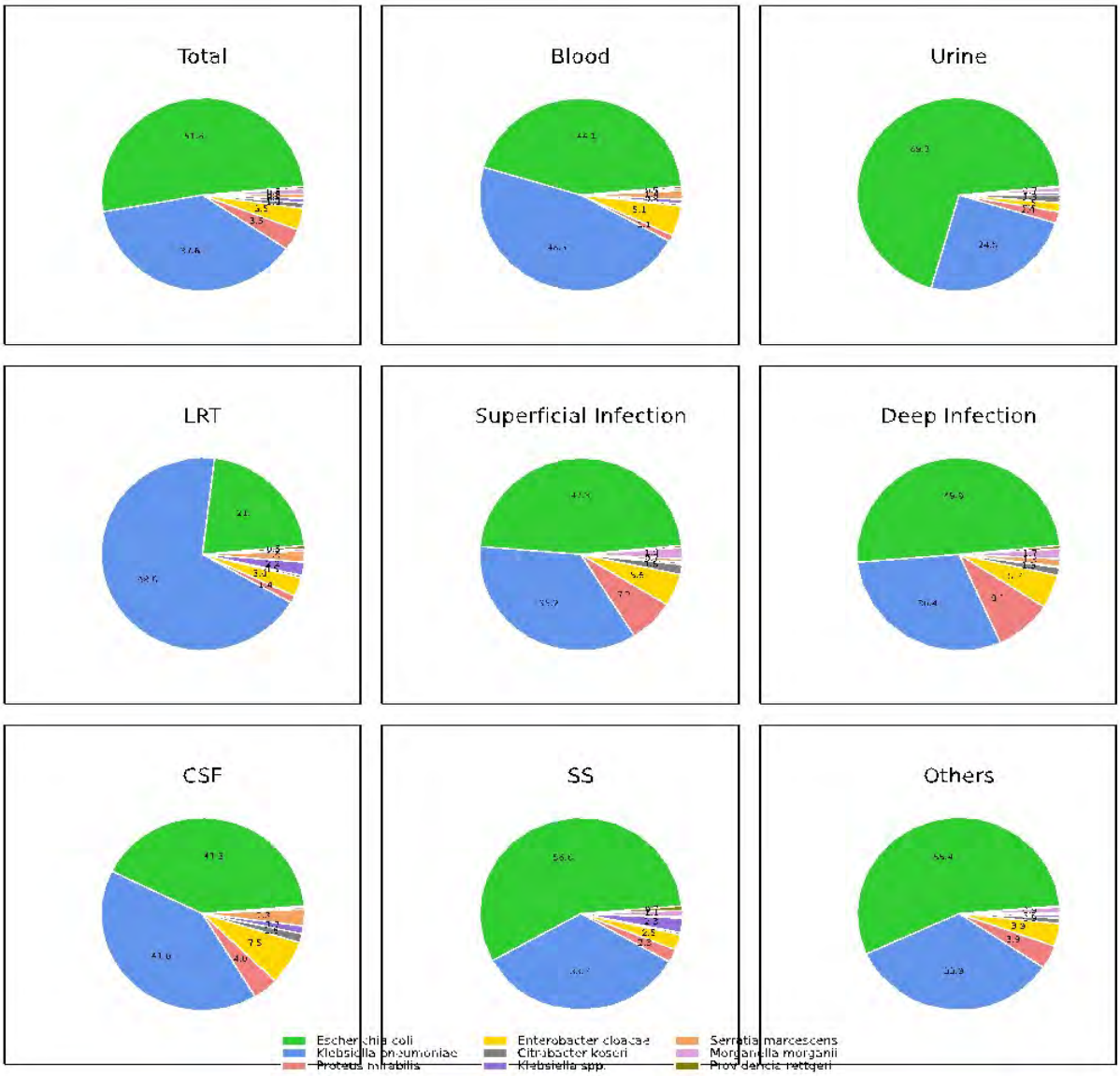


Figure 1.5b: Specimen wise distribution of major species of Family Enterobacterales except *Salmonella and Shigella*

Table 1.6: Regional centre wise distribution of major species of family Enterobacterales except Salmonella in Total (except Faeces) specimen type

Regional Centre	Total (except faeces) Isolates	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterobacter cloacae*	*Citrobacter koseri*	*Enterobacter spp.*	*Citrobacter freundii*	*Proteus vulgaris*	*Citrobacter spp.*
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC2	13597 (14.3)	2767 (20.4)	2095 (15.4)	302 (2.2)	372 (2.7)	34 (0.3)	84 (0.6)	20 (0.1)	0 (0)	76 (0.6)
RC4	13391 (14.1)	2936 (21.9)	2014 (15)	332 (2.5)	358 (2.7)	89 (0.7)	0 (0)	18 (0.1)	13 (0.1)	1 (0)
RC1	7327 (7.7)	1412 (19.3)	1251 (17.1)	45 (0.6)	97 (1.3)	13 (0.2)	10 (0.1)	15 (0.2)	1 (0)	6 (0.1)
RC14	6147 (6.5)	2229 (36.3)	1200 (19.5)	73 (1.2)	222 (3.6)	74 (1.2)	9 (0.1)	6 (0.1)	9 (0.1)	9 (0.1)
RC6	4987 (5.2)	1310 (26.3)	1205 (24.2)	122 (2.4)	83 (1.7)	24 (0.5)	0 (0)	22 (0.4)	10 (0.2)	0 (0)
RC15	4963 (5.2)	1056 (21.3)	1110 (22.4)	79 (1.6)	16 (0.3)	3 (0.1)	120 (2.4)	0 (0)	8 (0.2)	1 (0)
RC3	4698 (4.9)	856 (18.2)	605 (12.9)	51 (1.1)	38 (0.8)	5 (0.1)	96 (2)	5 (0.1)	4 (0.1)	22 (0.5)
RC13	4657 (4.9)	1262 (27.1)	828 (17.8)	32 (0.7)	11 (0.2)	6 (0.1)	69 (1.5)	2 (0)	16 (0.3)	12 (0.3)
RC10	4346 (4.6)	1120 (25.8)	794 (18.3)	91 (2.1)	84 (1.9)	53 (1.2)	3 (0.1)	8 (0.2)	9 (0.2)	6 (0.1)
RC20	3762 (4)	1160 (30.8)	637 (16.9)	70 (1.9)	0 (0)	7 (0.2)	0 (0)	8 (0.2)	24 (0.6)	0 (0)
RC7	3502 (3.7)	1149 (32.8)	1015 (29)	121 (3.5)	39 (1.1)	23 (0.7)	0 (0)	24 (0.7)	4 (0.1)	0 (0)
RC18	3145 (3.3)	656 (20.9)	698 (22.2)	19 (0.6)	82 (2.6)	29 (0.9)	0 (0)	19 (0.6)	9 (0.3)	0 (0)
RC5	3111 (3.3)	899 (28.9)	529 (17)	81 (2.6)	58 (1.9)	27 (0.9)	16 (0.5)	7 (0.2)	10 (0.3)	12 (0.4)
RC19	2937 (3.1)	555 (18.9)	419 (14.3)	37 (1.3)	13 (0.4)	1 (0)	4 (0.1)	2 (0.1)	1 (0)	1 (0)

RC9	2906 (3.1)	879 (30.2)	410 (14.1)	24 (0.8)	12 (0.4)	66 (2.3)	0 (0)	4 (0.1)	2 (0.1)	0 (0)
RC17	2903 (3.1)	1038 (35.8)	487 (16.8)	4 (0.1)	19 (0.7)	1 (0)	2 (0.1)	0 (0)	0 (0)	0 (0)
RC12	2443 (2.6)	703 (28.8)	438 (17.9)	34 (1.4)	66 (2.7)	12 (0.5)	9 (0.4)	14 (0.6)	5 (0.2)	2 (0.1)
RC16	2238 (2.4)	676 (30.2)	480 (21.4)	51 (2.3)	8 (0.4)	6 (0.3)	12 (0.5)	29 (1.3)	14 (0.6)	0 (0)
RC21	1444 (1.5)	353 (24.4)	394 (27.3)	12 (0.8)	2 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC11	531 (0.6)	100 (18.8)	143 (26.9)	2 (0.4)	3 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total National	95077	23629	17216	1611	1644	477	438	211	139	149

This distribution showed that isolates from the RC 6 had higher percentage isolate rate (5.5%) of *Salmonella* Typhi from blood than the rest of RCs (Table 1.7). *Salmonella* Paratyphi A isolate percentage was also more in RC 6 along with RC 10 (1.4%) as compared to other RCs. The relative distribution of Typhoidal *Salmonella* isolated from blood in the OPD, admitted to the wards and ICUs are presented in Table 1.8 and Figures 1.8. Typhoidal *Salmonella* was common isolate from the OPD (6%) followed by the wards and was least isolated from the ICU. (Table 1.8). Among Typhoidal Salmonella, *Salmonella* Typhi had higher percentage isolation rate than *Salmonella* Paratyphi A. Yearly isolation trends showed that there is a decline in isolation rates of *Salmonella* Typhi in 2021 from the last five years from all over India (Table 1.9 & Figure 1.9).

Table 1.7: Isolates percentages across Regional Centres of Typhoidal *Salmonella* isolated from Blood

Regional Centre	Total Blood Isolates n(%)	*Salmonella* Typhi n(%)	*Salmonella* Paratyphi A n(%)
RC2	2971 (15.6)	34 (1.1)	5 (0.2)
RC3	2418 (12.7)	33 (1.4)	17 (0.7)
RC4	2300 (12.1)	19 (0.8)	1 (0)
RC1	1683 (8.9)	20 (1.2)	3 (0.2)
RC6	904 (4.8)	67 (7.4)	13 (1.4)
RC17	893 (4.7)	7 (0.8)	0 (0)
RC10	880 (4.6)	37 (4.2)	12 (1.4)
RC15	783 (4.1)	8 (1)	1 (0.1)
RC19	780 (4.1)	0 (0)	0 (0)
RC14	696 (3.7)	17 (2.4)	1 (0.1)
RC8	621 (3.3)	0 (0)	1 (0.2)
RC5	615 (3.2)	27 (4.4)	3 (0.5)
RC13	590 (3.1)	3 (0.5)	0 (0)
RC9	576 (3)	3 (0.5)	0 (0)
RC18	572 (3)	0 (0)	0 (0)
RC21	435 (2.3)	0 (0)	0 (0)
RC7	409 (2.2)	0 (0)	0 (0)
RC12	392 (2.1)	12 (3.1)	0 (0)
RC11	192 (1)	0 (0)	0 (0)
RC20	160 (0.8)	5 (3.1)	1 (0.6)
RC16	118 (0.6)	1 (0.8)	0 (0)
Total National	18988	293	58

Table 1.8: Isolation Distribution of Typhoidal *Salmonella* from Blood location wise

Organism	Total	OPD	Ward	ICU
Total Typhoidal Salmonella	351/18988 (1.8)	162/2708 (6)	160/9377 (1.7)	29/6903 (0.4)
Salmonella Typhi	293/18988 (1.5)	132/2708 (4.9)	139/9377 (1.5)	22/6903 (0.3)
Salmonella Paratyphi A	58/18988 (0.3)	30/2708 (1.1)	21/9377 (0.2)	7/6903 (0.1)

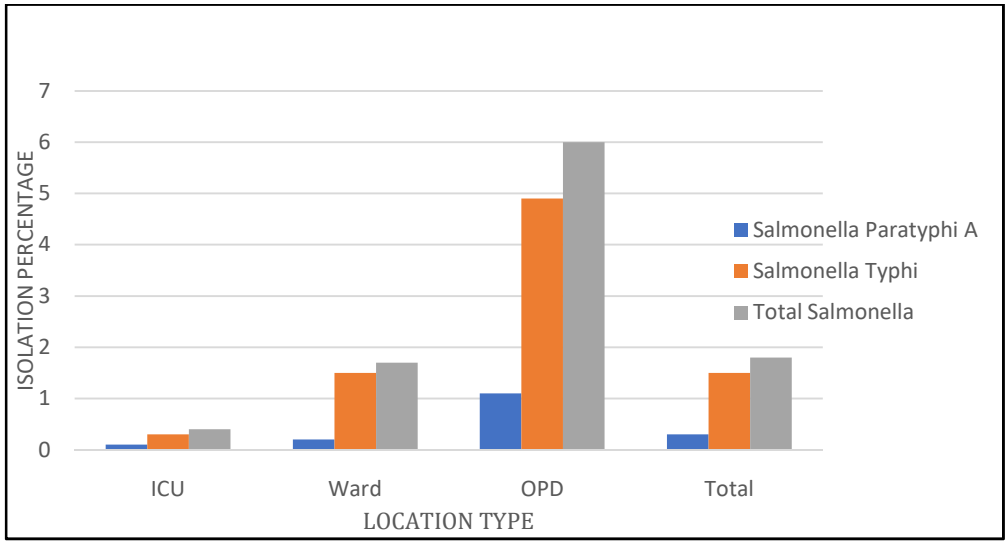


Figure 1.8: Location-wise Isolation pattern of Typhoidal *Salmonella* isolated from Blood across OPD, Ward and ICU

Table 1.9: Yearly-isolation trend of *Salmonella* Typhi from Blood across different regions

Years	2016	2017	2018	2019	2020	2021
North	12/636 (1.9%)	138/4272 (3.2%)	246/5248 (4.7%)	174/4533 (3.8%)	47/3479 (1.4%)	126/6498 (1.9%)
Central	0/0* (-)	0/0* (-)	12/110 (10.9%)	36/570 (6.3%)	14/448 (3.1%)	12/584 (2.1%)
East	0/0* (-)	0/171* (0%)	2/712 (0.3%)	4/1443 (0.3%)	1/935 (0.1%)	1/1746 (0.1%)
West	0/0* (-)	31/648 (4.8%)	116/2011 (5.8%)	164/2761 (5.9%)	41/2041 (2%)	41/2973 (1.4%)
South	25/989 (2.5%)	176/4400 (4%)	204/6018 (3.4%)	350/8033 (4.4%)	103/6206 (1.7%)	113/7187 (1.6%)
National	37/1625 (2.3%)	345/9491 (3.6%)	580/14099 (4.1%)	728/17340 (4.2%)	206/13109 (1.6%)	293/18988 (1.5%)

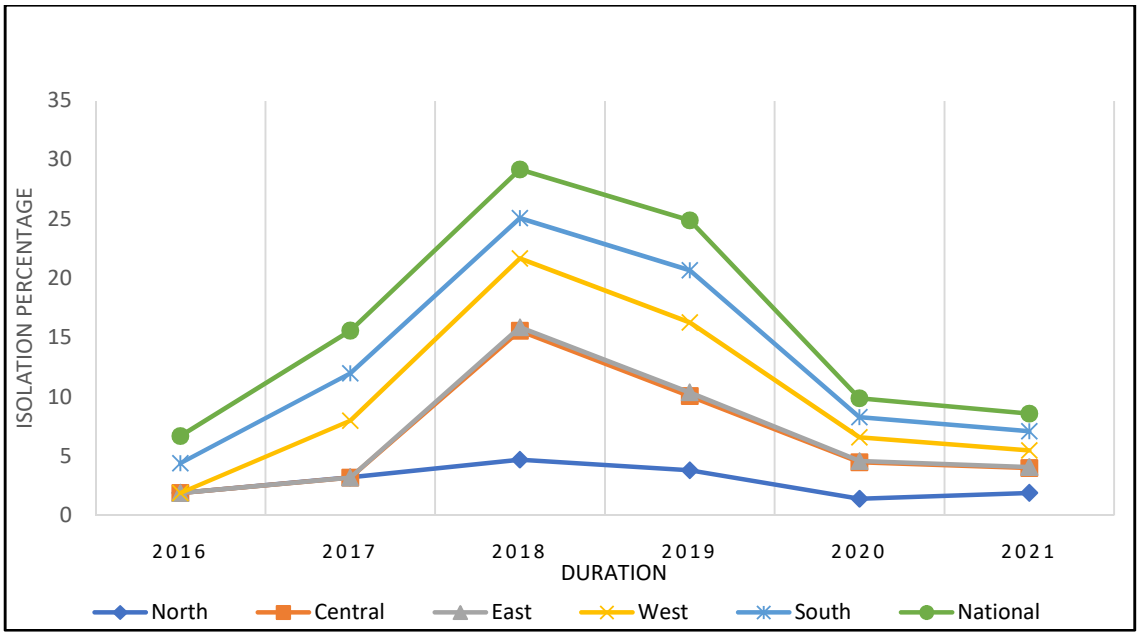


Figure 1.9: Yearly-isolation trends of *Salmonella* Typhi from Blood across different regions

Non-fermenting Gram negative bacteria

Non-fermenting Gram negative bacteria (NFGNB) constituted 27.4% of the total isolates (26,185 out of 95.728) (Table 1.10). Among the NFGNB, *Acinetobacter baumannii* was the commonest isolate (12.9%) followed by *Pseudomonas aeruginosa* (12.1%). *Stenotrophomonas maltophilia* and *Burkholderia cepacia* accounted for 0.8% and 0.3% of all isolates respectively. *Acinetobacter baumannii* was the predominant isolate from LRT (31.7%) and CSF (25.41%) followed by blood (13.9%). *Pseudomonas aeruginosa* was grossly predominant in LRT (19.7%) followed by superficial infection (15.6), deep infections (13.4%) and others (12.8) (Table 1.10 and Figure 1.10).

Regional center (RC) wise distribution showed that RC 11 had higher percentage isolate rate of *Acinetobacter baumannii* and RC 3 had higher percentage isolate rate of *Pseudomonas aeruginosa* than the rest of RCs (Table 1.11). Among clinical settings, *P. aeruginosa* was predominantly isolated in all ward, ICU and OPD (11.8-13.1%), while *A. baumannii* was predominant in ICU (25.5%), followed by ward (11.3%) and OPD (5.6%) respectively (Table 1.12a and Figure 1.11).

However, trend analysis over the years 2016 – 2021 has shown a stable pattern in the isolation rates of *P. aeruginosa* from 11.9% to 12.1% in 2016 to 2021, respectively (Table 1.12b). In contrast, isolation rates of *A. baumannii* increased from 5% to 12.9% between 2016 and 2021 respectively. No significant changes in the isolation rates of other pathogens such as *B. cepacia* and *S. maltophilia* have been noted (Figure 1.12).

Table 1.10: Specimen wise distribution of NFGNB

Isolate	Culture positive																			
	Total n=95728		Blood n=18988		Urine n=19319		LRT n=16746		Superficial Infection n=19592		Deep Infection n=8125		CSF n=995		SS n=2787		Faeces n=651		Others n=8525	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
NFGNB	26185 (27.4)	100	4720 (24.9)	18	1955 (10.1)	7.5	9161 (54.7)	35	5339 (27.3)	20.4	1987 (24.5)	7.6	437 (43.9)	1.7	650 (23.3)	2.5	0 (0)	0	1936 (22.7)	7.4
Acinetobacter baumannii	12393 (12.9)	100	2653 (13.9)	21.4	440 (2.3)	3.6	5313 (31.7)	42.9	1937 (9.9)	15.6	762 (9.4)	6.1	253 (25.4)	2	328 (11.8)	2.7	0 (0)	0	707 (8.3)	5.7
Pseudomonas aeruginosa	11622 (12.1)	100	1336 (7)	11.5	1398 (7.2)	12	3291 (19.7)	28.3	3066 (15.6)	26.4	1085 (13.4)	9.3	111 (11.2)	1	244 (8.8)	2.1	0 (0)	0	1091 (12.8)	9.4
Stenotrophomonas maltophilia	766 (0.8)	100	235 (1.2)	30.7	12 (0.1)	1.6	262 (1.6)	34.2	102 (0.5)	13.3	58 (0.7)	7.6	16 (1.6)	2.1	32 (1.1)	4.2	0 (0)	0	49 (0.6)	6.4
Burkholderia cepacia	247 (0.3)	100	147 (0.8)	59.5	9 (0)	3.6	61 (0.4)	24.7	8 (0)	3.2	5 (0.1)	2	0 (0)	0	6 (0.2)	2.4	0 (0)	0	11 (0.1)	4.5

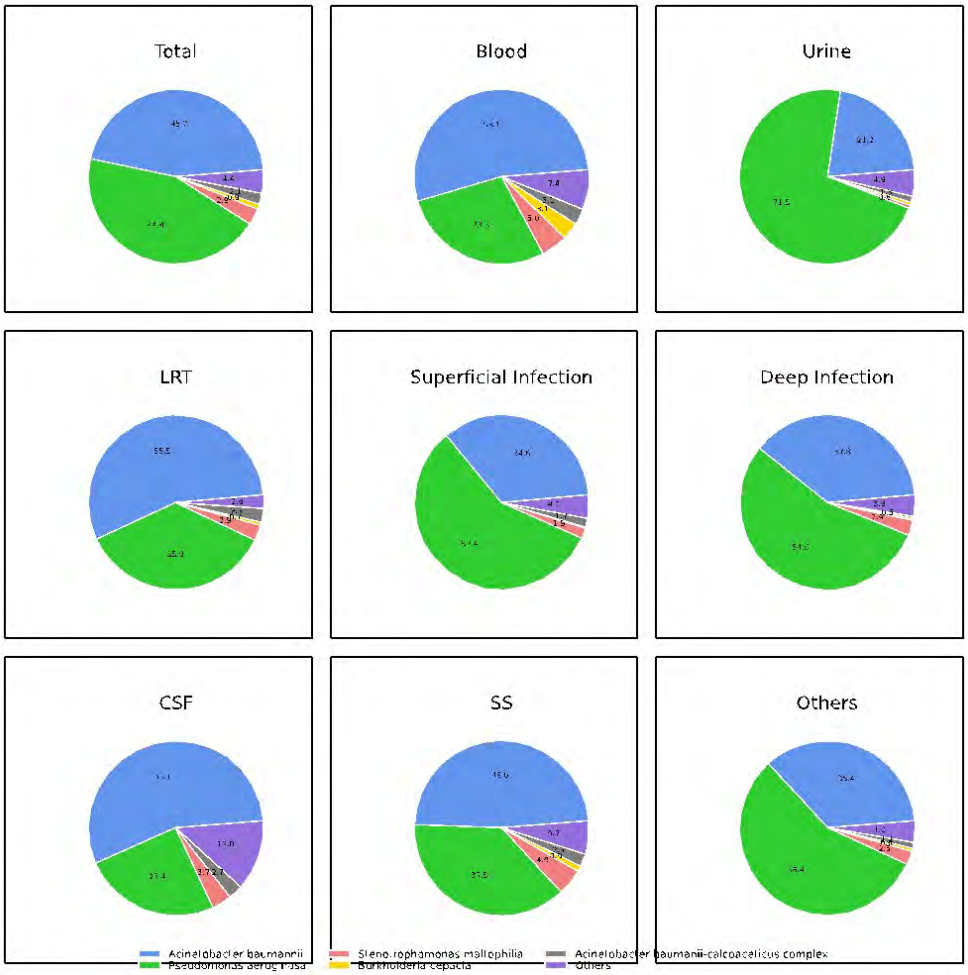


Figure 1.10: Specimen wise distribution of NFGNB (Percentage calculated from total of NFGNB isolates)

Table 1.11: Isolates percentages across Regional Centres of *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and *Burkholderia cepacia* from all specimens (except Faeces)

Regional Centre	Total Isolates	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Stenotrophomonas maltophilia*	*Burkholderia cepacia*
	n(%)	n(%)	n(%)	n(%)	n(%)
RC2	13597 (14.3)	2734 (20.1)	1037 (7.6)	93 (0.7)	46 (0.3)
RC4	13391 (14.1)	1690 (12.6)	1755 (13.1)	297 (2.2)	11 (0.1)
RC1	7327 (7.7)	1406 (19.2)	1053 (14.4)	203 (2.8)	64 (0.9)
RC14	6147 (6.5)	233 (3.8)	596 (9.7)	10 (0.2)	2 (0)
RC6	4987 (5.2)	399 (8)	847 (17)	40 (0.8)	11 (0.2)
RC15	4963 (5.2)	804 (16.2)	667 (13.4)	1 (0)	13 (0.3)
RC3	4698 (4.9)	704 (15)	875 (18.6)	0 (0)	0 (0)
RC13	4657 (4.9)	695 (14.9)	609 (13.1)	4 (0.1)	1 (0)
RC10	4346 (4.6)	253 (5.8)	517 (11.9)	19 (0.4)	15 (0.3)
RC20	3762 (4)	654 (17.4)	438 (11.6)	0 (0)	0 (0)
RC7	3502 (3.7)	203 (5.8)	611 (17.4)	0 (0)	27 (0.8)
RC18	3145 (3.3)	397 (12.6)	263 (8.4)	14 (0.4)	2 (0.1)
RC5	3111 (3.3)	76 (2.4)	478 (15.4)	36 (1.2)	15 (0.5)
RC19	2937 (3.1)	507 (17.3)	300 (10.2)	4 (0.1)	5 (0.2)
RC9	2906 (3.1)	311 (10.7)	381 (13.1)	0 (0)	0 (0)

RC17	2903 (3.1)	323 (11.1)	265 (9.1)	0 (0)	0 (0)
RC12	2443 (2.6)	337 (13.8)	248 (10.2)	25 (1)	10 (0.4)
RC16	2238 (2.4)	165 (7.4)	184 (8.2)	0 (0)	16 (0.7)
RC8	2042 (2.1)	135 (6.6)	326 (16)	2 (0.1)	3 (0.1)
RC21	1444 (1.5)	231 (16)	117 (8.1)	12 (0.8)	3 (0.2)
RC11	531 (0.6)	136 (25.6)	55 (10.4)	6 (1.1)	3 (0.6)
Total National	95077	12393	11622	766	247

Table 1.12a: Location-wise isolates percentage of *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and *Burkholderia cepacia* from all samples across OPD, Ward and ICU

Organism	Total	OPD	Ward	ICU
Acinetobacter baumannii	12393/95728 (12.9)	1331/23643 (5.6)	5842/51633 (11.3)	5220/20452 (25.5)
Pseudomonas aeruginosa	11622/95728 (12.1)	3098/23643 (13.1)	6099/51633 (11.8)	2425/20452 (11.9)
Stenotrophomonas maltophilia	766/95728 (0.8)	91/23643 (0.4)	414/51633 (0.8)	261/20452 (1.3)
Burkholderia cepacia	247/95728 (0.3)	27/23643 (0.1)	64/51633 (0.1)	156/20452 (0.8)

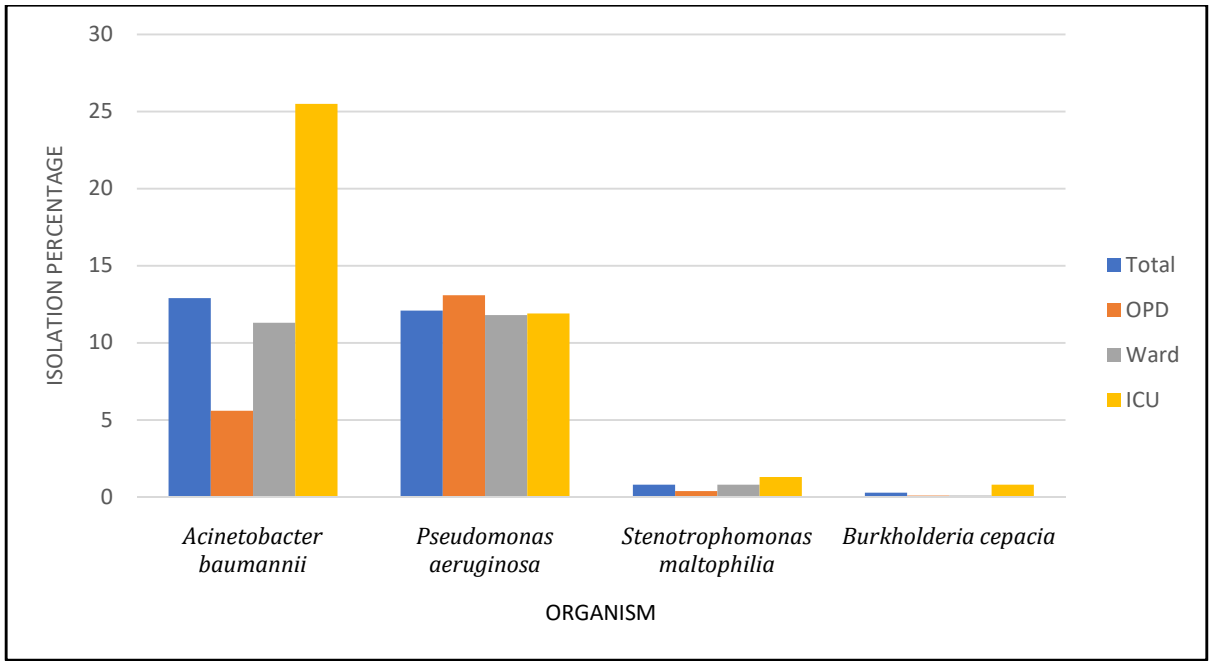


Figure 1.11: Location-wise isolation pattern of *A. baumannii, B. cepacia, P. aeruginosa,* and *S. maltophilia* isolated from all samples

Table 1.12b: Yearly Isolation trend of *P. aeruginosa, A. baumannii, S. maltophilia* and *B. cepacia* isolated from all samples

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)	Year-2021 (%)
Acinetobacter baumannii	556/11604 (4.8)	3361/45521 (7.4)	4550/74295 (6.1)	8533/108465 (7.9)	6851/65561 (10.4)	12393/95728 (12.9)
Pseudomonas aeruginosa	1380/11604 (11.9)	5689/45521 (12.5)	8883/74295 (12)	12638/108465 (11.7)	7843/65561 (12)	11622/95728 (12.1)
Stenotrophomonas maltophilia	33/11604 (0.3)	157/45521 (0.3)	310/74295 (0.4)	374/108465 (0.3)	360/65561 (0.5)	766/95728 (0.8)
Burkholderia cepacia	30/11604 (0.3)	112/45521 (0.2)	197/74295 (0.3)	181/108465 (0.2)	200/65561 (0.3)	247/95728 (0.3)

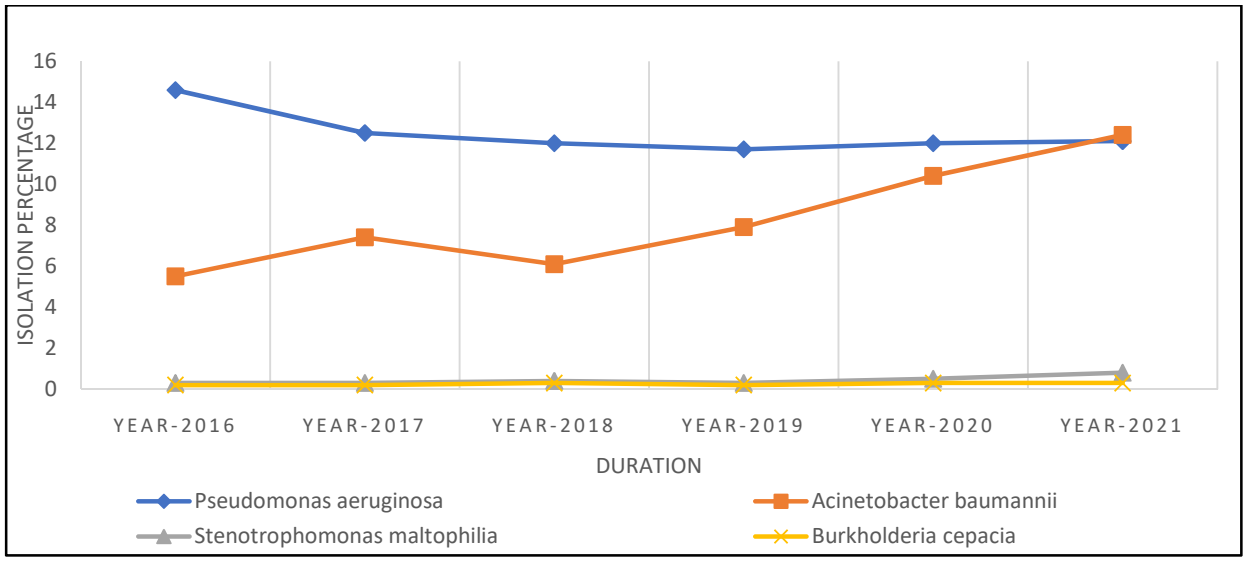


Figure 1.12: Yearly Isolation trend of *P. aeruginosa, A. baumannii, S. maltophilia* and *B. cepacia* isolated from all samples

Staphylococci

Staphylococci constituted 12% of the total isolates (Table 1.13). *Staphylococcus aureus* was the predominant species in the deep infections (19.2%), superficial infections (19%), miscellaneous infections (9.1%), blood (8.8%), LRT (4.2), sterile body fluids (3.9%), and urine (1.2%) (Table 1.13). Coagulase-negative staphylococci (CoNS) were the predominant isolates in blood (10.5%) and CSF (4.4%) reflecting the high incidence of shunt infections and intra vascular device associated infections respectively. In blood and CSF, *Staphylococcus epidermidis* isolation rate was 2.1% and 1.4% respectively, reflecting the ability of the species to form biofilms and high incidence of shunt associated and dialysis associated infections. Predominant percentage isolation of methicillin resistant *Staphylococcus aureus* (MRSA) was from the superficial infections (SI) 7.3%, followed by isolation from deep infection (DI)7% and 3.7% from blood. Methicillin sensitive *Staphylococcus aureus* (MSSA) were the predominant isolates from the Deep infections (DI) (12.1%) followed by isolation from superficial infection (SI) 11.5%, 5.8% and 5% from others and blood respectively (Figure 1.13). Amongst the coagulase-negative staphylococci (CoNS), *S. haemolyticus* (31.48%) were the commonest species followed by *S. epidermidis* (22.4%) and *S. hominis* (15.06 %) (Table1.13). Regional centre wise distribution showed the predominance of isolation of *Staphylococcus aureus* in RC18 (17%) with MRSA percentage isolation (11.7%). The least percentage isolation of *Staphylococcus aureus*and MRSA was found among RC 7 and RC 11 i.e., 4.1% and 1.9-2.4% respectively (Table 1.14).

Among clinical settings, *Staphylococcus aureus* was predominantly isolated in OPD (13.2%), followed by ward (8.9%) and ICU (5.5%), while the coagulase-negative staphylococci (CoNS) was predominant in ward (2.9%), followed by ICU and OPD (2.6%) (Table 1.15 and Figure 1.14). Trend analysis over the years 2016 – 2021 have shown a steady decline in the isolation rates of *Staphylococcus aureus* from 13% to 9.2% in 2017 to 2021respectively (Table 1.16 and Figure 1.15).

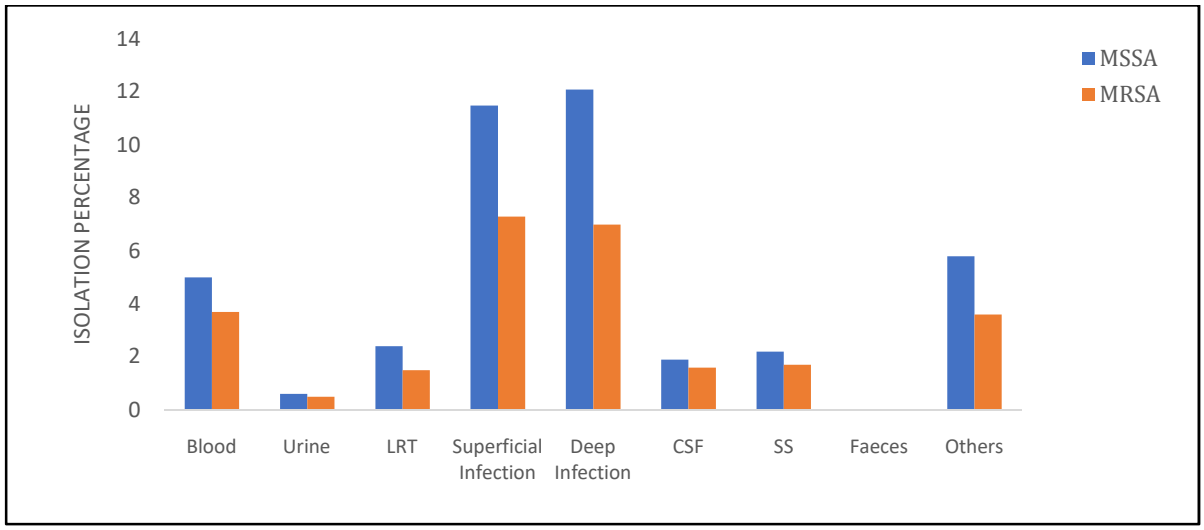


Figure1.13: Specimen wise relative distribution of MSSA and MRSA

Table 1.13: Specimen wise relative distribution of *S. aureus* and CoNS species

Isolate	Culture positive																			
	Total n=95728		Blood n=18988		Urine n=19319		LRT n=16746		Superficial Infection n=19592		Deep Infection n=8125		CSF n=995		SS n=2787		Faeces n=651		Others n=8525	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Staphylococcus aureus	8827 (9.2)	100	1663 (8.8)	18.8	230 (1.2)	2.6	701 (4.2)	7.9	3719 (19)	42.1	1563 (19.2)	17.7	35 (3.5)	0.4	109 (3.9)	1.2	0 (0)	0	807 (9.5)	9.1
MSSA	5273 (5.5)	100	944 (5)	17.9	113 (0.6)	2.1	410 (2.4)	7.8	2254 (11.5)	42.7	981 (12.1)	18.6	19 (1.9)	0.4	61 (2.2)	1.2	0 (0)	0	491 (5.8)	9.3
MRSA	3423 (3.6)	100	698 (3.7)	20.4	105 (0.5)	3.1	252 (1.5)	7.4	1434 (7.3)	41.9	566 (7)	16.5	16 (1.6)	0.5	46 (1.7)	1.3	0 (0)	0	306 (3.6)	8.9
CoNS	2655 (2.8)	100	1995 (10.5)	75.1	74 (0.4)	2.8	37 (0.2)	1.4	339 (1.7)	12.8	52 (0.6)	2	44 (4.4)	1.7	15 (0.5)	0.6	0 (0)	0	99 (1.2)	3.7
Staphylococcus haemolyticus	836 (0.9)	100	657 (3.5)	78.6	4 (0)	0.5	8 (0)	1	108 (0.6)	12.9	10 (0.1)	1.2	15 (1.5)	1.8	3 (0.1)	0.4	0 (0)	0	31 (0.4)	3.7
Staphylococcus epidermidis	595 (0.6)	100	391 (2.1)	65.7	5 (0)	0.8	8 (0)	1.3	138 (0.7)	23.2	7 (0.1)	1.2	14 (1.4)	2.4	2 (0.1)	0.3	0 (0)	0	30 (0.4)	5
Staphylococcus hominis	400 (0.4)	100	355 (1.9)	88.8	0 (0)	0	1 (0)	0.3	26 (0.1)	6.5	4 (0)	1	8 (0.8)	2	1 (0)	0.3	0 (0)	0	5 (0.1)	1.3
Staphylococcus spp.	669 (0.7)	100	497 (2.6)	74.3	28 (0.1)	4.2	20 (0.1)	3	53 (0.3)	7.9	29 (0.4)	4.3	6 (0.6)	0.9	8 (0.3)	1.2	0 (0)	0	28 (0.3)	4.2
Staphylococci	11482 (12)	100	3658 (19.3)	31.9	304 (1.6)	2.6	738 (4.4)	6.4	4058 (20.7)	35.3	1615 (19.9)	14.1	79 (7.9)	0.7	124 (4.4)	1.1	0 (0)	0	906 (10.6)	7.9

Table 1.14 Isolates percentages across Regional Centres of *S. aureus*, MRSA, MSSA and CoNS species isolated from all samples (Except Faeces)

Regional Centre	Total Isolates	*S. aureus*	MRSA	MSSA	*Staphylococcus haemolyticus*	*Staphylococcus epidermidis*	*Staphylococcus hominis*	*Staphylococcus lugdunensis*	*Staphylococcus saprophyticus*	*Staphylococcus spp.*
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC2	13597 (14.3)	1675 (12.3)	377 (2.8)	1255 (9.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC4	13391 (14.1)	1376 (10.3)	325 (2.4)	1050 (7.8)	51 (0.4)	38 (0.3)	8 (0.1)	3 (0)	0 (0)	6 (0)
RC1	7327 (7.7)	482 (6.6)	202 (2.8)	279 (3.8)	266 (3.6)	239 (3.3)	152 (2.1)	1 (0)	3 (0)	7 (0.1)
RC14	6147 (6.5)	826 (13.4)	351 (5.7)	475 (7.7)	0 (0)	8 (0.1)	0 (0)	0 (0)	20 (0.3)	0 (0)
RC6	4987 (5.2)	254 (5.1)	144 (2.9)	110 (2.2)	27 (0.5)	42 (0.8)	18 (0.4)	1 (0)	0 (0)	0 (0)
RC15	4963 (5.2)	525 (10.6)	176 (3.5)	348 (7)	2 (0)	0 (0)	1 (0)	0 (0)	0 (0)	17 (0.3)
RC3	4698 (4.9)	337 (7.2)	119 (2.5)	218 (4.6)	2 (0)	5 (0.1)	0 (0)	0 (0)	0 (0)	389 (8.3)
RC13	4657 (4.9)	309 (6.6)	141 (3)	135 (2.9)	7 (0.2)	4 (0.1)	3 (0.1)	0 (0)	0 (0)	131 (2.8)
RC10	4346 (4.6)	359 (8.3)	105 (2.4)	242 (5.6)	6 (0.1)	15 (0.3)	2 (0)	2 (0)	1 (0)	4 (0.1)
RC20	3762 (4)	366 (9.7)	289 (7.7)	73 (1.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC7	3502 (3.7)	142 (4.1)	65 (1.9)	66 (1.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
RC18	3145 (3.3)	536 (17)	369 (11.7)	167 (5.3)	13 (0.4)	2 (0.1)	7 (0.2)	0 (0)	1 (0)	0 (0)
RC5	3111 (3.3)	291 (9.4)	98 (3.2)	189 (6.1)	31 (1)	71 (2.3)	32 (1)	2 (0.1)	2 (0.1)	30 (1)
RC19	2937 (3.1)	167 (5.7)	88 (3)	79 (2.7)	269 (9.2)	104 (3.5)	132 (4.5)	0 (0)	4 (0.1)	7 (0.2)
RC9	2906 (3.1)	273 (9.4)	103 (3.5)	161 (5.5)	52 (1.8)	18 (0.6)	21 (0.7)	110 (3.8)	1 (0)	2 (0.1)
RC17	2903 (3.1)	267 (9.2)	134 (4.6)	133 (4.6)	57 (2)	2 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)

RC12	2443 (2.6)	200 (8.2)	80 (3.3)	112 (4.6)	0 (0)	2 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
RC16	2238 (2.4)	221 (9.9)	163 (7.3)	56 (2.5)	0 (0)	1 (0)	0 (0)	0 (0)	1 (0)	66 (2.9)
RC8	2042 (2.1)	104 (5.1)	33 (1.6)	71 (3.5)	33 (1.6)	33 (1.6)	23 (1.1)	0 (0)	2 (0.1)	0 (0)
RC21	1444 (1.5)	95 (6.6)	48 (3.3)	47 (3.3)	18 (1.2)	11 (0.8)	1 (0.1)	1 (0.1)	0 (0)	10 (0.7)
RC11	531 (0.6)	22 (4.1)	13 (2.4)	7 (1.3)	2 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total National	95077	8827	3423	5273	836	595	400	120	35	669

Table 1.15: Location-wise isolates percentage of *S. aureus*, MSSA, MRSA and CoNS from all samples across OPD, Ward and ICU

Organism	Total	OPD	Ward	ICU
Total staphylococci	11482/95728 (12)	3742/23643 (15.8)	6078/51633 (11.8)	1662/20452 (8.1)
Staphylococcus aureus	8827/95728 (9.2)	3132/23643 (13.2)	4573/51633 (8.9)	1122/20452 (5.5)
MSSA	5273/95728 (5.5)	1965/23643 (8.3)	2602/51633 (5)	706/20452 (3.5)
MRSA	3423/95728 (3.6)	1125/23643 (4.8)	1916/51633 (3.7)	382/20452 (1.9)
CoNS	2655/95728 (2.8)	610/23643 (2.6)	1505/51633 (2.9)	540/20452 (2.6)

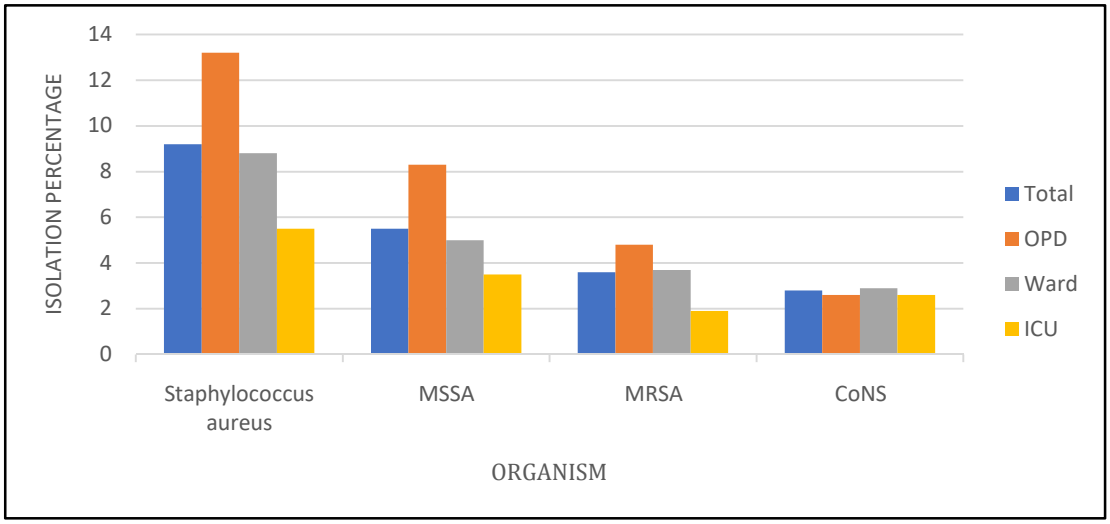


Figure 1.14: Location-wise Isolation pattern of *Staphylococcus aureus*, CoNS, MRSA, MSSA isolated from all samples

Table 1.16: Yearly isolation trend of *Staphylococcus* species

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)	Year-2021 (%)
Total staphylococci	2723/11604 (23.5)	8564/45714 (18.7)	12950 (17.2)	16277/110264 (14.8)	5163/65561 (12.7)	11482/95728 (12)
S. aureus	1978/11604 (17)	5722/45714 (12.5)	8782/75182 (11.8)	12623/110264 (11.4)	6293/65561 (9.6)	8827/95728 (9.2)
MRSA	1362/11604 (11.7)	1874/45714 (4.1)	3549 (4.7)	5353/110264 (4.9)	2622/65561 (4)	3423/95728 (3.6)
MSSA	612/11604 (5.3)	3820/45714 (8.4)	5233 (7)	7149/110264 (6.5)	3671/65561 (5.6)	5273/95728 (5.5)
CoNS	745/11604 (6.4)	2842/45714 (6.2)	4076 (5.4)	3654/110264 (3.3)	1966/65561 (3)	2655/95728 (2.8)
S. haemolyticus	46/11604 (0.4)	634/45714 (1.4)	871/75182 (1.2)	827/110264 (0.8)	626/65561 (0.9)	836/95728 (0.9)
S. epidermidis	87/11604 (0.7)	579/45714 (1.3)	912/75182 (1.2)	730/110264 (0.7)	397/65561 (0.6)	595/95728 (0.6)
S. hominis	34/11604 (0.3)	383/45714 (0.8)	490/75182 (0.7)	451/110264 (0.4)	313/65561 (0.5)	400/95728 (0.4)

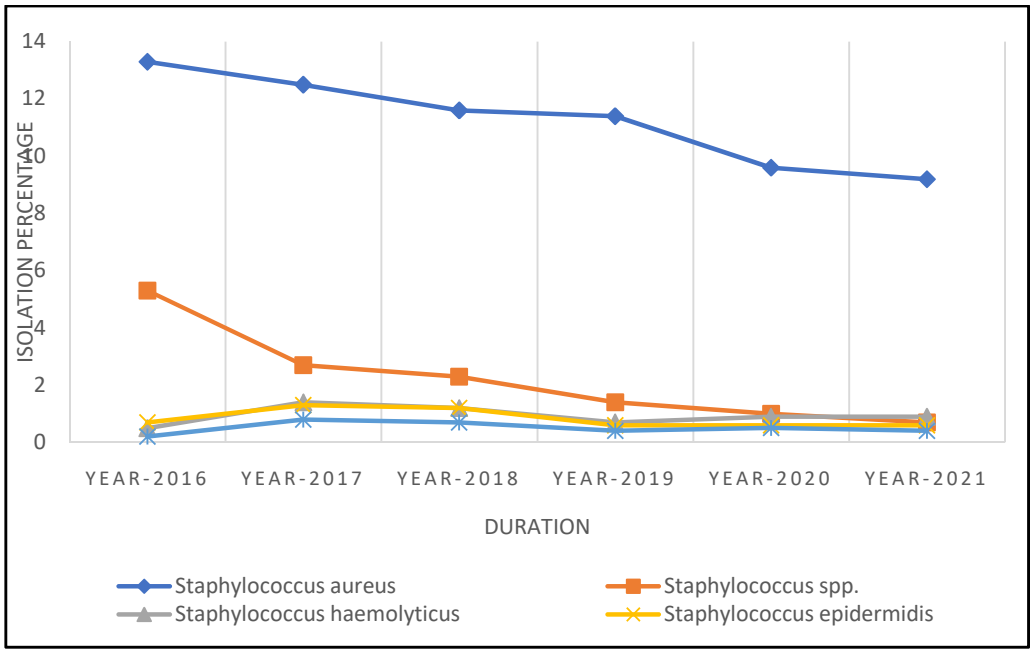


Figure 1.15 Yearly isolation trends of *Staphylococcus* species

Enterococci

Enterococci constituted overall 5.9% of all the isolates (Table 1.17). Among the *Enterococcus* species, *E. faecalis* and *E. faecium* accounted for 85% of all the total isolates, both *E. faecium* (42.89%) and *E. faecalis* (42.02%) were the predominant species. *E. faecium* was relatively more frequent in the CSF (4.6 %) and SS (4.4%) while *E. faecalis* was more frequent in the urine (4.5%) and CSF (3.5%) (Table 1.17 and Figure 1.16). Regional centre wise distribution showed the predominance of isolation of *E. faecalis* in RC10 (7.6%) and *E. faecium*in RC18 (6.2%) (Table 1.18).

The trend analysis over the years have shown a stable trend in the isolation rates of *E. faecium* from 2.5% to 2.5% in 2016 to 2021 and in *E. faecalis* from 2% to 3.2% in 2016 to 2020 respectively with a slight decrease from last year 3.2% in 2020 to 2.5% in 2021 to 2.5 % (Table 1.19 and Figure 1.17).

Table 1.17: Specimen wise distribution of *Enterococcus* species

	Total n=95728		Blood n=18988		Urine n=19319		LRT n=16746		Superficial Infection n=19592		Deep Infection n=8125		CSF n=995		SS n=2787		Faeces n=651	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enterococci	5647 (5.9)	100	1332 (7)	23.6	1939 (10)	34.3	65 (0.4)	1.2	1072 (5.5)	19	309 (3.8)	5.5	101 (10.2)	1.8	257 (9.2)	4.6	572 (6.7)	10.1
Enterococcus faecium	2422 (2.5)	100	700 (3.7)	28.9	811 (4.2)	33.5	20 (0.1)	0.8	402 (2.1)	16.6	109 (1.3)	4.5	46 (4.6)	1.9	124 (4.4)	5.1	210 (2.5)	8.7
Enterococcus faecalis	2373 (2.5)	100	472 (2.5)	19.9	871 (4.5)	36.7	14 (0.1)	0.6	546 (2.8)	23	129 (1.6)	5.4	35 (3.5)	1.5	66 (2.4)	2.8	240 (2.8)	10.1
Enterococcus spp.	852 (0.9)	100	160 (0.8)	18.8	257 (1.3)	30.2	31 (0.2)	3.6	124 (0.6)	14.6	71 (0.9)	8.3	20 (2)	2.3	67 (2.4)	7.9	122 (1.4)	14.3

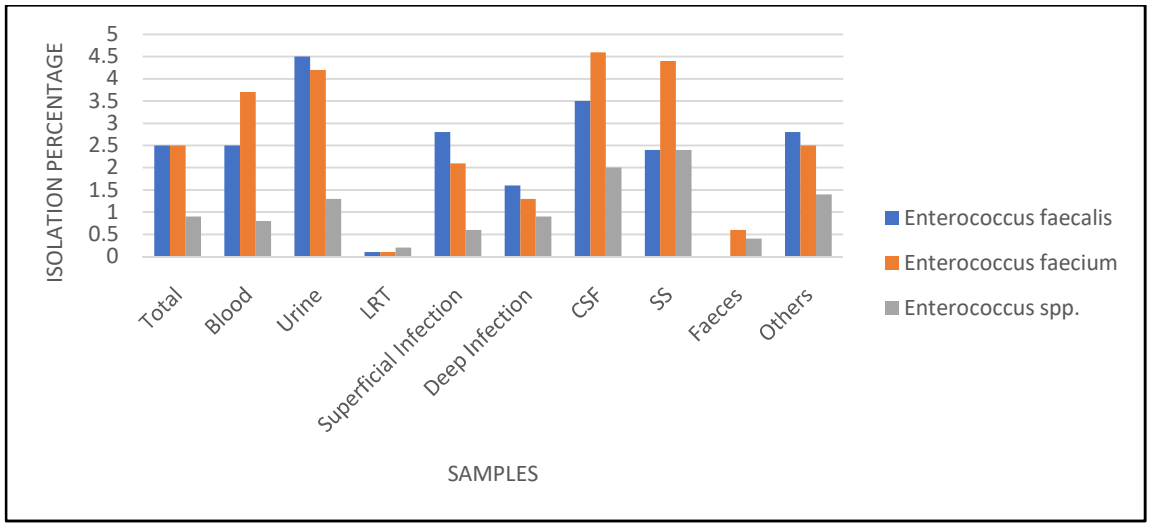


Figure 1.16: Specimen wise distribution of *Enterococcus* species

Table 1.18a. Location-wise isolation of *Enterococcus faecalis, Enterococcus faecium, Enterococcus* spp. from all Specimens (Except Faeces)

Organism	Total	OPD	Ward	ICU
Enterococcus faecalis	2373/95728 (2.5)	671/23643 (2.8)	1339/51633 (2.6)	363/20452 (1.8)
Enterococcus faecium	2422/95728 (2.5)	311/23643 (1.3)	1482/51633 (2.9)	629/20452 (3.1)
Enterococcus spp.	852/95728 (0.9)	179/23643 (0.8)	532/51633 (1)	141/20452 (0.7)

Table 1.18b. Isolates percentages across Regional Centres of *Enterococcus faecalis, Enterococcus faecium, Enterococcus* spp. from All Specimen (Except Faeces)

Regional Centre	Total Isolates (n=95077)	*Enterococcus faecalis* (n=2373)	*Enterococcus faecium* (n=2422)	Enterococcus spp. (n=852)
	n(%)	n(%)	n(%)	n(%)
RC2	13597 (14.3)	47 (0.3)	69 (0.5)	45 (0.3)
RC4	13391 (14.1)	731 (5.5)	476 (3.6)	114 (0.9)
RC1	7327 (7.7)	120 (1.6)	273 (3.7)	19 (0.3)
RC14	6147 (6.5)	124 (2)	64 (1)	14 (0.2)
RC6	4987 (5.2)	65 (1.3)	188 (3.8)	0 (0)
RC15	4963 (5.2)	26 (0.5)	67 (1.3)	22 (0.4)
RC3	4698 (4.9)	64 (1.4)	123 (2.6)	74 (1.6)
RC13	4657 (4.9)	6 (0.1)	25 (0.5)	342 (7.3)
RC10	4346 (4.6)	332 (7.6)	169 (3.9)	28 (0.6)
RC20	3762 (4)	98 (2.6)	65 (1.7)	116 (3.1)
RC7	3502 (3.7)	12 (0.3)	7 (0.2)	1 (0)
RC18	3145 (3.3)	102 (3.2)	194 (6.2)	0 (0)
RC5	3111 (3.3)	67 (2.2)	61 (2)	13 (0.4)
RC19	2937 (3.1)	156 (5.3)	133 (4.5)	2 (0.1)
RC9	2906 (3.1)	160 (5.5)	68 (2.3)	2 (0.1)
RC17	2903 (3.1)	80 (2.8)	109 (3.8)	1 (0)
RC12	2443 (2.6)	32 (1.3)	108 (4.4)	3 (0.1)
RC16	2238 (2.4)	119 (5.3)	109 (4.9)	20 (0.9)
RC8	2042 (2.1)	22 (1.1)	58 (2.8)	1 (0)
RC21	1444 (1.5)	7 (0.5)	32 (2.2)	35 (2.4)
RC11	531 (0.6)	3 (0.6)	24 (4.5)	0 (0)
Total	95077	2373	2422	852

Table 1.19: Yearly isolation trend of *Enterococcus* species

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)	Year-2021 (%)
Total Enterococcus	670/11604 (5.8)	2403/45521 (5.3)	4256/74295 (5.7)	6766/108465 (6.1)	4941/65561 (7.5)	5647/95728 (5.9)
Enterococcus faecium	288/11604 (2.5)	937/45521 (2.1)	1476/74295 (2)	2700/108465 (2.5)	1994/65561 (3)	2422/95728 (2.5)
Enterococcus faecalis	229/11604 (2)	1034/45521 (2.3)	2014/74295 (2.7)	2895/108465 (2.7)	2101/65561 (3.2)	2373/95728 (2.5)
Enterococcus spp.	153/11604 (1.3)	421/45521 (0.9)	711/74295 (1)	1079/108465 (1)	703/65561 (1.1)	852/95728 (0.9)

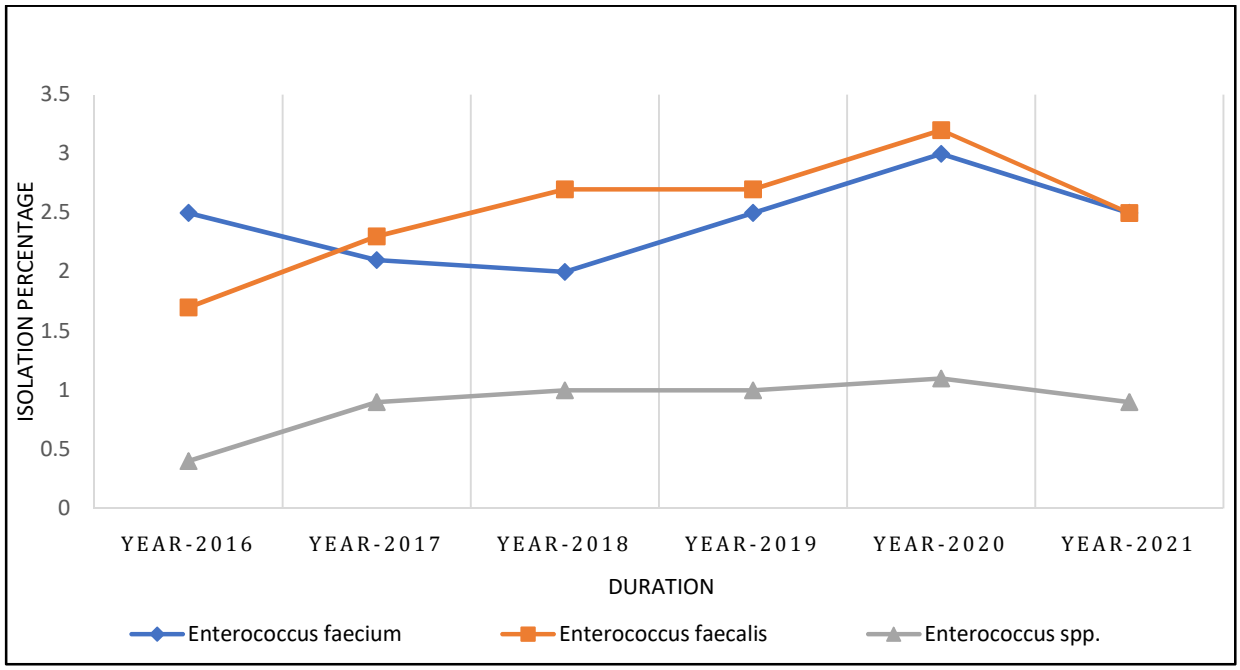


Figure 1.17 Yearly isolation trends of *Enterococcus* species

Fungal species

Total number of yeast isolates studied during the year 2021 was 2605, of those 53.2% (1386) were isolated from blood. Majority of the isolates were from *Candida tropicalis* (n=796) followed by *Candida albican*s (n=662) (Table 1.20). In the distribution of fungi species in different specimens, *C. tropicalis* was the predominant isolates in the genital (4.3%) followed by blood (2.3%), *Candida albican*s was also the predominant isolates in the genital (34.8%) followed by others (2.5) and blood (0.9%) (Table 1.20). Among clinical settings, in ICUs, *C. tropicalis and* were common isolates from the ICU (1.1%) and *C. albican*s from the ward (0.8%) (Table 1.21 and Figure 1.18).

Yearly isolation trend showed that there is a steady decline in isolation of *C. tropicalis* from 1.7% in 2016 to 0.8% in 2021, with a slight increase from last year 0.76 in 2020 to 0.8 in 2021. Yearly isolation trend *of Candida albican*s showed a steady decline from 1.2% in 2016 to 0.7 in 2021with a slight increase from last year 0.56 in 2020 to 0.7 in 2021. Both *C. auris and C. parapsilosis* isolates showed an increased trend from 2016 to 2021 (Table 1.22 & Figure 1.19).

Table 1.20. *Candida* species isolated from different sample types except faeces

Isolate	Total n=95728		Blood n=18988		Urine n=19319		LRT n=16746		Superficial Infection n=19592		Deep Infection n=8125		CSF n=995		Genital n=23		Others n=9497	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Fungal isolates	3452 (3.6)	100	1485 (7.8)	43	264 (1.4)	7.6	383 (2.3)	11.1	175 (0.9)	5.1	107 (1.3)	3.1	39 (3.9)	1.1	20 (87)	0.6	979 (10.3)	26.9
Candida tropicalis	796 (0.8)	100	445 (2.3)	55.9	103 (0.5)	12.9	30 (0.2)	3.8	53 (0.3)	6.7	14 (0.2)	1.8	0 (-)	0	1 (4.3)	0.1	115 (1.2)	14.4
Candida albicans	662 (0.7)	100	173 (0.9)	26.1	90 (0.5)	13.6	71 (0.4)	10.7	53 (0.3)	8	12 (0.1)	1.8	0 (-)	0	8 (34.8)	1.2	240 (2.5)	36.3
Candida glabrata	314 (0.3)	100	126 (0.7)	40.1	25 (0.1)	8	11 (0.1)	3.5	14 (0.1)	4.5	10 (0.1)	3.2	0 (-)	0	11 (47.8)	3.5	101 (1.1)	32.2
Candida parapsilosis	279 (0.3)	100	204 (1.1)	73.1	19 (0.1)	6.8	8 (0)	2.9	13 (0.1)	4.7	9 (0.1)	3.2	0 (-)	0	0 (0)	0	19 (0.2)	6.8
Candida auris	194 (0.2)	100	150 (0.8)	77.3	18 (0.1)	9.3	2 (0)	1	3 (0)	1.5	10 (0.1)	5.2	0 (-)	0	0 (0)	0	7 (0.1)	3.6
Candida utilis	174 (0.2)	100	172 (0.9)	98.9	1 (0)	0.6	0 (0)	0	0 (0)	0	0 (0)	0	0 (-)	0	0 (0)	0	1 (0)	0.6
Candida krusei	82 (0.1)	100	40 (0.2)	48.8	1 (0)	1.2	11 (0.1)	13.4	5 (0)	6.1	1 (0)	1.2	0 (-)	0	0 (0)	0	20 (0.2)	24.4
Candida pelliculosa	22 (0)	100	22 (0.1)	100	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (-)	0	0 (0)	0	0 (0)	0
Candida kefyr	13 (0)	-	4 (0)	-	6 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (-)	0	0 (0)	-	3 (0)	-
Candida lusitaniae	16 (0)	-	11 (0.1)	-	0 (0)	-	0 (0)	-	3 (0)	-	0 (0)	-	0 (-)	0	0 (0)	-	1 (0)	-
Candida	2605 (2.7)	100	1386 (7.3)	53.2	263 (1.4)	10.1	136 (0.8)	5.2	147 (0.8)	5.6	58 (0.7)	2.2	0 (-)	0	20 (87)	0.8	511 (5.4)	19.6

Notes:

1. Percentages are out of particular specimen (column).
2. Percentages in rows below Culture positive are out of Culture positive in respective columns.
3. **Blood** includes: Blood-central catheter, Blood-peripheral and Peripheral catheter-blood.
4. **LRT** (Lower Respiratory Tract) includes: BAL, Sputum, Lung aspirate, Endotracheal aspirate (ETA) and Lobectomy tissue (Lung tissue).
5. **Superficial Infection** includes: SST (Skin & Soft Tissue), Pus/exudate, Wound swab, Superficial Biopsy and Superficial Tissue.
6. **Deep Infection** includes: Abscess aspirate, Pus aspirate, Deep Biopsy and Deep Tissue.
7. **SS** (Sterile sites) includes: Fluid from sterile spaces, Abdominal fluid, Intracostal tube fluid, Pancreatic drain fluid, Pericardial fluid, Peritoneal fluid and Pleural fluid.

Table 1.21. *Candida* species isolated from all samples across OPD, Ward and ICUs

Organism	Total	OPD	Ward	ICU
Candida tropicalis	796/95728 (0.8)	83/23643 (0.4)	494/51633 (1)	219/20452 (1.1)
Candida albicans	662/95728 (0.7)	105/23643 (0.4)	409/51633 (0.8)	148/20452 (0.7)
Candida glabrata	314/95728 (0.3)	44/23643 (0.2)	194/51633 (0.4)	76/20452 (0.4)
Candida parapsilosis	279/95728 (0.3)	34/23643 (0.1)	158/51633 (0.3)	87/20452 (0.4)
Candida auris	194/95728 (0.2)	13/23643 (0.1)	100/51633 (0.2)	81/20452 (0.4)
Candida utilis	174/95728 (0.2)	3/23643 (0)	129/51633 (0.2)	42/20452 (0.2)
Candida krusei	82/95728 (0.1)	8/23643 (0)	60/51633 (0.1)	14/20452 (0.1)
Candida pelliculosa	22/95728 (0)	0/0 (-)	3/51633 (0)	19/20452 (0.1)
Candida lusitaniae	16/95728 (0)	1/23643 (0)	10/51633 (0)	5/20452 (0)
Candida kefyr	13/95728 (0)	1/23643 (0)	11/51633 (0)	1/20452 (0)

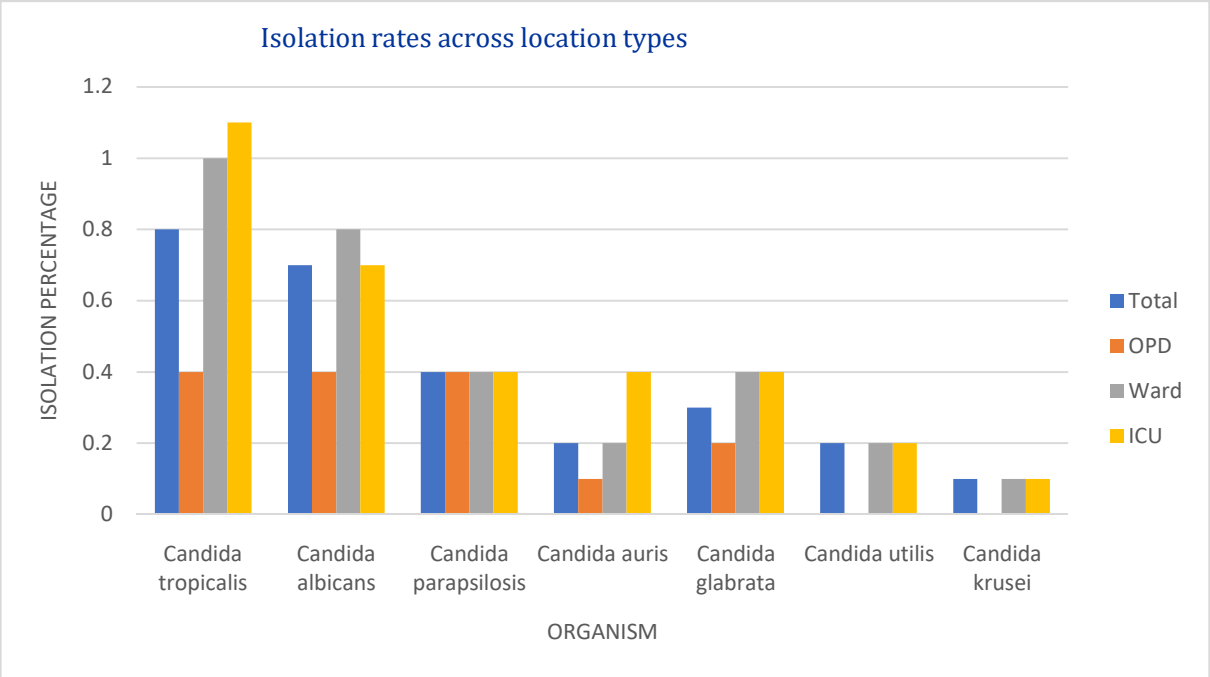


Figure 1.18. Location-wise pattern of *Candida* species isolated from all samples across OPD, Wards and ICUs.

Table 1.22 Yearly trends for isolation of *Candida* species isolated from all samples

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)	Year-2021 (%)
Total Candida	432/11604 (3.7)	1498/45521 (3.3)	1704/74295 (2.3)	2403/108465 (2.2)	1869/65561 (2.8)	2605/95728 (2.7)
Candida tropicalis	201/11604 (1.7)	628/45521 (1.38)	494/74295 (0.66)	621/108465 (0.57)	500/65561 (0.76)	796/95728 (0.8)
Candida albicans	145/11604 (1.2)	452/45521 (0.99)	560/74295 (0.75)	652/108465 (0.60)	364/65561 (0.56)	662/95728 (0.7)
Candida glabrata	47/11604 (0.4)	136/45521 (0.30)	179/74295 (0.24)	185/108465 (0.17)	113/65561 (0.17)	314/95728 (0.3)
Candida parapsilosis	25/11604 (0.2)	105/45521 (0.23)	134/74295 (0.18)	232/108465 (0.21)	189/65561 (0.29)	279/95728 (0.3)
Candida auris	0/11604 (0)	17/45521 (0.04)	55/74295 (0.07)	117/108465 (0.11)	121/65561 (0.18)	194/95728 (0.2)

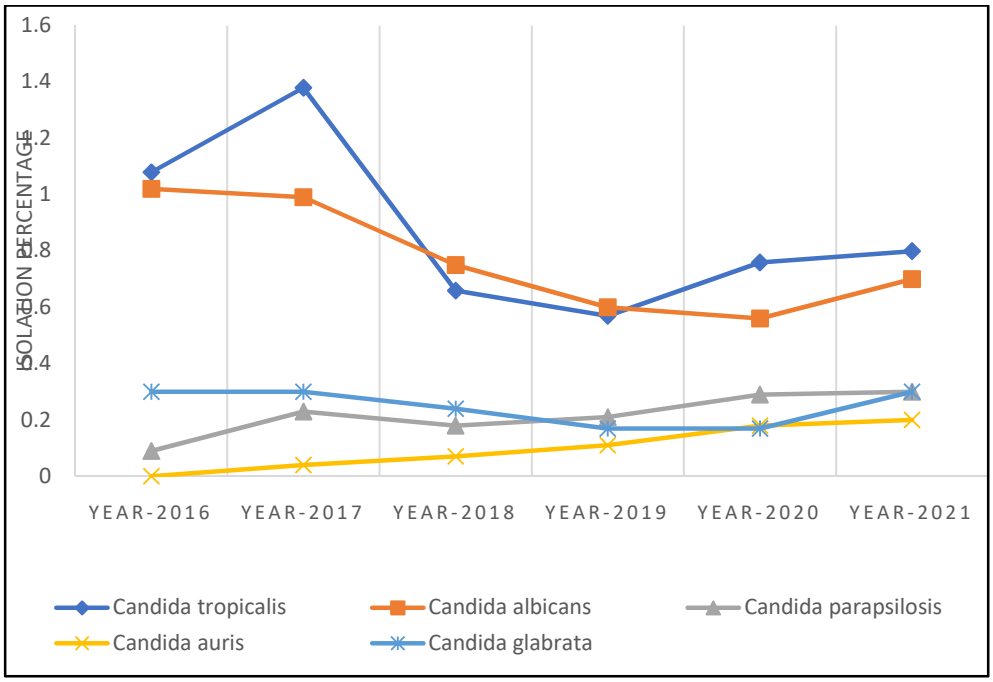


Figure 1.19: Yearly trends for isolation of *Candida* species isolated from all samples

Table 1.23 Isolation pattern of *Aspergillus* species from all specimens

Organism	Total n=95728
Aspergillus flavus	243 (0.3)
Aspergillus fumigatus	154 (0.2)
Aspergillus terreus	16 (0)
Aspergillus niger	12 (0)
Aspergillus versicolor	2 (0)

Diarrheal pathogens

A total of 714 diarrheal pathogen isolates were studied during the year 2021 which constituted 0.7% of total isolates (Table 1.1). The predominant species among diarrheal pathogens isolated from faeces sample identified was Salmonella spp Faecal (27.8%) followed by *Aeromonas spp* (27.5%), *Escherichia coli Diarrheagenic* (13.5%), *Shigella* (13.2%) and *Vibrio spp*(11.5%) (Table 1.24). From non-faecal specimens, *Aeromonas spp* was isolated (n=57) and constituted 0.1% of total cultures (Table 1.25).

Table 1.24: Isolation rates of faecal isolates from Faeces sample

Isolates	n	% Isolation from Faecal isolates (n= 651)	% Isolation from total positive cultures (n=95728)
Non Typhoidal Salmonella	222	34.1	0.23
Salmonella spp. Faecal	180	27.8	0.19
Aeromonas spp.	179	27.5	0.19
Escherichia coli Diarrhoeagenic	88	13.5	0.09
Shigella	86	13.2	0.09
Vibrio	74	11.5	0.08
Vibrio cholerae	58	8.9	0.06
Shigella sonnei	41	6.2	0.04
Salmonella Typhimurium Faecal	38	6	0.04
Shigella flexneri	37	5.7	0.03
Vibrio spp.	16	2.6	0.02
Salmonella Enteritidis	5	0.8	0
Shigella spp	4	0.6	0
Shigella boydii	4	0.6	0

Table 1.25 Isolation rates of Diarrhoeagenic pathogens from non-faecal specimen isolated in 2021

Isolates	n	% Isolation from total positive cultures except faeces (n=95077)
Aeromonas spp.	57	0.1
Escherichia coli Diarrhoeagenic	0	0
Shigella	2	0
Vibrio	1	0
Non Typhoidal *Salmonella*	3	0

Diarrheagenic pathogens were predominantly isolated from patients in OPD and wards (Table 1.26).Non Typhoidal Salmonella was mainly isolated in ICU (75%) followed by ward (40%) and OPD (25.2%).*Escherichia coli* Diarrheagenic was mainly isolated in OPD (23.7%) followed by ward (6%), while the *Aeromonas spp* was predominant in ward (28.2%), followed by OPD (26.6%) and ICU (25%)(Table 1.26 and Figure 1.20). *Shigella flexneri* was predominant in OPD and *Vibrio cholerae* in ward. The isolation trend over the period of five years (2016– 2021) showed a decreasing trend in the isolation of *Aeromonas spp*. whereas, the isolation trend of Non Typhoidal Salmonella and *Vibrio spp* showed an increasing trend from last year (Table 1.27and Figure 1.21).

Table 1.26: Location-wise Isolation pattern of top 5 faecal isolates isolated from Faeces across OPD, Ward and ICU

Organism	Total	OPD	Ward	ICU
Non Typhoidal Salmonella	222/651 (34.1)	70/278 (25.2)	146/365 (40)	6/8 (75)
Aeromonas spp.	179/651 (27.5)	74/278 (26.6)	103/365 (28.2)	2/8 (25)
Escherichia coli Diarrhoeagenic	88/651 (13.5)	66/278 (23.7)	22/365 (6)	0/0 (-)
Vibrio cholerae	58/651 (8.9)	10/278 (3.6)	48/365 (13.2)	0/0 (-)
Shigella flexneri	37/651 (5.7)	19/278 (6.8)	18/365 (4.9)	0/0 (-)

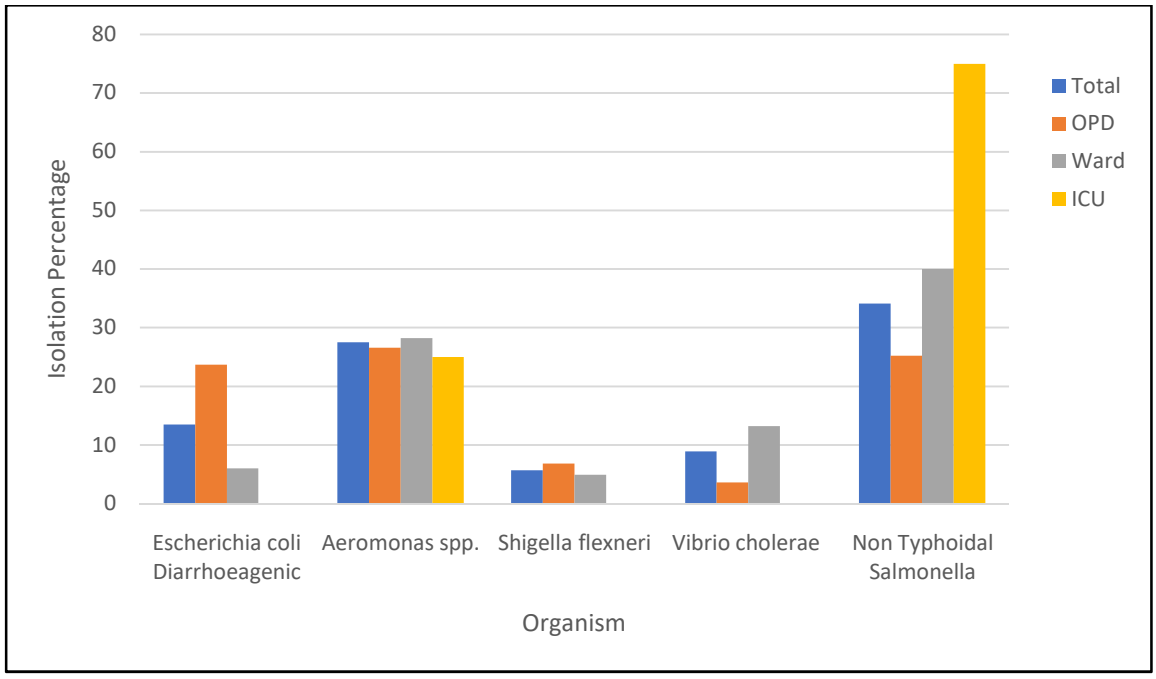


Figure 1.20: Location-wise Isolation pattern of top 5 faecal isolates isolated from Faeces across OPD, Ward and ICU

Table 1.27. Yearly Isolation trends of top 5 faecal isolates isolated from Faeces

Bacteria	Year-2016 (%)	Year-2017 (%)	Year-2018 (%)	Year-2019 (%)	Year-2020 (%)	Year-2021 (%)
Escherichia coli Diarrhoeagenic	0/55 (0)	0/501 (0)	0/621 (0)	134/1063 (12.6)	102/572 (17.8)	88/651 (13.5)
Aeromonas spp.	21/55 (38.2)	131/501 (26.1)	114/621 (18.4)	170/1063 (16)	77/572 (13.5)	179/651 (27.5)
Shigella flexneri	7/55 (12.7)	89/501 (17.8)	47/621 (7.6)	95/1063 (8.9)	55/572 (9.6)	37/651 (5.7)
Vibrio cholerae	1/55 (1.8)	24/501 (4.8)	25/621 (4)	39/1063 (3.7)	31/572 (5.4)	58/651 (8.9)
Non Typhoidal Salmonella	0/55 (0)	20/501 (4)	39/621 (6.3)	60/1063 (5.6)	24/572 (4.2)	222/651 (34.1)

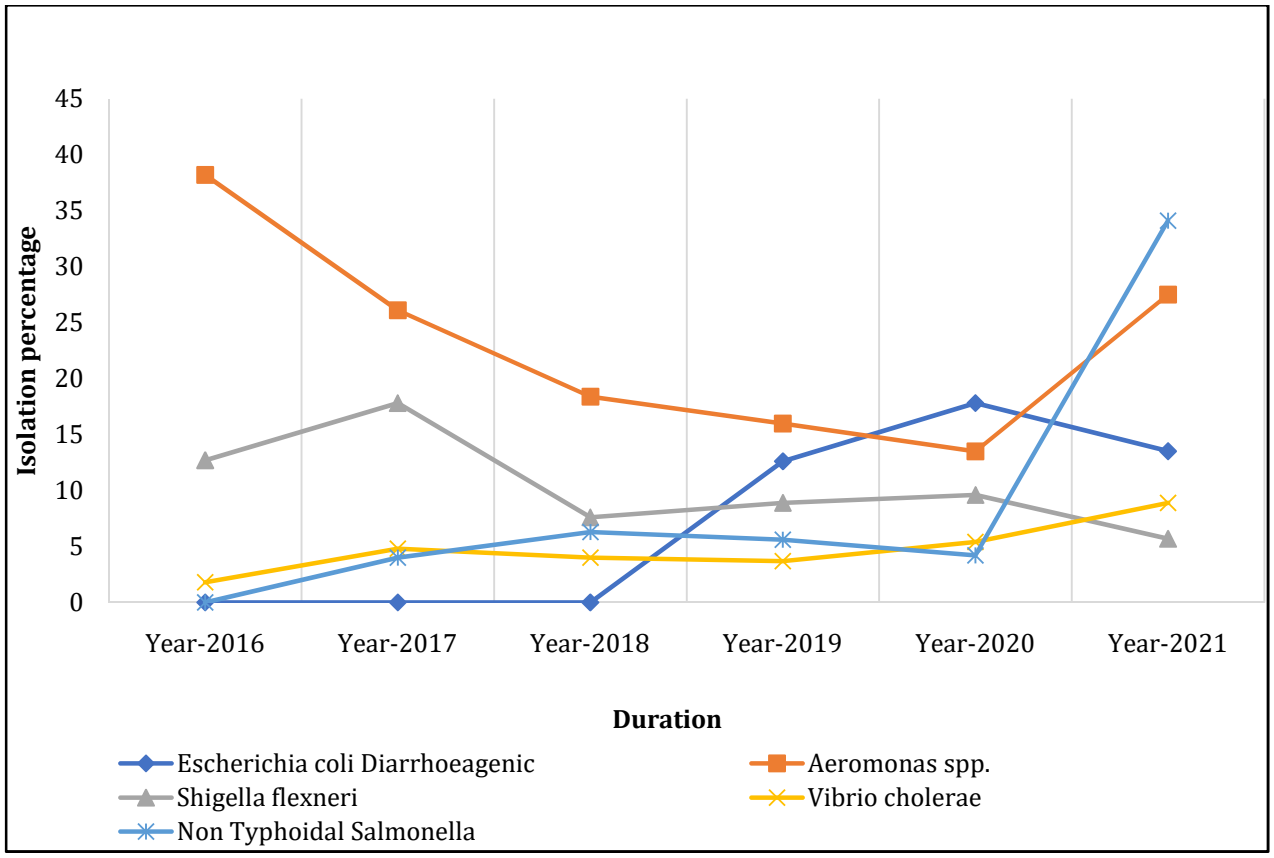


Figure 1.21: Yearly Isolation trends of top 5 faecal isolates isolated from Faeces

Streptococcus species

Total number of *Streptococcus* isolates studied during the year 2021 was 377, of those 1.3% (68) were isolated from the upper respiratory tract. Majority of the isolates were from *Streptococcus agalactiae* (n=148) followed by *Streptococcus pyogenes* (n=135) and *Streptococcus pneumoniae* (n=90) (Table 1.28). Among clinical settings, *Streptococcus* isolates were common isolates from the OPD (0.7%) followed by ward and ICU (Table 1.29 and Figure 1.22).

Table 1.28: Sample-wise Isolation pattern of *Streptococcus* species

Organism	All Specimens	Blood	LRT	Superficial Infection	Deep Infection	SS	Faeces	Urine	Upper respiratory tract	Others
Streptococcus	377/95728 (0.4)	41/18988 (0.2)	40/16746 (0.2)	137/19587 (0.7)	58/8125 (0.7)	1/2787 (0)	0/0 (-)	68/19319 (0.4)	5/382 (1.3)	27/9794 (0.3)
Streptococcus agalactiae	148/95728 (0.2)	9/18988 (0)	1/16746 (0)	49/19587 (0.3)	13/8125 (0.2)	0/0 (-)	0/0 (-)	60/19319 (0.3)	0/382 (0)	16/9794 (0.2)
Streptococcus pyogenes	135/95728 (0.1)	5/18988 (0)	1/16746 (0)	84/19587 (0.4)	37/8125 (0.5)	1/2787 (0)	0/0 (-)	0/0 (-)	3/382 (0.7)	4/9794 (0)
Streptococcus pneumoniae	90/95728 (0.1)	26/18988 (0.1)	38/16746 (0.2)	3/19587 (0)	7/8125 (0.1)	0/0 (-)	0/0 (-)	7/19319 (0)	2/382 (0.5)	7/9794 (0.1)
Streptococcus viridans	4/95728 (0)	1/18988 (0)	0/0 (-)	1/19587 (0)	1/8125 (0)	0/0 (-)	0/0 (-)	1/19319 (0)	0/382 (0)	0/9794 (0)

Table 1.29: Location-wise Isolation pattern of *Streptococcus* isolated from all specimens across OPD, Ward and ICU

Organism	Total	OPD	Ward	ICU
Streptococcus	377/95728 (0.4)	159/23643 (0.7)	166/51633 (0.3)	52/20452 (0.3)
Streptococcus agalactiae	148/95728 (0.2)	90/23643 (0.4)	44/51633 (0.1)	14/20452 (0.1)
Streptococcus pyogenes	135/95728 (0.1)	43/23643 (0.2)	71/51633 (0.1)	21/20452 (0.1)
Streptococcus pneumoniae	90/95728 (0.1)	24/23643 (0.1)	50/51633 (0.1)	16/20452 (0.1)
Streptococcus viridans	4/95728 (0)	2/23643 (0)	1/51633 (0)	1/20452 (0)

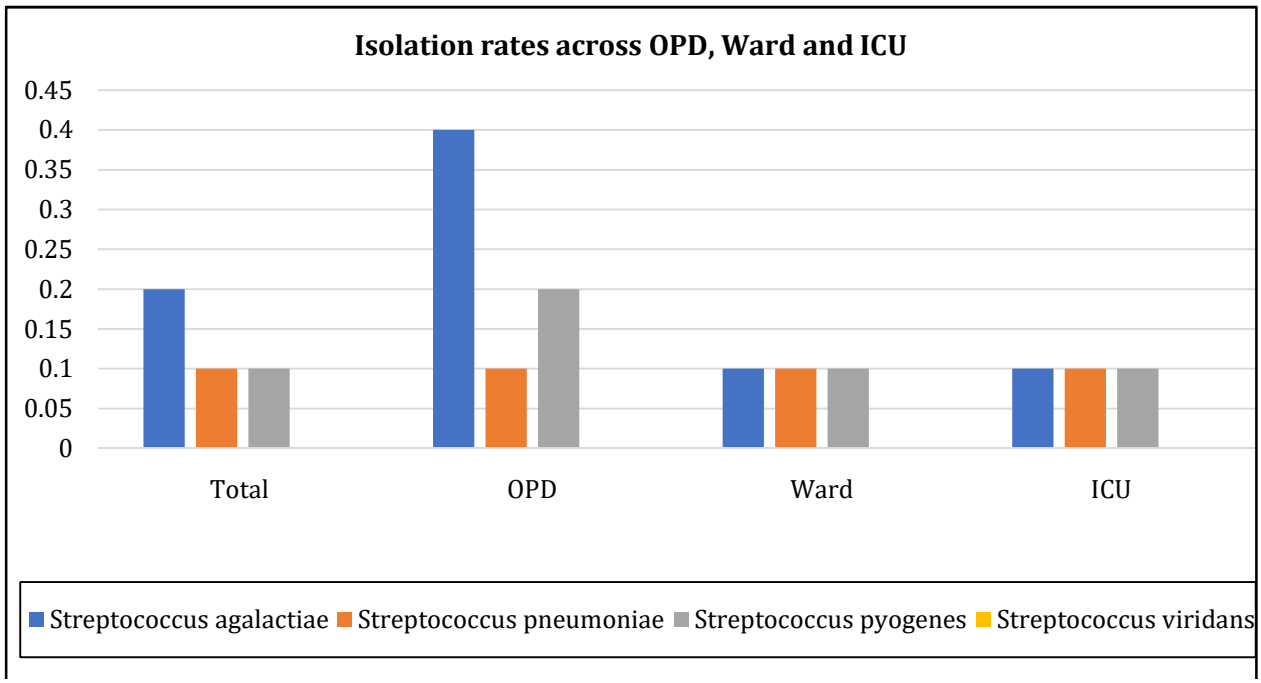


Figure 1.22: Location wise Isolation of *streptococcus* species

Chapter 2 Enterobacterales

Species wise susceptibility of Enterobacterales isolated from of all specimens except urine and faeces

In the year 2021, a total of 47,399 clinical isolates belonging to various genera and species of family Enterobacterales from 21 participating centers were included in the analysis. The isolates belonged to various specimens including blood (7307), sterile body fluids including cerebrospinal fluid (338), pus, wound swabs and aspirates (4158) and respiratory tract specimens (6353).

Significant clinical isolates from all specimens (except urine and faeces) were tested for susceptibility to 10 antibiotics including aminoglycoside (amikacin), cephalosporins (cefotaxime and ceftazidime), fluoroquinolones (ciprofloxacin and levofloxacin), beta lactam and beta-lactamase inhibitor combination (piperacillin-tazobactam), carbapenems (imipenem, meropenem and ertapenem) and polymyxin (colistin). Susceptibility was tested following CLSI guidelines using disc diffusion or automated systems except colistin where micro-broth dilution test was used.

Susceptibilities of different species to the antibiotics are presented in Table 2.1, Figure 2.1 and 2.2. Colistin susceptibility (tested in limited number of species) overall was 97% (marginally lower than previous 4 years); *Enterobacter cloacae* showed 100% susceptibility followed by *Escherichia coli*(99%), *Klebsiella pneumoniae*, and*Citrobacter* spp.(96% each).

Table 2.1. Species wise susceptibility of Enterobacterales isolated from of all specimens except urine and faeces

	Pip-taz		Cefotax		Ceftazid		Ertapen		Imipen		Meropen		Colistin		Amikacin		Ciproflox		Levoflox	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
C. freundii	153	56	132	33	110	36	128	66	152	63	144	71			157	75	145	57	97	55
C. koseri	275	73	241	63	157	52	195	80	271	75	265	79			278	82	264	73	118	63
Citrobacter spp	114	64	87	40	48	31	93	87	111	64	131	62	48	96	128	70	121	60	34	79
K. oxytoca	233	49	185	35	145	29	175	61	223	52	221	64			232	70	220	47	149	31
K. pneumoniae	13185	33	10878	20	7507	19	8297	42	12660	43	12677	45	4696	96	13451	46	11712	31	6101	30
Klebsiella spp	265	43	198	34	176	35	246	50	87	39	278	50			240	51	161	43	141	41
Enterobacter cloacae	1381	61	1218	42	801	40	896	76	1362	69	1359	71	165	100	1429	76	1361	62	451	74
Enterobacter spp	369	63	290	27	251	27	216	79	281	68	378	69			371	72	272	69	170	66
K. (E.) aerogenes	133	78	133	44	109	45	43	79	130	78	133	84			135	81	100	62	52	50
P. mirabilis	1293	92	978	58	859	54	621	86	1195	50	1280	84			1308	68	1170	42	587	56
P. rettgeri	100	52	45	36	65	31	37	57	92	22	101	39			100	54	90	40	56	55
P. stuartii	167	54	126	34	146	36	46	74	169	51	174	67			177	54	175	38	59	68
E. coli	12936	47	10613	16	6786	18	7932	67	12339	64	12775	69	3895	99	13210	78	12014	19	5142	17
M. morganii	313	87	277	60	166	60	209	90	292	53	322	84			313	87	294	47	107	50
S. marcescens	271	79	274	61	195	55	263	89	239	86	339	83			327	82	276	77	191	70
Overall	31188	**45**	25675	**23**	17521	**23**	19397	**58**	29603	**55**	30577	**60**	8877	**97**	31856	**64**	28375	**30**	13455	**30**

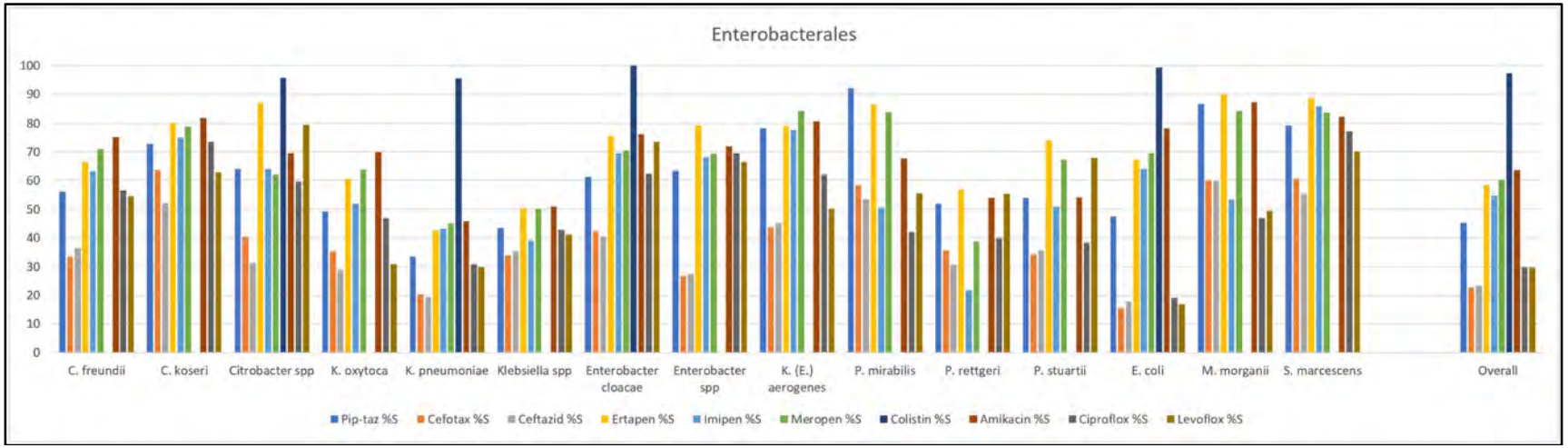


Figure 2.1. Species wise susceptibility of Enterobacterales isolated from of all specimens except urine and faeces

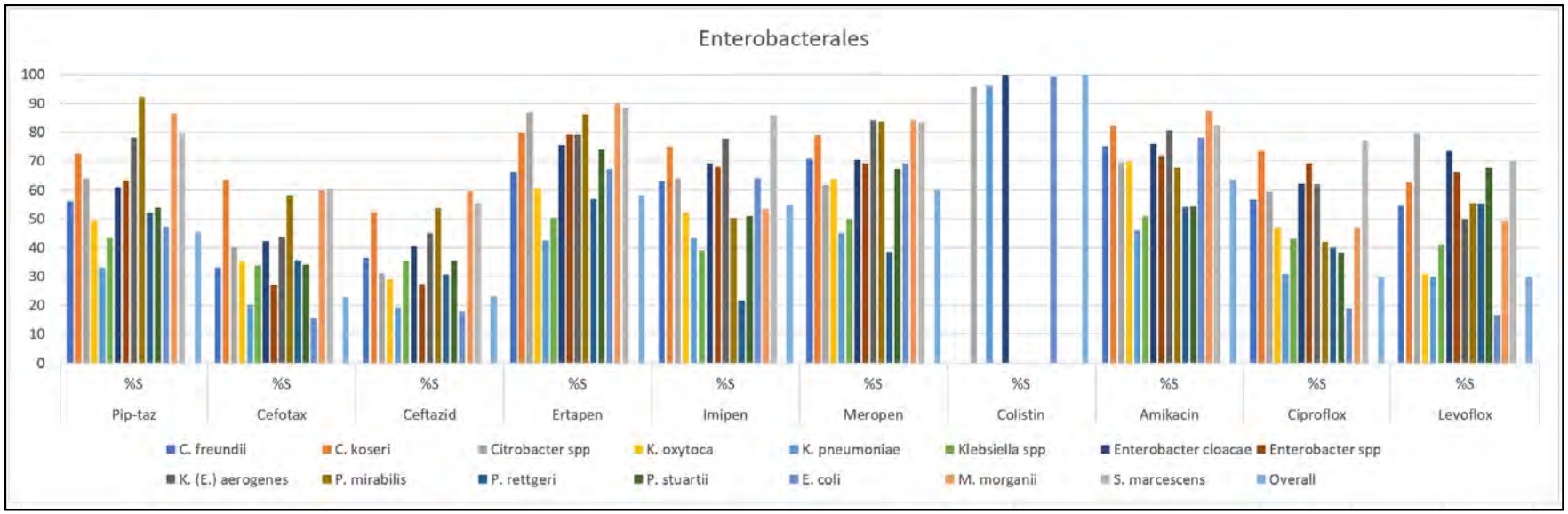


Figure 2.2. Antibiotic wise susceptibility of species of Enterobacterales isolated from of all specimens except urine and faeces

Out of the carbapenems, overall, meropenem showed 60% susceptibility followed by ertapenem (58%) and imipenem (55%). *K. aerogenes* (84%), *P. mirabilis* (84%), *M. morganii* (84%) and *S. marcescens* (83%) showed highest susceptibility to meropenem followed by *C. koseri* (79%), *C. freundii* (71%), *E. cloacae* (71%), *E. coli* (69%), *Enterobacter* spp. (69%), *P. stuartii* (67%), *K. oxytoca* (64%) and *Citrobacter* spp. (62%). Least susceptibility was shown by *K. pneumoniae* and *Klebsiella* spp (45-50%) and *P. rettgeri* (39%).

Piperacillin-tazobactam susceptibility was overall 45%. Maximum susceptibility was found in *Proteus mirabilis* (92%), *Morganella morganii* (87%), *Serratia marcescens* (79%), and *K. aerogenes* (78%). *C. koseri, Citrobacter* spp., *Enterobacter* spp, *E. cloacae, C. freundii*, *P. stuartii*, and *P. rettgeri* showed susceptibilities between 52% and 73% with *K. oxytoca* (49%), *E. coli* (47%), *Klebsiella* spp. (43%), and *K. pneumoniae* (33%) showing the least. Overall, less than one third (30%) of isolates showed fluoroquinolone susceptibility. *Citrpbacter* spp (79%) and *E. cloacae* (74%) showed maximum susceptibility to levofloxacin. *E. coli* showed the lowest susceptibility to levofloxacin (17%). Ciprofloxacin and levofloxacin showed similar patterns of resistance for most species tested.

Third generation cephalosporins, cefotaxime and ceftazidime showed comparable susceptibility in 23% of isolates overall. *C. koseri* (63%), *S. marcescens* (61%), *M. morganii* (60%) and *P. mirabilis* (58%) showed susceptibility in half of the isolates or more. Overall, two thirds (64%) of the isolates were susceptible to amikacin. *M. morganii* (87%), followed by *S. marcescens* (82%), *C. koseri* (82%), *K. aerogenes* (81%), *E. coli* (78%), *E. cloacae* (76%), and *C. freundii* (75%) showed better susceptibility than other species.

Comparison of susceptibility of isolates from OPD, ward and ICU

Overall, for all the drugs tested, *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Enterobacter cloacae* isolated from out-patients were more susceptible than those from in-patients and among in-patients, isolates from wards were more susceptible than those from ICU (Tables 2.2 to 2.5, Figures 2.3 to 2.6). The differences were more marked for *E. coli*, and *K. pneumoniae* and *Enterobacter cloacae*, and *Citrobacter koseri*.

Table 2.2. Comparison of susceptibility of *Escherichia coli* isolated from OPD, ward and ICU

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	2504	84	8724	78	1981	70	13209	78
Cefotaxime	1992	22	7010	14	1611	13	10613	16
Ceftazidime	1207	27	4631	17	948	13	6786	18
Ciprofloxacin	2317	23	7993	18	1703	17	12013	19
Colistin	810	99	2587	99	498	100	3895	99
Ertapenem	1567	78	5073	66	1293	58	7933	67
Imipenem	2346	71	8136	63	1856	58	12338	64
Levofloxacin	899	24	3465	15	779	15	5143	17
Meropenem	2370	78	8506	69	1898	60	12774	69
Pip-taz	2426	58	8595	46	1914	40	12935	47

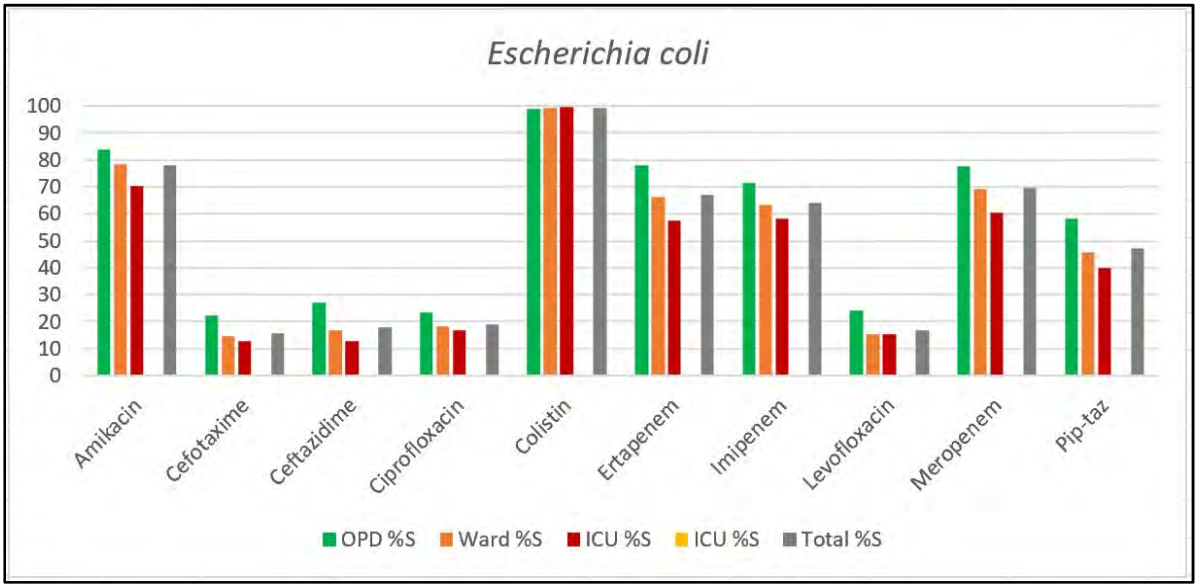


Figure 2.3. Comparison of susceptibility of *Escherichia coli* isolated from OPD, ward and ICU

Table 2.3. Comparison of susceptibility of *Klebsiella pneumoniae* isolated from OPD, ward and ICU

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	2066	66	7465	47	3920	33	13451	46
Cefotaxime	1745	37	5932	20	3202	12	10879	20
Ceftazidime	1153	38	4324	19	2030	9	7507	19
Ciprofloxacin	1904	50	6667	31	3141	20	11712	31
Colistin	596	98	2689	97	1411	92	4696	96
Ertapenem	1326	65	4424	44	2548	28	8298	42
Imipenem	1961	59	7063	45	3636	31	12660	43
Levofloxacin	861	48	3410	30	1830	22	6101	30
Meropenem	1940	66	7168	46	3570	31	12678	45
Pip-taz	2023	51	7290	34	3872	23	13185	33

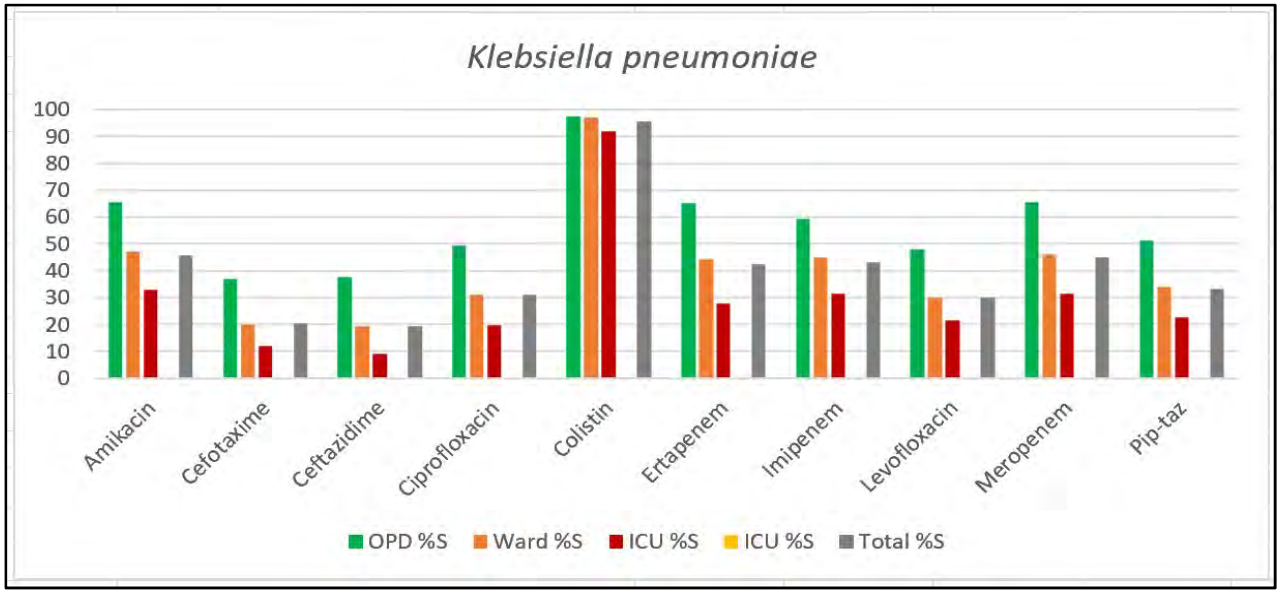


Figure 2.4. Comparison of susceptibility of *Klebsiella pneumoniae* isolated from OPD, ward and ICU

Table 2.4. Comparison of susceptibility of *Citrobacter koseri* isolated from OPD, ward and ICU

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	93	89	152	79	33	76	278	82
Cefotaxime	85	79	127	58			241	63
Ceftazidime	46	70	94	49			157	52
Ciprofloxacin	87	85	150	70			264	73
Ertapenem	68	84	102	78			195	80
Imipenem	90	82	149	72	32	66	271	75
Levofloxacin	34	76	63	63			118	63
Meropenem	85	88	150	76	30	67	265	79
Pip-taz	93	83	151	68	31	65	275	73

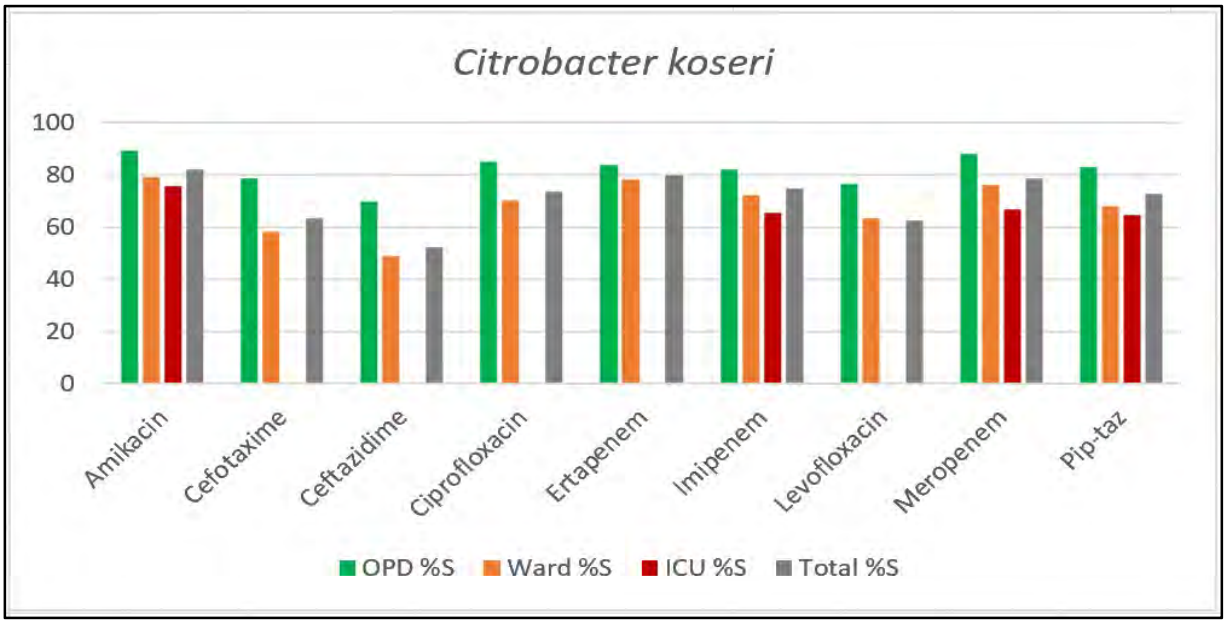


Figure 2.5. Comparison of susceptibility of *Citrobacter koseri* isolated from OPD, ward and ICU

Table 2.5. Comparison of susceptibility of *Enterobacter cloacae* isolated from OPD, ward and ICU

	OPD		Ward		ICU		Total	
	n	%S	n	%S	n	%S	n	%S
Amikacin	378	86	780	73	270	72	1428	76
Cefotaxime	325	53	666	39	226	38	1217	42
Ceftazidime	214	52	451	36	136	36	801	40
Ciprofloxacin	363	72	759	59	238	58	1360	62
Ertapenem	257	84	476	74	162	67	895	76
Imipenem	360	79	751	66	250	65	1361	69
Levofloxacin	132	81	229	72	90	68	451	74
Meropenem	355	79	758	70	245	61	1358	71
Pip-taz	363	75	761	56	256	58	1380	61

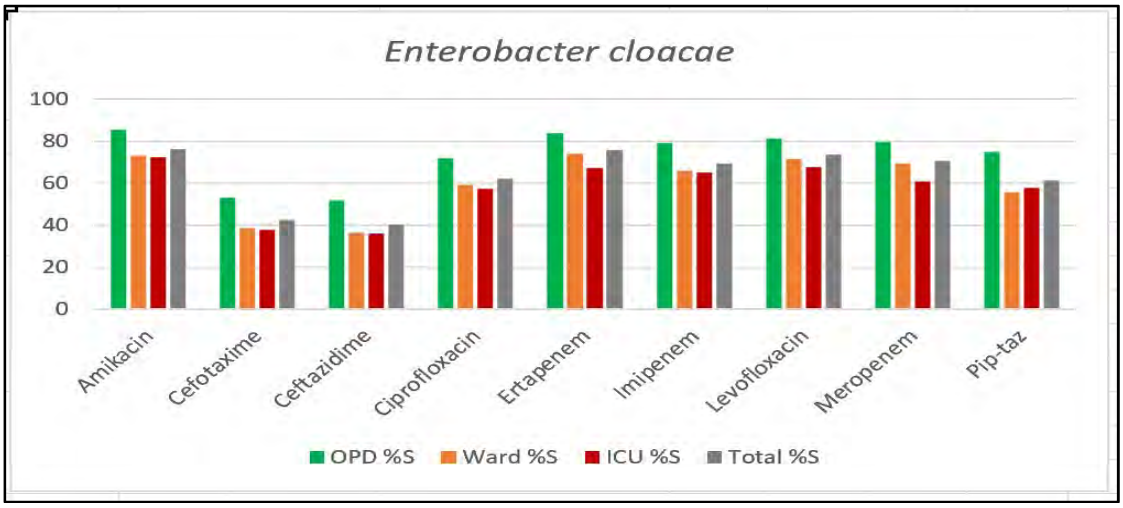


Figure 2.6. Comparison of susceptibility of *Enterobacter cloacae* isolated from OPD, ward and ICU

Susceptibility trends of various species over time

Over the last six years, imipenem susceptibility of *E. coli* dropped steadily from 86% in 2016 to 64% in 2021 (Table 2.6, Figure 2.7) and that of *Klebsiella pneumoniae* dropped steadily from 65% in 2016 to 43% in 2021 (Table 2.7, Figure 2.8). The drop in meropenem susceptibility was modest and inconsistent. There was an increase in susceptibility of *Citrobacter* species to amikacin from 53% in 2016 to 70% in 2021 and to ciprofloxacin from 37% in 2016 to 60% in 2021 (51% in 2017 to 79% in 2021 for levofloxacin) (Table 2.8, Figure 2.9). There was an increase in susceptibility of *Enterobacter* species to

ciprofloxacin from 46% in 2016 to 70% in 2021 (Table 2.9, Figure 2.10). Susceptibility to other antibiotics didn't show much change over the last six years.

Table 2.6. Yearly susceptibility trend of *E. coli* isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=1018	Year-2017 (S%) Total n=6282	Year-2018 (S%) Total n=9187	Year-2019 (S%) Total n=13133	Year-2020 (S%) Total n=8198	Year-2021 (S%) Total n=13533
Piperacillin-tazobactam	607/1009 (60.2)	3424/6030 (56.8)	4857/8961 (54.2)	6620/12121 (54.6)	4211/7890 (53.4)	6126/12935 (47.4)
Cefazolin	*0/0	*0/8	*2/6	*0/1	*0/4	*0/1
Cefotaxime	165/928 (17.8)	879/5747 (15.3)	1274/7817 (16.3)	1537/10646 (14.4)	1063/6835 (15.6)	1656/10613 (15.6)
Ceftazidime	244/977 (25)	1295/5513 (23.5)	1398/5956 (23.5)	1501/7540 (19.9)	943/5072 (18.6)	1220/6786 (18)
Ertapenem	514/705 (72.9)	3104/4605 (67.4)	4528/6877 (65.8)	6633/9335 (71.1)	4067/5729 (71)	5334/7933 (67.2)
Imipenem	699/814 (85.9)	4699/5773 (81.4)	6453/8874 (72.7)	6497/10254 (63.4)	5176/7191 (72)	7903/12338 (64.1)
Meropenem	792/981 (80.7)	4158/5678 (73.2)	5873/8404 (69.9)	9110/12167 (74.9)	5683/7499 (75.8)	8872/12774 (69.5)
Amikacin	796/961 (82.8)	4788/6048 (79.2)	7071/8912 (79.3)	9936/12549 (79.2)	6451/7935 (81.3)	10326/13209 (78.2)
Ciprofloxacin	151/745 (20.3)	1028/5368 (19.2)	1889/8451 (22.4)	2427/11700 (20.7)	1580/7092 (22.3)	2287/12013 (19)
Levofloxacin	*2/4	140/889 (15.7)	600/3493 (17.2)	1145/6050 (18.9)	717/3762 (19.1)	866/5143 (16.8)

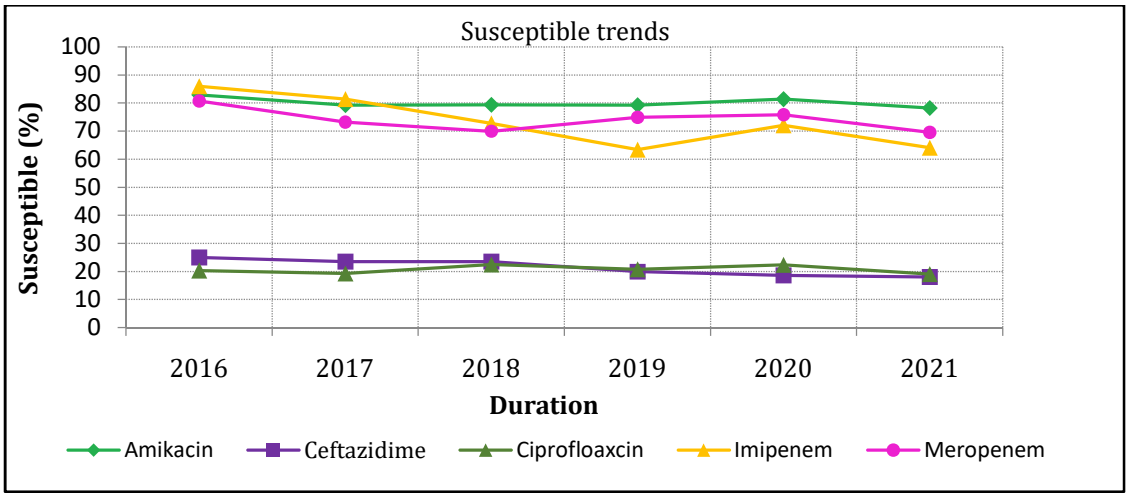


Figure 2.7. Yearly susceptibility trend of *E. coli* isolated from all samples (except faeces and urine)

Table 2.7. Yearly susceptibility trend of *Klebsiella pneumoniae* isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=875	Year-2017 (S%) Total n=5389	Year-2018 (S%) Total n=8394	Year-2019 (S%) Total n=13381	Year-2020 (S%) Total n=8932	Year-2021 (S%) Total n=13633
Piperacillin-tazobactam	364/871 (41.8)	2207/5179 (42.6)	3256/8223 (39.6)	4872/12502 (39)	3165/8669 (36.5)	4393/13185 (33.3)
Cefazolin	*0/0	*0/3	*0/0	*0/1	*0/3	*1/3
Cefotaxime	170/831 (20.5)	1109/5092 (21.8)	1577/7158 (22)	2400/11292 (21.3)	1472/7658 (19.2)	2217/10879 (20.4)
Ceftazidime	213/853 (25)	1320/4790 (27.6)	1488/5503 (27)	1985/7908 (25.1)	1147/5334 (21.5)	1452/7507 (19.3)
Ertapenem	317/690 (45.9)	2022/4456 (45.4)	3189/6667 (47.8)	4362/9650 (45.2)	2560/6255 (40.9)	3526/8298 (42.5)
Imipenem	566/874 (64.8)	3136/5360 (58.5)	4257/8223 (51.8)	5039/11031 (45.7)	3771/8392 (44.9)	5474/12660 (43.2)
Meropenem	436/847 (51.5)	2478/5147 (48.1)	3832/7591 (50.5)	6081/12164 (50)	3660/7771 (47.1)	5707/12678 (45)
Amikacin	396/848 (46.7)	2583/5286 (48.9)	4204/8276 (50.8)	6507/13018 (50)	4171/8828 (47.2)	6174/13451 (45.9)
Ciprofloxacin	243/838 (29)	1667/5213 (32)	2766/7688 (36)	4144/11560 (35.8)	2420/7218 (33.5)	3621/11712 (30.9)
Levofloxacin	*1/1	254/898 (28.3)	967/3333 (29)	2596/7432 (34.9)	1391/4913 (28.3)	1830/6101 (30)

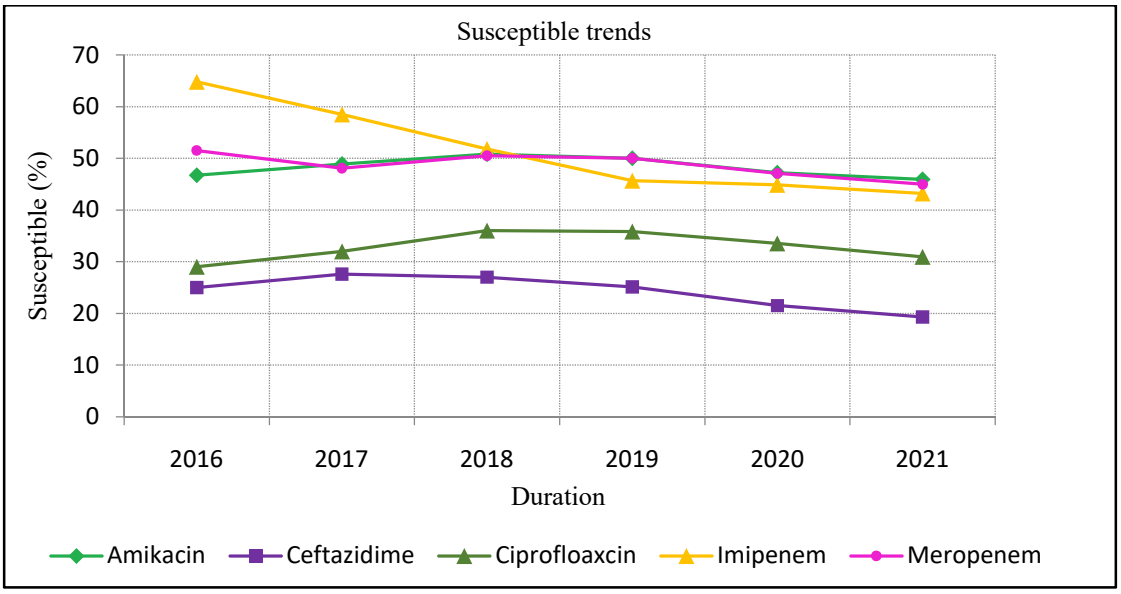


Figure 2.8. Yearly susceptibility trend of *Klebsiella pneumoniae* isolated from all samples (except faeces and urine)

Table 2.8. Yearly susceptibility trend of *Citrobacter* species isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=49	Year-2017 (S%) Total n=321	Year-2018 (S%) Total n=613	Year-2019 (S%) Total n=796	Year-2020 (S%) Total n=447	Year-2021 (S%) Total n=136
Piperacillin-tazobactam	31/48 (64.6)	178/308 (57.8)	365/603 (60.5)	458/760 (60.3)	252/427 (59)	73/114 (64%)
Cefazolin	*0/0	*0/0	*0/0	*0/0	*0/0	*0/0
Cefotaxime	5/46 (10.9)	94/306 (30.7)	193/556 (34.7)	228/654 (34.9)	144/388 (37.1)	35/87 (40.2%)
Ceftazidime	13/47 (27.7)	110/285 (38.6)	168/474 (35.4)	201/577 (34.8)	105/295 (35.6)	15/48 (31.3%)
Ertapenem	25/46 (54.3)	161/263 (61.2)	336/522 (64.4)	381/597 (63.8)	224/334 (67.1)	81/93 (87.1%)
Imipenem	39/46 (84.8)	198/303 (65.3)	369/594 (62.1)	403/679 (59.4)	270/421 (64.1)	71/111 (64%)
Meropenem	33/49 (67.3)	187/284 (65.8)	396/580 (68.3)	505/765 (66)	299/427 (70)	81/131 (61.8%)
Amikacin	25/47 (53.2)	212/318 (66.7)	416/604 (68.9)	509/763 (66.7)	312/438 (71.2)	89/128 (69.5%)
Ciprofloxacin	18/49 (36.7)	138/295 (46.8)	324/599 (54.1)	430/740 (58.1)	256/410 (62.4)	72/121 (59.5%)
Levofloxacin	*0/0	44/86 (51.2)	145/319 (45.5)	296/512 (57.8)	132/236 (55.9)	27/34 (79.4%)

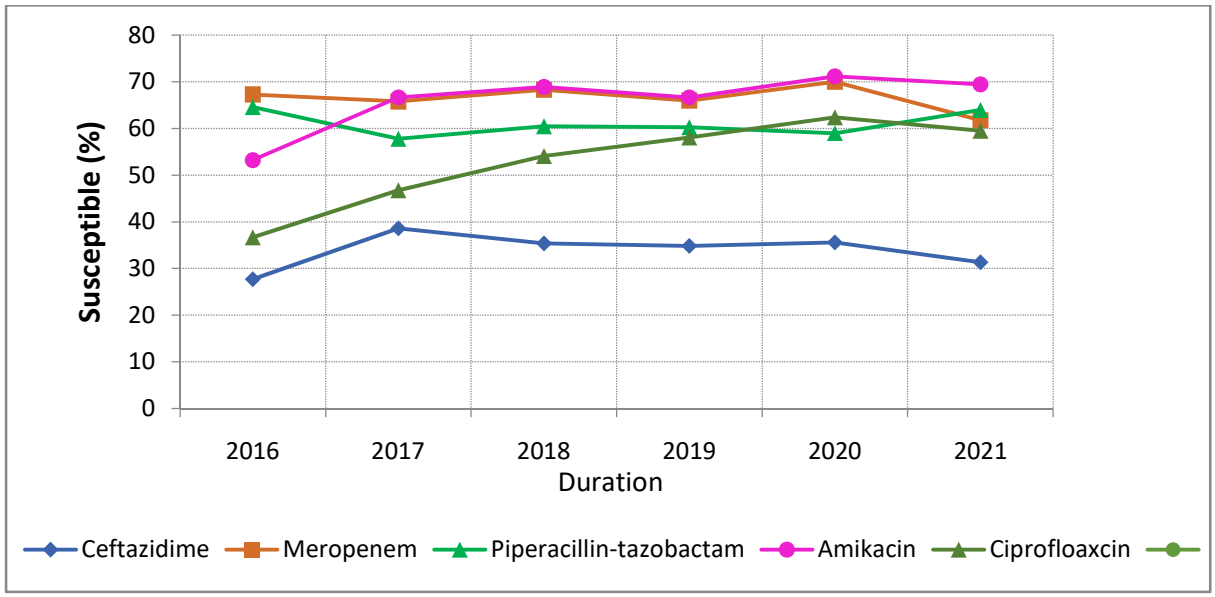


Figure 2.9. Yearly susceptibility trend of *Citrobacter* species isolated from all samples (except faeces and urine)

Table 2.9. Yearly susceptibility trend of *Enterobacter* species isolated from all samples (except faeces and urine)

AMA	Year-2016 (S%) Total n=222	Year-2017 (S%) Total n=1140	Year-2018 (S%) Total n=1600	Year-2019 (S%) Total n=2071	Year-2020 (S%) Total n=1287	Year-2021 (S%) Total n=393
Piperacillin-tazobactam	123/216 (56.9)	682/1092 (62.5)	961/1567 (61.3)	1253/1908 (65.7)	781/1225 (63.8)	234/369 (63.4%)
Cefazolin	*0/0	*0/0	*0/0	*0/0	*0/0	*0/0
Cefotaxime	55/214 (25.7)	310/1093 (28.4)	448/1423 (31.5)	576/1590 (36.2)	391/1094 (35.7)	78/290 (26.9%)
Ceftazidime	71/216 (32.9)	363/1013 (35.8)	424/1159 (36.6)	494/1305 (37.9)	281/823 (34.1)	69/251 (27.5%)
Ertapenem	117/187 (62.6)	613/929 (66)	855/1170 (73.1)	950/1281 (74.2)	562/783 (71.8)	171/216 (79.2%)
Imipenem	174/219 (79.5)	851/1133 (75.1)	1111/1575 (70.5)	1117/1662 (67.2)	826/1148 (72)	191/281 (68%)
Meropenem	150/215 (69.8)	735/1051 (69.9)	1068/1503 (71.1)	1497/1990 (75.2)	918/1211 (75.8)	262/378 (69.3%)
Amikacin	139/193 (72)	734/1059 (69.3)	1119/1572 (71.2)	1446/1965 (73.6)	948/1250 (75.8)	267/371 (72%)
Ciprofloxacin	98/213 (46)	578/1088 (53.1)	837/1369 (61.1)	1147/1836 (62.5)	699/1080 (64.7)	189/272 (69.5%)
Levofloxacin	*0/0	93/150 (62)	289/550 (52.5)	587/959 (61.2)	334/554 (60.3)	113/170 (66.5%)

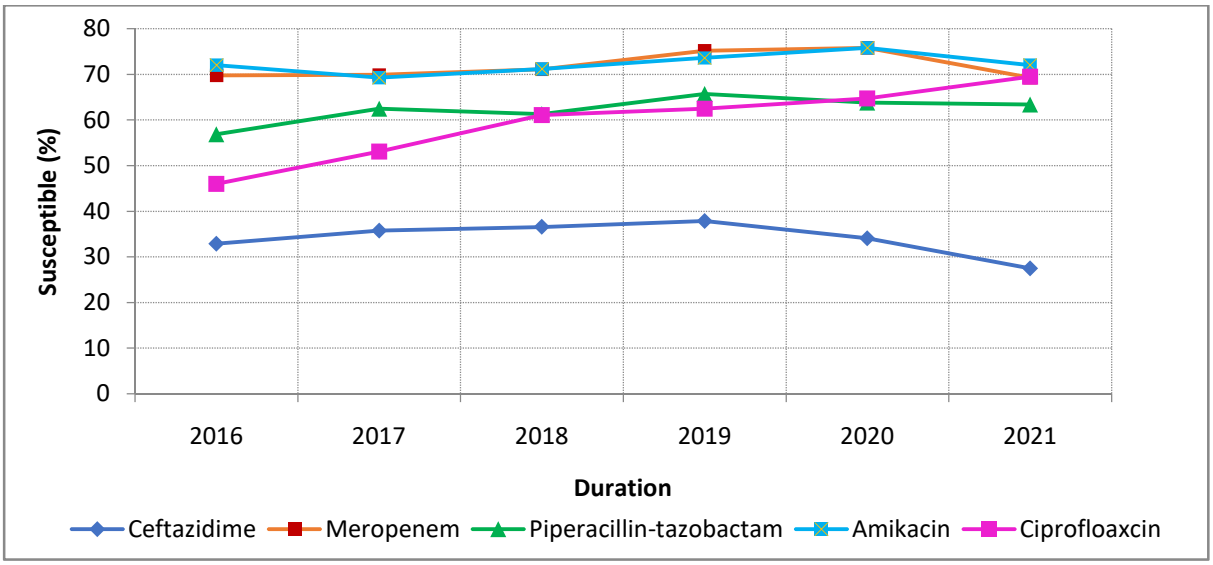


Figure 2.10. Yearly susceptibility trend of *Enterobacter* species isolated from all samples (except faeces and urine)

Table 2.10. Susceptibilities of carbapenem susceptible (CS) and carbapenem resistant (CR) isolates of *E. coli* and *K. pneumoniae* to all antibiotics

	E. coli CS (%S)	*E. coli* CR (%R)	*K. pneum* CS (%S)	*K. pneum* CR (%R)
Pip-taz	70	7	77	3
Cefotaxime	23	2	44	1
Ceftazidime	27	2	42	2
Ertapenem	98	16	98	5
Imipenem	97	9	97	7
Meropenem	98	18	98	6
Amikacin	92	54	89	16
Ciprofloxacin	28	4	66	6
Levofloxacin	26	7	70	11
Cotrimoxazole	52	16		
NFT	94	86		

Relative susceptibilities of carbapenem susceptible and carbapenem resistant isolates of *E. coli* and *K. pneumoniae*:

Overall, carbapenem susceptible isolates showed higher susceptibility to all the antibiotics tested, than carbapenem resistant (resistant to at least one of the carbapenems tested) isolates (Table 2.10 and Figure 2.11). The difference was more in *K. pneumoniae* than *E. coli*

indicating that carbapenem resistant *K. pneumoniae* isolates were more resistant to all the antibiotics than carbapenem resistant *E. coli* isolates. In *E. coli*, the differences in susceptibility were high for carbapenems and piperacillin (range of differences 63-87%) and moderate for other antibiotics (range of differences 19-39%). In *K. pneumoniae*, the differences were high for all the antibiotics tested (range of differences 41-92%).

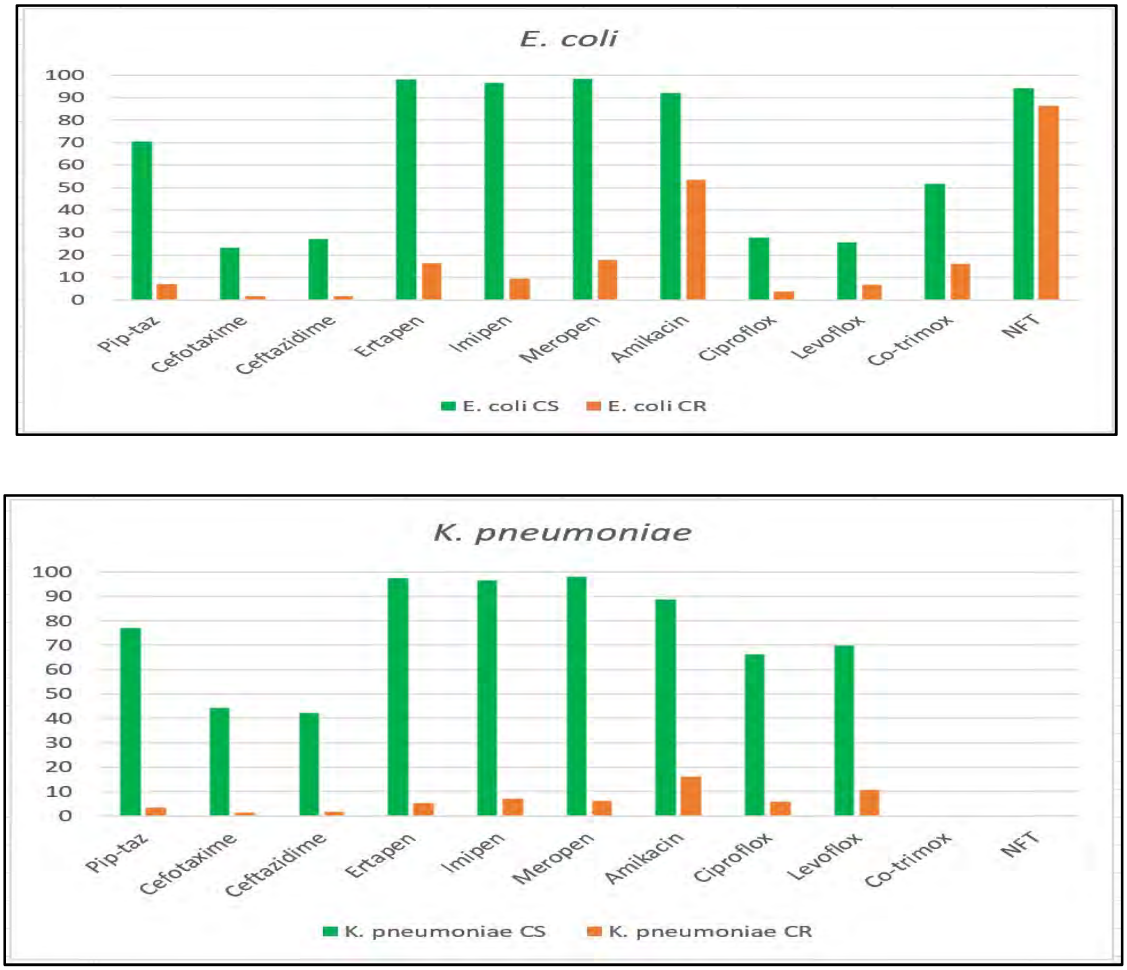


Figure 2.11. Susceptibilities of carbapenem susceptible (CS) and carbapenem resistant (CR) isolates of *E. coli* and *K. pneumoniae* to all antibiotics

Analysis of results from individual Regional Centers

21 Regional Centers (RCs) from various parts of the country, both public and private sectors, participated in surveillance. The results of all centers for the designated organisms and the designated antibiotics were used for overall susceptibility but only those drug-

pathogen combinations where the number tested was 30 or more were used for RC wise analyses. The susceptibility profiles showed considerable variation between the RCs.

Species wise susceptibility of Enterobacterales isolated from urine

Fosfomycin showed 92% susceptibility to *E. coli* isolated from urine (Table 2.11 and figure 2.12 and 2.13). Overall, the isolates from urine showed good susceptibility to amikacin (76%), meropenem (73%), imipenem (71%) and ertapenem (71%), followed by nitrofurantoin (66%) and piperacillin-tazobactam (59%). Species wise, *C. koseri* was the most susceptible followed by *E. cloacae* and *M. morganii*. *P. rettgeri* was the least susceptible showing susceptibility of 18 percent or less to all antibiotics tested. Comparison of overall susceptibilities of urinary isolates and non-urinary isolates of Enterobacterales showed marginally better susceptibility in the former (Figure 2.14).

Table 2.11. Susceptibility of species of Enterobacterales isolated from urine to antibiotics, overall and species wise

	E. coli		K. pneumoniae		K. oxytoca		Klebsiella spp		E. cloacae		Enterobacter spp		P. mirabilis		C. koseri		C. freundii		M. morganii		P. rettgeri		Overall	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
Pip-taz	9648	63	3403	41	46	59	30	47	190	64	42	62	281	90	91	91	46	59	85	81	38	16	14001	59
Cefazolin	3169	21	3560	55	46	74	31	52					79	34	40	40							6942	39
Cefotaxime	7852	25	2653	28					171	46			247	59	79	79	28	36	75	65	32	9	11292	28
Ceftazid																								
Ertapenem	7881	78	2834	51	37	68			149	66			222	86	88	88	42	67	68	78	31	16	11448	71
Imipenem	9595	77	3387	54	45	51	31	55	201	68	39	77	250	54	89	89	46	57	81	42	37	14	13898	71
Meropenem	9122	78	3180	56	42	67	31	55	192	73	41	73	279	89	92	92	39	64	87	77	38	18	13228	73
Amikacin	10043	83	3560	55	46	74	31	52	205	81	40	75	285	71	91	91	48	79	89	82	39	18	14582	76
Ciprofloxacin	8986	28	3047	37	41	41			189	62			240	48	84	84	42	55	82	51	37	11	12903	32
Levofloxacin	4408	25	1687	29					70	60			135	37	63	63							6496	27
Cotrimoxazole	8506	43	3117	40	41	49			147	63			242	28	78	78	46	61	74	54	34	15	12406	43
Fosfomycin	4319	97	1524	80					61	87			104	90	93	93							6180	92
NFT	9064	83	3123	27	43	60			179	35	30	30	217	0	77	77	41	73	65	2			12994	66

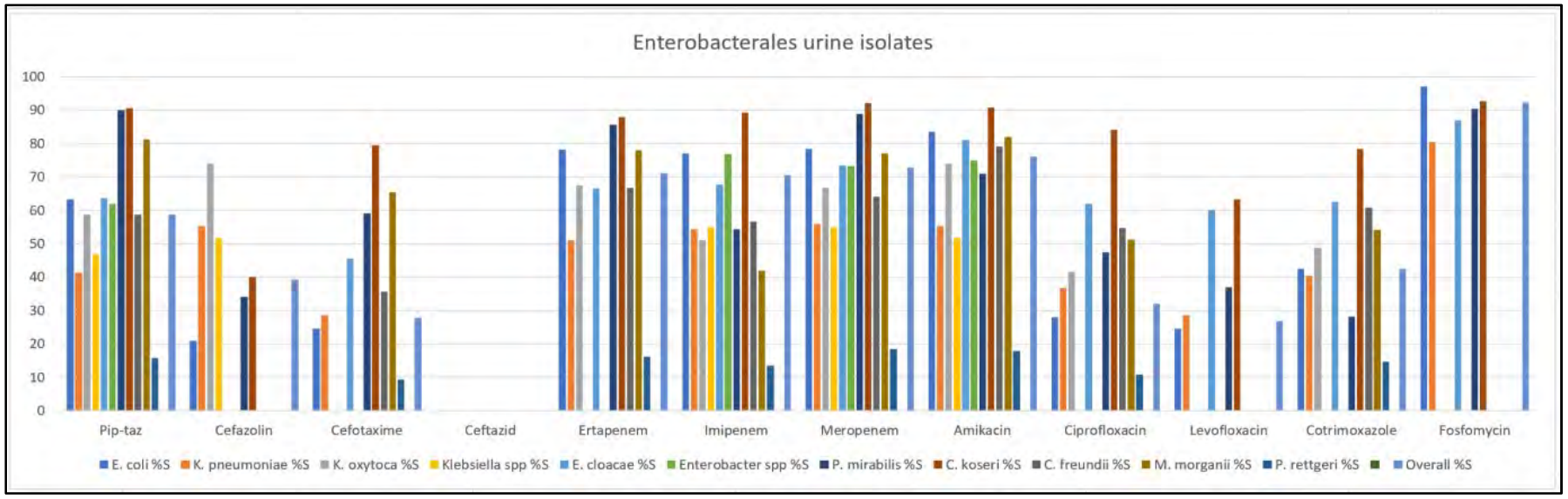


Figure 2.12. Susceptibility of Enterobacterales isolated from urine, antibiotic wise

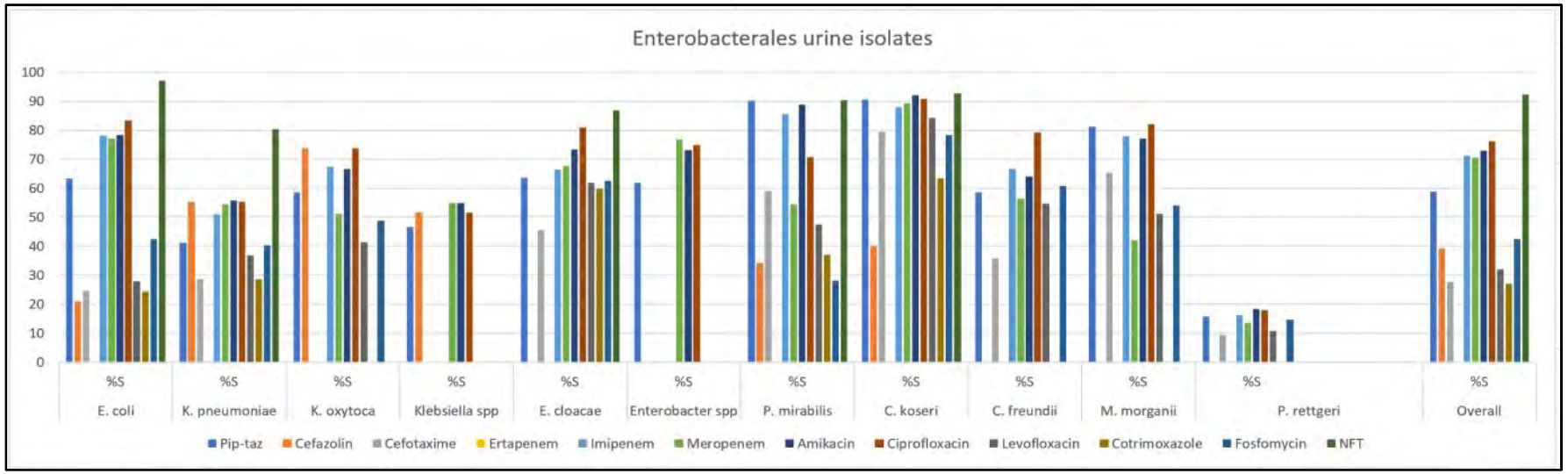


Figure 2.13. Susceptibility of Enterobacterales isolated from urine, overall and species wise

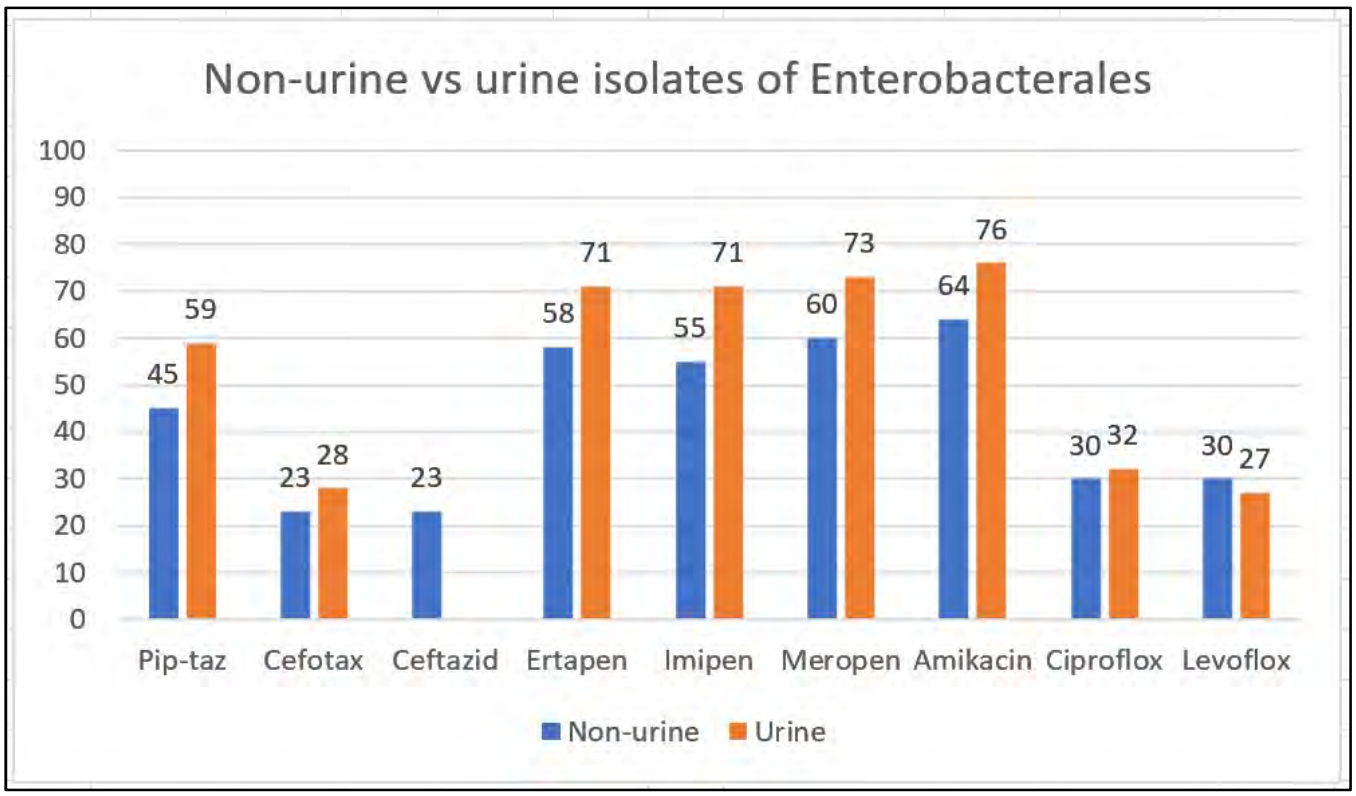


Figure 2.14. Overall susceptibility of non-urinary versus urinary isolates of Enterobacterales to the common antibiotics tested

Comparison of susceptibilities of *E. coli* and *K. pneumoniae* showed that the former is more susceptible than the latter to all antibiotics except cefazolin and fluoroquinolones (Table 2.12 and Figure 2.15). RC wise susceptibility of *E. coli* and *K. pneumoniae* showed similar variations as the non-urine isolates except in *E. coli* for fosfomycin and nitrofurantoin. RC 21 showed unusually low susceptibility for most antibiotics tested (Table 2.13 and 2.14).

Table 2.12. Comparison of susceptibility of *E. coli* and *K. pneumoniae* from urine

	E. coli	K. pneumoniae
Pip-taz	63	41
Cephazolin	21	55
Cefotaxime	25	28
Ertapen	78	51
Imipen	77	54
Meropen	78	56
Amikacin	83	55
Ciproflox	28	37
Levoflox	25	29
Cotrimox	43	40
NFT	83	27

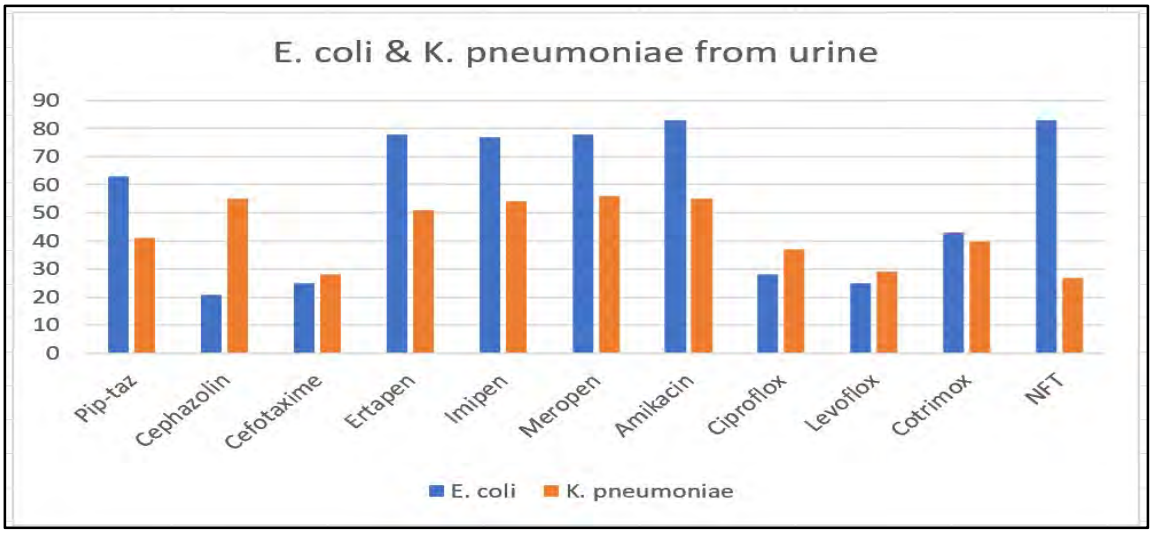


Figure 2.15. Comparison of susceptibility of *E. coli* and *K. pneumoniae* from urine

Table 2.13. Susceptibility of *E. coli* isolated from urine, overall and RC wise

	Pip-taz		Cephazolin		Cefotaxime		Ertapen		Imipen		Meropen		Amikacin		Ciproflox		Levoflox		Cotrimox		Phosphomycin		NFT	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
RC 01	655	54	652	23	655	22	650	69	655	72	655	69	655	62	655	18	655	19	652	26	647	97	652	89
RC 02																								
RC 03																								
RC 04	1165	60			1136	32			1161	84	1172	84	1172	84	1172	28							1117	88
RC 05	513	77			541	28	533	87	541	90	541	90	541	93	541	31			540	47			536	81
RC 06	639	62			680	19	680	76	679	78	160	78	680	85	129	11	552	19	680	33			679	75
RC 07	728	72					613	87	453	82	458	81	762	88	754	35	149	28	762	48	251	96	654	81
RC 08	195	72			187	26	198	80	200	84	199	84	200	86	199	22	200	22	199	44	200	100		
RC 09	500	77	480	35	500	39	498	83	504	81	503	86	503	89	481	42	482	42	499	51	413	100	459	90
RC 10	537	80			560	33	555	91	591	92	591	92	582	93	578	33			416	51	555	99	349	85
RC 11																								
RC 12	386	48	419	22	238	7	416	66	431	63	429	67	430	75	431	20	354	27	417	34	263	97	411	51
RC 13	385	57					143	55	456	63	468	61	457	80	165	20	345	23	307	35	329	97	329	93
RC 14	1351	80			1414	35	1413	88	1414	90	1413	90	1414	95	1414	41			1409	52			1414	85
RC 15	311	64	311	19	300	20			311	96	311	94	311	84	149	24	162	26	302	37			311	86
RC 16	430	40	92	9	399	14	318	71	321	69	364	82	437	87	430	23	296	25	426	39	404	97	424	88
RC 17	611	71					630	84	653	87	653	87	653	91	653	19			650	50			486	82
RC 18	403	35	403	33	403	11	403	65	403	45	403	71	403	59	403	32	403	37	403	46	403	94	403	85
RC 19	187	43	183	8	186	7	185	52	187	50	169	46	187	67	177	13	158	20	186	34	185	100	185	90
RC 20	452	40	454	7	455	9	446	85	449	30	447	91	455	87	453	12	454	13	455	33	455	93	451	84
RC 21	178	43	170	0	178	2	177	16	173	69	173	55	179	25	180	5	166	14	180	29	174	99	179	40
Overall	9648	63	3169	21	7852	25	7881	78	9595	77	9122	82	10043	83	8986	28	4408	25	8509	43	4319	97	9064	83

Table 2.14. Susceptibility of *K. pneumoniae* isolated from urine, overall and RC wise

		Pip-taz		Cephazol		Cefotax		Ertapen		Imipen		Meropen		Amikacin		Ciproflox		Levoflox		Cotrimox		NFT	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
RC 01	324	24	324	12	324	10	323	37	324	48	324	39	324	25	324	19	324	18	324	35	323	12		
RC 02																								
RC 03																								
RC 04	250	51			244	42			252	64	250	65	253	66	253	43			239	33				
RC 05	202	58			211	36	208	63	211	68	211	67	211	73	211	45			211	13	211	51		
RC 06	312	25			323	17	323	30	323	33	70	43	323	41	53	28	270	21	323	9	322	28		
RC 07	385	40					321	64	299	52	297	52	402	59	400	35	62	19	343	26	404	44		
RC 08	87	41			83	31	87	43	88	45	89	46	89	51	89	35	88	38			89	36		
RC 09	108	66	106	39	108	50	107	64	109	68	108	66	109	70	107	60	108	60	98	52	105	51		
RC 10	195	53			203	43	202	62	204	68	210	65	206	66	207	42			104	30	151	50		
RC 11																								
RC 12	154	32	160	21	95	11	153	35	163	36	164	36	164	46	164	27	130	26	160	11	156	33		
RC 13	206	28					80	26	249	28	257	25	255	35	87	16	179	29	143	27	149	32		
RC 14	365	73			393	55	393	79	392	85	393	85	393	87	393	61			393	27	390	69		
RC 15	143	57	143	20	142	23			143	80	143	77	143	61	76	49	72	40	143	28	140	42		
RC 16	151	17			142	18	108	51	105	59	138	59	154	59	150	28	81	23	148	43	146	32		
RC 17	138	46					144	51	148	59	148	59	148	60	148	32			116	14	148	49		
RC 18	140	27	140	30	140	13	140	51	140	40	140	58	140	56	140	39	140	45	140	54	140	49		
RC 19	62	26	62	10	62	3	62	27	61	28	58	17	62	37	61	15	58	21	59	39	61	20		
RC 20	126	13	118	8	127	13	123	45	127	29	128	52	128	39	128	24	128	23	121	26	124	27		
RC 21	46	50	41	0	47	2	46	11	45	62	45	47	47	30	47	9	41	46	47	11	47	43		
Overall	3403	41	1120	18	2653	28	2834	51	3387	54	3177	56	3560	55	3047	37	1687	29	3123	27	3117	40		

Clinical implications

The relative frequency of isolation of various species and their susceptibility trends has an important role in deciding empiric antibiotic policies in hospitals. The trends of change in susceptibility indicate behaviour of organisms over time and alert us to take appropriate preventive measures.

Colistin, as expected, was the most effective antibiotic with an overall susceptibility of near 100% with most species tested except *Citrobacter* species showing more than very high susceptibility. With increasing use over the last five years, colistin resistance is emerging and the recent removal by CLSI of susceptible category from colistin indicates that there are strains of organisms without any detectable resistance mechanism (wild strains) which may not respond to therapy with this drug. Systemic therapy with colistin has also been mentioned as not adequate for treating respiratory tract infections. The fact that, in tertiary care facilities, many isolates from hospital-acquired and ventilator-associated pneumonias are carbapenem resistant, colistin therapy, if required, should be supplemented with nebulized colistin through inhalation. The removal of the susceptible category from colistin also indicates that, in all situations, therapy with colistin may have unpredictable outcomes and therefore should be highly restricted.

Carbapenem (meropenem) resistance was very high in *P. rettgeri* (61%), *Klebsiella pneumoniae* (55%), and *K. oxytoca* (36%), with an overall all-species susceptibility of 60%. Carbapenems have been mainstay in empiric therapy in tertiary care ICU settings. Though there was good susceptibility in *P. mirabilis* (84%), *M. morganii* (84%), *K. aerogenes* (84%) and *S. marcescens* (83%), the efficacy of this drug as empiric therapy protocol should depend on relative distribution of the various species in a particular set up. This also demands regular surveillance of carbapenem resistant Enterobacterales by molecular detection of various genes.

Piperacillin-tazobactam susceptibility overall was alarmingly low at 45%. Though the drug showed good susceptibility in *Proteus mirabilis* (92%), *M. morganii* (87%), *Serratia marcescens* (79%), and *K. aerogenes* (78%), it showed high resistance in commonly isolated species like *Klebsiella pneumoniae* (susceptibility 33%) and *E. coli* (susceptibility 47%) and therefore should be used only when an isolate is tested susceptible. Third generation cephalosporins and fluoroquinolones have susceptibilities far below the level to consider them appropriate for use in serious patients. Extensive use and abuse of these two groups over the last three decades have resulted in high prevalence of extended-spectrum beta lactamases and carbapenemases against oxyimino-cephalosporins and multiple mutations in organisms against fluoroquinolones making them nearly unusable as empiric therapy in seriously ill patients in tertiary care practices.

The differences in susceptibility of various organisms isolated from patients in OPD, indoor wards and ICU practices are clearly an outcome of the extent of use of the antibiotics in

these areas and the consequent selection pressure. While OPD patients are usually put on oral antibiotics, the indoor patients are frequently on parenteral antibiotics and the ICU patients are usually exposed to the highest and broad-spectrum antibiotics, often multiple. Resistance of an organism to an antibiotic is a direct outcome of the frequency of isolation of the organisms and the selection pressure of the antibiotic load used to treat it. Over the last two decades, use of carbapenems have increased many folds and the same is reflected in imipenem susceptibility of *E. coli* dropping steadily from 86% in 2016 to 64% in 2021 and that of *Klebsiella pneumoniae* dropping steadily from 65% in 2016 to 43% in 2021. The increase in susceptibility of amikacin and ciprofloxacin in *Citrobacter* species and ciprofloxacin in *Enterobacter* species may reflect drop in use of the same.

Molecular tests

Materials and methods

Molecular mechanism of antimicrobial resistance in clinical isolates

Three multiplex PCRs were performed (as described by Dallenne *et al*.) to detect resistance mechanisms in representative indicator organisms (*E. coli*, *K. pneumoniae*) (Table 2.15).

Table 2.15 PCR gene targets and primers used

PCR name	Beta lactamase targeted	Primers	Product size (bp)
Multiplex I TEM,SHV and OXA-1	Tem variants including TEM1 and TEM 2 Oxa1,4 and 30	F:CATTTCCGTGTCGCCCTTATTC R:CGTTCATCCATAGTTGCCTGAC F:AGCCGCTTGAGCAATTAAAC R:ATCCCGCAGATAAATCACCAC F:GGCACCAGATTCAACTTTCAAG R:GACCCCAAGTTTCCTGTAAGTG	800 713 564
Multiplex II CTXM1,2 and 9	Variants of CTXM group 1, M3 and 15 Variants of CTXM group 2 and variants of CTXM group 9 and CTXM14	F:TTAGGAARTGTGCCGCTGYA R:CGATATCGTTGGTGGTRCCCAT F:CGTTAACGGCACGATGAC R:CGATATCGTTGGTGGTRCCAT F:TCAAGCCTGCCGATCTGGT R:TGATTCTCGCCGCTGAAG	688 404 561
Multiplex IV Metallo beta lactamases and carbapenamases	IMP,VIM and KPC	F:TTGACACTCCATTTACDG R:GATYGAGAATTAAGCCACYCT F:GATGGTGTTTGGTCGCATA R:CGAATGCGCAGCACCAG F:CATTCAAGGGCTTTCTTGCTGC R:ACGACGGCATAGTCATTTGC	139 390 538

Multiplex III ACC, FOX, MOX, DHA, CIT and EBC	AmpC beta lactamases ACC1 and2 FOX1 to 5, MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11and CMY19 DHA-1 and DHA-2 LAT-1 to LAT-3, BIL-1, CMY-2 to CMY-7, CMY-12 to CMY-18 andCMY-21 to CMY-23 ACT-1 and MIR-1	F:CACCTCCAGCGACTTGTTAC R:GTTAGCCAGCATCACGATCC F:CTACAGTGCGGGTGGTTT R:CTATTTGCGGCCAGGTGA F:GCAACAACGACAATCCATCCT R:GGGATAGGCGTAACTCTCCCA F:TGATGGCACAGCAGGATATTC R:GCTTTGACTCTTTCGGGTATTCG F:CGAAGAGGCAATGACCAGAC R:ACGGACAGGGTTAGGTTAGGATAGY F: CGGTAAAGCCGATGTTGCG R: AGCCTAACCCCTGATACA	346 162 895 997 538 683
Simplex	NDM-1 CTXM-15 OXA-48	F:GGTTTGGCGATCTGGTTTTC R:CGGAATGGCTCATCACGATC F:AGAATAAGGAATCCCATGGTT R:ACCGTCGGTGACGATTTTAG F:TATATTGCATTAAGCAAGGG R: CACACAAATACGCGCTAACC	621 913 800

E. coli

Total two hundred and seventy three *E. coli* isolates were subjected to three multiplex PCRs and two monoplex PCRs for CTXM-15 and NDM. Overall, CTXM-15 (47%) was the most common, followed by TEM (37%), IMP (37%) and CIT (36%) (Table 2.16 and Figures 2.16 and 2.17). In RC-02, *E. coli* isolates positive for IMP were maximum (77%), followed by TEM (57%) and CTX-M15 (50%). In RC-3 isolates, CIT (65%) was the most common, followed by CTX-M15 (52%), NDM (48%) and TEM (48%). RC-05 isolates showed IMP (82%) followed by CIT (79%) and CTX-M15 (42%).In RC-07, CTX-M15 was detected in 50% isolates whereas other genes were in low prevalence. In RC-14 isolates, CTXM-1 and CIT were the commonest (70% each) followed by IMP (57%) andCTX-M1 (47%). In RC-19 isolates, CTXM-15 and OXA-48 were commonest (50% each) followed by TEM (47%). In RC-21 isolates, CIT was commonest (50%) followed by NDM and TEM (38% each).

Table 2.16. Showing positivity of various genes in *E. coli* isolates from various centers, center wise and overall

	RC-02		RC-03		RC-05		RC-07		RC-14		RC-19		RC-21		Overall	
Gene	n	%+	n	%+	n	%+	n	%+	n	%+	n	%+	n	%+	n	%+
NDM	30	33	31	48	33	9	28	7	30	23	30	13	34	38	273	31
TEM	30	57	31	48	33	12	28	11	30	23	30	47	34	38	273	37
SHV	30	3	31	0	33	0	28	4	30	7	30	0	34	0	273	2
OXA	30	33	31	19	33	24	28	4	30	30	30	40	34	24	273	30
VIM	30	13	31	6	33	6	28	11	30	30	30	3	34	0	273	9
KPC	30	7	31	6	33	3	28	0	30	23	30	0	34	0	273	5
IMP	30	77	31	42	33	82	28	0	30	57	30	17	34	6	273	37
CTX-M15	30	50	31	52	33	42	28	50	30	70	30	50	34	15	273	47
OXA-48	30	17	31	13	33	6	28	7	30	10	30	50	34	24	273	18
CTXM 1	30	13	31	23	33	0	28	7	30	47	30	17	34	6	273	19
CTXM 2	30	0	31	0	33	3	28	0	30	0	30	27	34	0	273	4
CTX-M 9	30	17	31	0	33	3	28	0	30	7	30	0	34	6	273	4
CTX-M 8/25	30	3	31	0	33	3	28	0	30	0	30	0	34	0	273	1
CIT	30	0	31	65	33	79	28	0	30	70	30	0	34	50	273	36
MOX	30	0	31	0	33	18	28	0	30	10	30	0	34	0	273	4
DHA	30	0	31	0	33	6	28	0	30	10	30	0	34	0	273	3
ACC	30	0	31	3	33	6	28	0	30	13	30	0	34	12	273	5
EBC	30	0	31	6	33	9	28	0	30	7	30	0	34	0	273	3
FOX	30	0	31	0	33	0	28	0	30	10	30	0	34	0	273	3

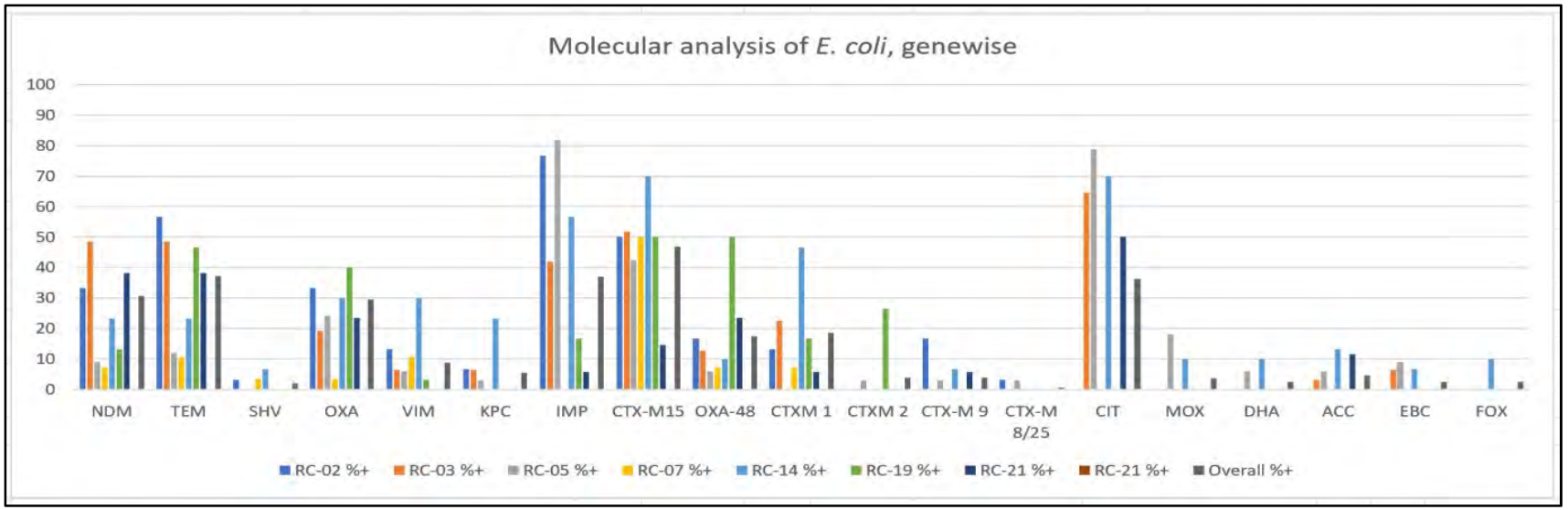


Figure 2.16. Showing positivity of various genes in E. coli isolates from various centers, gene wise and overall

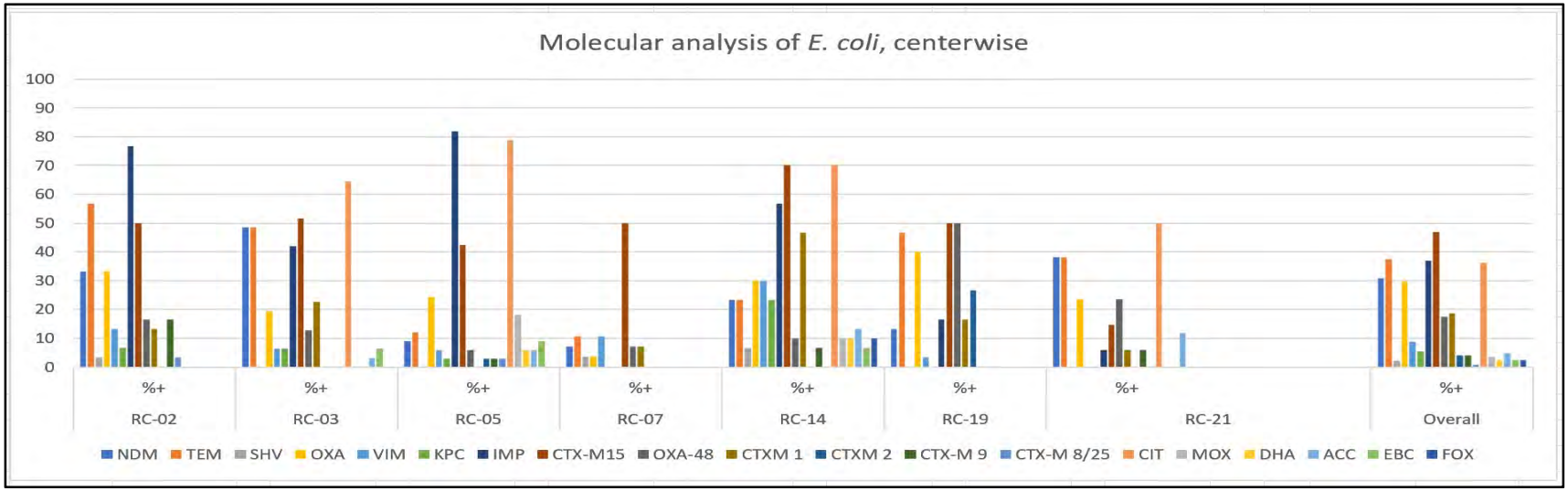


Figure 2.17. Showing positivity of various genes in E. coli isolates from various centers, gene wise and overall

K. pneumoniae

Two hundred and nine *K. pneumoniae* isolates were subjected to same PCR protocols as *E. coli*. Overall, SHV (72%) was the most commonly detected, followed by CTXM-15 (53%), TEM (46%), NDM (40%) and OXA-48 (39%) (Table 2.17 and Figure 2.18 and 2.19). In RC-02, CTX-M15 (80%) was the commonest, followed by TEM (73%), OXA-48 (63%), CTX-M1 (63%), and NDM (53%). In RC-03, SHV (100%) was detected in all tested, followed by CTX-M15 (50%), CTX-M1 (43%), and OXA-48 (40%). In RC-04, SHV (89%) was the most prevalent, followed by NDM (44%), and CTX-M15 (33%). In RC-05, SHV (70%) was followed by CTX-M15 (33%). In RC-21, SHV was the most prevalent (65%), followed by OXA-48 and TEM (60% each), CTXM-15 (55%) and OXA (50%). The center wise distribution of genes in *E. coli* and *K. pneumoniae* is shown in tables 2.18 to 2.21.

Table 2.17. Showing positivity of various genes in *K. pneumoniae* isolates from various centers, center wise and overall

	RC-02		RC-03		RC-04		RC-05		RC-21	
	n	%+	n	%+	n	%+	n	%+	n	%+
NDM	30	53	30	27	27	44	30	3	20	45
TEM	30	73	30	37	27	22	30	17	20	60
SHV	30	43	30	100	27	89	30	70	20	65
OXA	30	40	30	23	27	4	30	23	20	50
VIM	30	10	30	0	27	0	30	0	20	0
KPC	30	50	30	23	27	0	30	7	20	5
IMP	30	10	30	13	27	15	30	13	20	5
CTX-M15	30	80	30	50	27	33	30	33	20	55
OXA-48	30	63	30	40	27	26	30	17	20	60
CTXM 1	30	63	30	43	27	19	30	0	20	0
CTXM 2	30	0	30	0	27	0	30	0	20	0
CTX-M 9	30	0	30	0	27	0	30	0	20	5
CTX-M 8/25	30	3	30	0	27	0	30	0	20	0
CIT	30	0	30	7	27	19	30	23	20	5
MOX	30	0	30	3	27	11	30	13	20	0
DHA	30	0	30	3	27	0	30	7	20	0
ACC	30	0	30	3	27	7	30	10	20	0
EBC	30	0	30	0	27	0	30	3	20	0
FOX	30	0	30	0	27	7	30	0	20	0

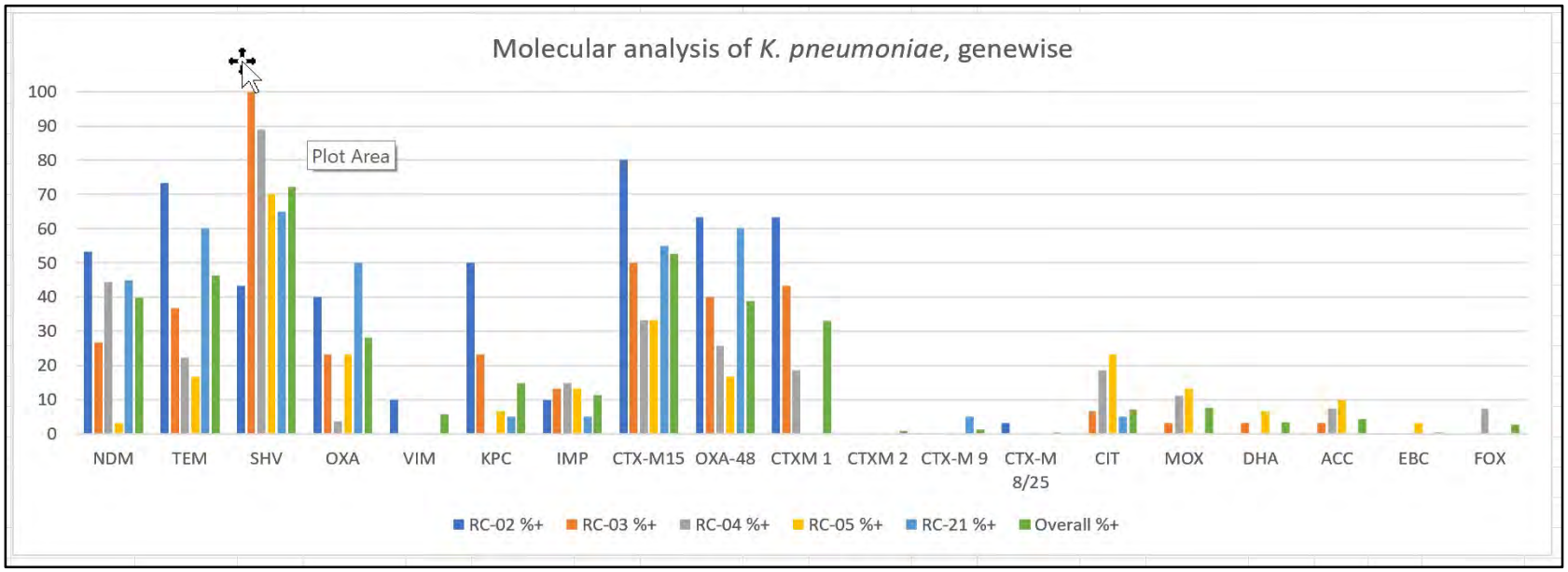


Figure 2.18. Showing positivity of various genes in *K. pneumoniae* isolates from various centers, gene wise and overall

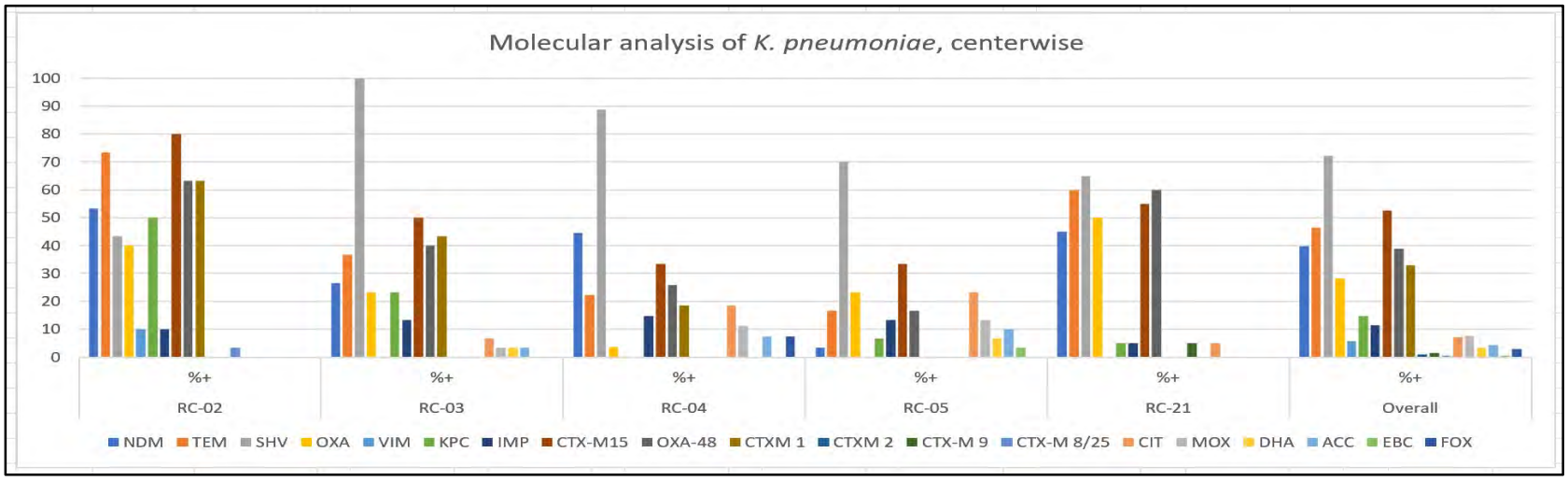


Figure 2.19. Showing positivity of various genes in *E. coli* isolates from various centers, gene wise and overall

Table 2.18. Relative prevalence of genes in *E. coli*, gene wise

Gene	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
NDM	RC-03	RC-05, RC-07, RC-19
TEM	RC-02	RC-05, RC-07
SHV	RC-14	RC-03, RC-05, RC-19, RC-21
OXA-1		RC-07
VIM	RC-14	RC-21
KPC	RC-14	RC-07, RC-19, RC-21
IMP	RC-02, RC-05	RC-07, RC-21
CTX-M15	RC-14	RC-21
OXA-48	RC-19	
CTX-M1	RC-14	RC-05
CTX-M2	RC-19	RC-02, RC-03, RC-05, RC-07, RC-14, RC-21
CTX-M9	RC-02	RC-03, RC-07, RC-19
CTX-M8/25	RC-02, RC-05	RC-03, RC-07, RC-14, RC-19, RC-21
CIT	RC-05, RC-14	RC-02, RC-07, RC-19
MOX	RC-05	RC-02, RC-03, RC-07, RC-19, RC-21
DHA	RC-14	RC-02, RC-03, RC-07, RC-19, RC-21
ACC	RC-14, RC-21	RC-02, RC-07, RC-19
EBC	RC-05, RC-14	RC-02, RC-07, RC-19, RC-21
FOX	RC-14	RC-02, RC-03, RC-05, RC-07, RC-19, RC-21

Table 2.19. Relative prevalence of genes in *E. coli*, center wise

Center	Relative high prevalence(> Mean+1SD)	Relative low prevalence(< Mean-1SD)
RC-02	TEM, IMP, CTX-M9, CTX-M8/25	CTX-M2, CIT, MOX, DHA, ACC, EBC, FOX
RC-03	NDM	SHV, CTX-M2, CTX-M9, CTX-M8/25, MOX, DHA, FOX
RC-05	IMP, CTX-M8/25, CIT, MOX, EBC	NDM, TEM, SHV, CTX-M1, CTX-M2, FOX
RC-07		NDM, TEM, OXA-1, KPC, IMP, CTX-M2, CTX-M9, CTX-M8/25, CIT, MOX, DHA, ACC, EBC, FOX
RC-14	SHV, VIM, KPC, CTX-M15, CTX-M1, CIT, DHA, ACC, EBC, FOX	CTX-M2, CTX-M8/25
RC-19	OXA-48	NDM, SHV, KPC, CTX-M9, CTX-M8/25, CIT, MOX, DHA, ACC, EBC, FOX
RC-21	ACC	SHV, KPC, IMP, CTX-M15, CTX-M2, CTX-M8/25, MOX, DHA, EBC, FOX

Table 2.20. Relative prevalence of genes in *K. pneumoniae*, gene wise

Gene	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
NDM		RC-05
TEM	RC-02	RC-04, RC-05
SHV	RC-03	
OXA-1	RC-21	RC-04
VIM	RC-02	RC-03, RC-04, RC-05, RC-21
KPC	RC-02	RC-04, RC-05, RC-21
IMP	RC-04	RC-21
CTX-M15	RC-02	RC-04, RC-05
OXA-48	RC-02, RC-21	RC-05
CTXM-1	RC-02	RC-05, RC-21
CTX-M2		
CTX-M9	RC-21	
CTX-M8/25	RC-02	
CIT	RC-04, RC-05	
MOX		
DHA	RC-05	
ACC	RC-05	
EBC		
FOX	RC-04	

Table 2.21. Relative prevalence of genes in *K. pneumoniae*, center wise

Center	Relative high prevalence (> Mean+1SD)	Relative low prevalence (< Mean-1SD)
RC-02	TEM, CTX-M15, CTX-M1, OXA-48, KPC, VIM	
RC-03	SHV	VIM
RC-04	IMP, CIT	TEM, OXA-1, VIM, KPC, CTX-M15
RC-05	CIT, DHA, ACC	NDM, TEM, VIM, KPC, CTX-M15, CTX-M1, OXA-48
RC-21	OXA-1, OXA-48	VIM, KPC, IMP, CTX-M1, CTX-M9

Chapter 3. Non fermenting Gram Negative Bacteria (NFGNB)

Among the non-fermenting gram negative bacteria, *Acinetobacter baumannii* (49.5%) was more common followed by *Pseudomonas aeruginosa* (46.4%), *Stenotrophomonas maltophilia* (3%) and *Burkholderia cepacia* (1%). *A. baumannii and P. aeruginosa* causes serious healthcare associated infections such as pneumonia, bloodstream infections and postoperative wound infections.

Acinetobacter baumannii

Isolation rate of *A. baumannii* was found to be higher in wards and ICUs (Table 3.1), denotes the persistence of these pathogens in healthcare settings. Increased efforts are therefore needed for infection control practices to prevent outbreaks. Susceptibility to all the tested antibiotics was lower (Table 3.1). The antimicrobial resistance phenotype in *A. baumannii* was similar, irrespective of the location and clinical source of the isolation (Table 3.1 and Table 3.2). *A. baumannii* (87.5%) are increasingly resistant to carbapenems (Table 3.1), limiting the availability of adequate treatment. There is no significant change in the trend of *A. baumannii* susceptibility to all the tested antibiotics (Table 3.3 and Figure 3.1). Therefore, combination therapy of colistin or polymyxin B or tigecycline with meropenem or a triple regimen of meropenem with polymyxin B and/or ampicillin–sulbactam is preferred.

Table 3.1: Location-wise susceptible percentage of *A. baumannii* isolated from all samples except faeces across OPD, Ward and ICU

AMA	Total n=12393 (S%)	OPD n=1331 (S%)	Ward n=5842 (S%)	ICU n=5220 (S%)
Piperacillin-tazobactam	1327/12052 (11)	273/1278 (21.4)	744/5681 (13.1)	310/5093 (6.1)
Cefepime	1086/11986 (9.1)	239/1281 (18.7)	601/5658 (10.6)	246/5047 (4.9)
Ceftazidime	890/10395 (8.6)	192/1133 (16.9)	484/4661 (10.4)	214/4601 (4.7)
Imipenem	1445/11934 (12.1)	284/1260 (22.5)	844/5648 (14.9)	317/5026 (6.3)
Meropenem	1516/12083 (12.5)	315/1274 (24.7)	861/5711 (15.1)	340/5098 (6.7)
Colistin*	4553/4758 (95.7)	390/421 (92.6)	2220/2292 (96.9)	1943/2045 (95)
Amikacin	1925/10734 (17.9)	329/1173 (28)	1040/4959 (21)	556/4602 (12.1)
Minocycline	5547/10185 (54.5)	600/1118 (53.7)	2644/4616 (57.3)	2303/4451 (51.7)
Levofloxacin	1382/9919 (13.9)	236/1069 (22.1)	796/4758 (16.7)	350/4092 (8.6)

*Colistin represents percentage Intermediate susceptibility of *Acinetobacter* spp.

Table 3.2: Sample-wise susceptible percentage of *A. baumannii*

AMA	Blood	LRT	Superficial infection	Deep infection	CSF	Urine
	n=2653	n=5313	n=1937	n=762	n=253	n=440
Piperacillin-tazobactam	397/2593 (15.3)	365/5124 (7.1)	210/1919 (10.9)	71/746 (9.5)	41/251 (16.3)	103/414 (24.9)
Cefepime	320/2574 (12.4)	304/5163 (5.9)	168/1902 (8.8)	59/732 (8.1)	35/249 (14.1)	74/396 (18.7)
Ceftazidime	292/2426 (12)	245/4474 (5.5)	149/1735 (8.6)	40/510 (7.8)	27/186 (14.5)	49/273 (17.9)
Imipenem	407/2563 (15.9)	414/5100 (8.1)	253/1904 (13.3)	85/737 (11.5)	40/242 (16.5)	97/398 (24.4)
Meropenem	410/2611 (15.7)	446/5140 (8.7)	262/1905 (13.8)	83/737 (11.3)	39/250 (15.6)	116/428 (27.1)
Colistin*	1056/1124 (94%)	1700/1763 (96.4%)	557/574 (97%)	454/481 (94.4%)	82/92 (89.1%)	110/110 (100%)
Amikacin	497/2281 (21.8)	607/4664 (13)	319/1772 (18)	116/615 (18.9)	42/181 (23.2)	109/361 (30.2)
Minocycline	1439/2352 (61.2)	1987/4413 (45)	958/1580 (60.6)	340/518 (65.6)	118/219 (53.9)	195/300 (65)
Levofloxacin	421/2140 (19.7)	381/4369 (8.7)	259/1645 (15.7)	67/464 (14.4)	36/185 (19.5)	72/327 (22)

*Colistin represents percentage Intermediate susceptibility of *Acinetobacter* spp.

Table 3.3: Yearly susceptible trend of *A. baumannii* isolated from all samples except faeces

AMA	Year -2016 Total=396	Year -2017 Total=3359	Year -2018 Total=4549	Year -2019 Total=8531	Year -2020 Total=6849	Year -2021 Total=12393
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Piperacillin-tazobactam	94/335 (28.1)	484/3187 (15.2)	760/4494 (16.9)	1245/8010 (15.5)	770/6724 (11.5)	1327/12052 (11)
Cefepime	67/318 (21.1)	368/3300 (11.2)	587/4457 (13.2)	1040/8271 (12.6)	587/6571 (8.9)	1086/11986 (9.1)
Ceftazidime	56/328 (17.1)	355/3202 (11.1)	575/4164 (13.8)	905/7453 (12.1)	546/6441 (8.5)	890/10395 (8.6)
Imipenem	104/334 (31.1)	501/3346 (15)	818/4517 (18.1)	1098/7272 (15.1)	744/6702 (11.1)	1445/11934 (12.1)
Meropenem	100/331 (30.2)	615/3287 (18.7)	953/4178 (22.8)	1742/8399 (20.7)	779/6747 (11.5)	1516/12083 (12.5)
Colistin*	*0/0	28/31 (90.3)	36/38 (94.7)	103/108 (95.4)	91/94 (96.8)	4553/4758 (95.7)
Amikacin	102/347 (29.4)	638/3312 (19.3)	877/3795 (23.1)	1429/7016 (20.4)	1014/5863 (17.3)	1925/10734 (17.9)
Minocycline	*0/0	926/1380 (67.1)	2393/3725 (64.2)	3893/6431 (60.5)	2794/5139 (54.4)	5547/10185 (54.5)
Levofloxacin	104/312 (33.3)	886/3040 (29.1)	959/4047 (23.7)	1500/7841 (19.1)	825/6181 (13.3)	1382/9919 (13.9)

*Colistin represents percentage Intermediate susceptibility of *Acinetobacter* spp.

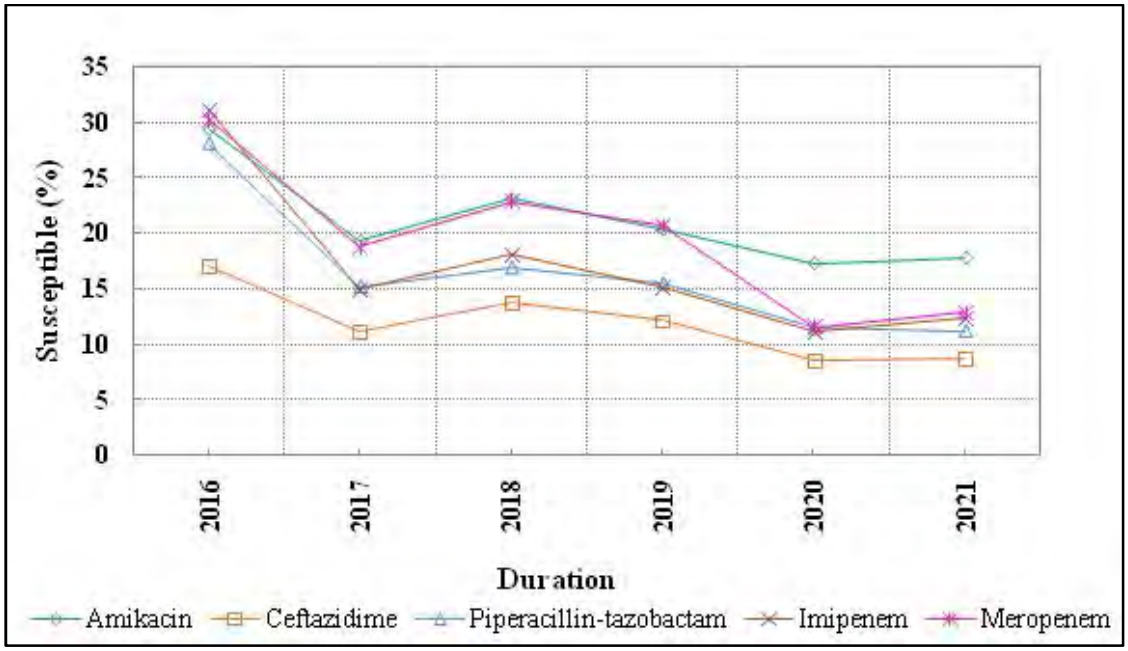


Figure 3.1: Yearly susceptible trend of *A. baumannii* isolated from all samples except faeces

Pseudomonas aeruginosa

P. aeruginosa is an opportunistic pathogen and cause infection in hospitalised patients indicated by the higher rate of isolation in wards and ICUs, compared to OPD (Table 3.4). However, there is no statistically significant difference in the susceptibility rates between the isolates from OPD and ward/ICU which represent the increasing prevalence of multi-drug resistance in *P. aeruginosa* (Table 3.4). The frequency of susceptibility to anti-pseudomonal cephalosporin such as ceftazidime (61.7% vs 54.9%) and cefepime (64.5% vs 55.5%) were higher in ward population, compared to ICU. Overall, 35% of *P. aeruginosa* isolates were resistant to carbapenems and the rate of resistance was higher in ICU population (45%) to ward. More than 60% of susceptibility to various aminoglycosides such as amikacin, gentamicin and tobramycin and fluroquinolones such as ciprofloxacin and levofloxacin were seen (Table 3.4). Higher rate of resistance to piperacillin-tazobactam (51.6%), ceftazidime (45.7%), cefepime (46.8%), meropenem (53.6%), amikacin (54.5%), gentamicin (50.6%), tobramycin (50.4%), ciprofloxacin (44.6%), levofloxacin (39.2%) was seen in those *P. aeruginosa* that were isolated from urine samples.

There is no significant difference in the susceptibility rates of *P. aeruginosa* isolated from blood and LRTI samples (Table 3.5). There is no significant change in the trend of susceptibility in *P. aeruginosa* isolated during 2016 to 2021 (Table 3.6 and Figure 3.2). For multidrug resistant *P. aeruginosa*, ceftazidime-avibactam can be considered as carbapenem sparing antibiotic and there are no defined treatment options for treating carbapenem

resistant *P. aeruginosa* infections. Colistin based combination therapy is preferred for treating *P. aeruginosa* infections.

Table 3.4: Location-wise susceptible percentage of *Pseudomonas aeruginosa* isolated from all samples (except faeces) across OPD, Ward and ICU

AMA	Total n=11622	OPD n=3098	Ward n=6099	ICU n=2425
	(S %)	(S %)	(S %)	(S %)
Piperacillin-tazobactam	7548/10835 (69.7)	2235/2907 (76.9)	3937/5671 (69.4)	1376/2257 (61)
Cefepime	7263/11233 (64.7)	2134/2954 (72.2)	3837/5953 (64.5)	1292/2326 (55.5)
Ceftazidime	6914/11028 (62.7)	2107/2978 (70.8)	3529/5724 (61.7)	1278/2326 (54.9)
Imipenem	6749/10389 (65)	1948/2722 (71.6)	3737/5627 (66.4)	1064/2040 (52.2)
Meropenem	7581/11280 (67.2)	2268/2980 (76.1)	4025/5953 (67.6)	1288/2347 (54.9)
Colistin*	2226/2298 (96.9)	491/509 (96.5)	1285/1317 (97.6)	450/472 (95.3)
Amikacin	7990/11480 (69.6)	2311/3074 (75.2)	4206/6004 (70.1)	1473/2402 (61.3)
Gentamicin	5277/8311 (63.5)	1554/2272 (68.4)	2781/4367 (63.7)	942/1672 (56.3)
Tobramycin	4148/6015 (69)	1125/1467 (76.7)	2265/3306 (68.5)	758/1242 (61)
Ciprofloxacin	6126/10159 (60.3)	1781/2728 (65.3)	3229/5408 (59.7)	1116/2023 (55.2)
Levofloxacin	5863/10123 (57.9)	1674/2686 (62.3)	3199/5442 (58.8)	990/1995 (49.6)

*Colistin represents percentage Intermediate susceptibility

Table 3.5: Sample-wise susceptible percentage of *Pseudomonas aeruginosa*

AMA	Blood	LRT	Superficial Infection	Deep Infection	CSF	Urine
	n=1336	n=3291	n=3066	n=1085	n=111	n=1398
Piperacillin-tazobactam	903/1236 (73.1%)	2313/3206 (72.1%)	2060/2888 (71.3%)	655/896 (73.1%)	47/105 (44.8%)	677/1311 (51.6%)
Cefepime	877/1304 (67.3%)	2196/3177 (69.1%)	1984/3008 (66%)	666/1034 (64.4%)	40/109 (36.7%)	624/1332 (46.8%)
Ceftazidime	833/1272 (65.5%)	2205/3250 (67.8%)	1888/2952 (64%)	550/923 (59.6%)	39/107 (36.4%)	585/1279 (45.7%)
Imipenem	717/1112 (64.5%)	1743/2612 (66.7%)	2114/2977 (71%)	609/1018 (59.8%)	31/103 (30.1%)	687/1336 (51.4%)
Meropenem	845/1271 (66.5%)	2250/3200 (70.3%)	2157/3004 (71.8%)	644/1031 (62.5%)	33/108 (30.6%)	730/1363 (53.6%)
Colistin*	302/311 (97.1%)	434/444 (97.7%)	556/570 (97.5%)	349/375 (93.1%)	29/29 (100%)	303/313 (96.8%)
Amikacin	944/1325 (71.2%)	2489/3280 (75.9%)	2127/3047 (69.8%)	748/1072 (69.8%)	35/96 (36.5%)	754/1383 (54.5%
Gentamicin	604/962 (62.8%)	1395/2025 (68.9%)	1398/2161 (64.7%)	667/987 (67.6%)	22/84 (26.2%)	617/1219 (50.6%)
Tobramycin	474/712 (66.6%)	1611/2158 (74.7%)	1220/1783 (68.4%)	176/246 (71.5%)	20/44 (45.5%)	258/512 (50.4%)
Ciprofloxacin	708/1110 (63.8%)	1677/2561 (65.5%)	1782/2808 (63.5%)	607/1041 (58.3%)	27/94 (28.7%)	587/1316 (44.6%)
Levofloxacin	625/1130 (55.3%)	1981/3023 (65.5%)	1660/2719 (61.1%)	416/791 (52.6%)	37/102 (36.3%)	463/1182 (39.2%)

*Colistin represents percentage Intermediate susceptibility

Table 3.6: Yearly susceptible trend of *Pseudomonas aeruginosa* isolated from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=1056	Total n=5687	Total n=8880	Total n=12634	Total n=7839	Total n=11622
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Piperacillin-tazobactam	705/1036 (68.1)	3757/5450 (68.9)	6034/8499 (71)	8416/11430 (73.6)	5012/7418 (67.6)	7548/10835 (69.7)
Cefepime	585/981 (59.6)	3074/5003 (61.4)	5259/8284 (63.5)	7660/12038 (63.6)	4497/7355 (61.1)	7263/11233 (64.7)
Ceftazidime	624/1035 (60.3)	3602/5504 (65.4)	5663/8598 (65.9)	7545/11977 (63)	4647/7635 (60.9)	6914/11028 (62.7)
Imipenem	809/1016 (79.6)	4059/5514 (73.6)	5627/8377 (67.2)	6425/10230 (62.8)	4411/7036 (62.7)	6749/10389 (65)
Meropenem	650/969 (67.1)	3490/5083 (68.7)	5736/8292 (69.2)	8255/12242 (67.4)	4955/7661 (64.7)	7581/11280 (67.2)
Colistin*	711/723 (98.3)	1727/1738 (99.4)	983/1075 (91.4)	1767/1899 (93)	1291/1355 (95.3)	2226/2298 (96.9)
Amikacin	693/1030 (67.3)	3864/5609 (68.9)	6019/8747 (68.8)	8340/12329 (67.6)	5276/7723 (68.3)	7990/11480 (69.6)
Gentamicin	402/776 (51.8)	2526/4249 (59.4)	4077/6462 (63.1)	5820/9383 (62)	3241/5341 (60.7)	5277/8311 (63.5)
Tobramycin	579/957 (60.5)	2954/4365 (67.7)	3809/5603 (68)	4627/6783 (68.2)	2907/4331 (67.1)	4148/6015 (69)
Ciprofloxacin	436/842 (51.8)	2930/5069 (57.8)	4814/8026 (60)	6281/10945 (57.4)	3768/6541 (57.6)	6126/10159 (60.3)
Levofloxacin	536/958 (55.9)	3236/5351 (60.5)	4794/8217 (58.3)	6148/10922 (56.3)	3771/6743 (55.9)	5863/10123 (57.9)

*Colistin represents percentage Intermediate susceptibility

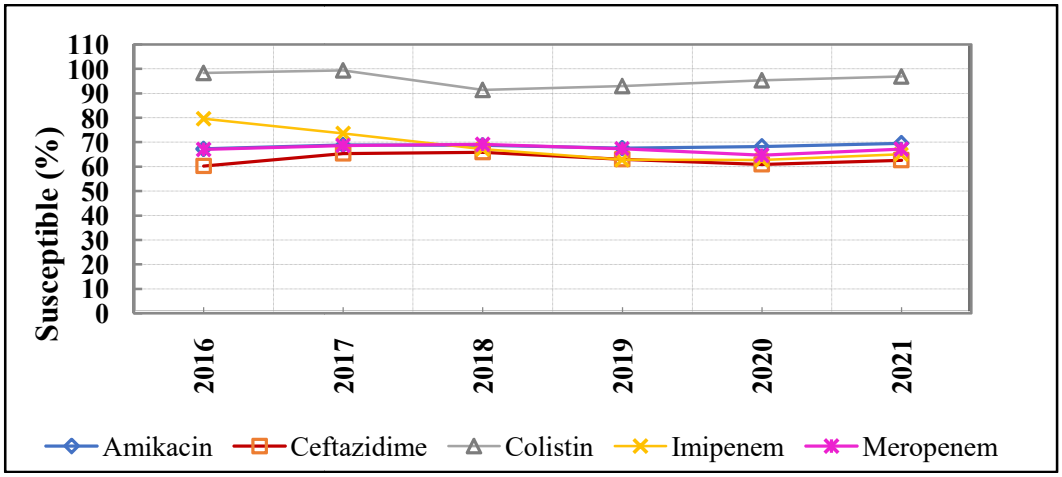


Figure 3.2. Yearly susceptible trend of *Pseudomonas aeruginosa* isolated from all samples.

Stenotrophomonas maltophilia

The rate of *S. maltophilia* isolation was <5%. Overall, *S. maltophilia* were highly susceptible to minocycline (97%), levofloxacin (90.8%) and trimethoprim-sulfamethoxazole (88.1%). Nearly, half of the tested isolates were resistant to ceftazidime (Table 3.7). There is no significant difference in the susceptibility profile of *S. maltophilia* isolated from blood and lower respiratory samples (Table 3.8). There is a little decrease in the susceptibility to ceftazidime from 2018 to 2021 and there is no significant change in the trend of susceptibility with the other tested antibiotics (Table 3.9 and Figure 3.3). *S. maltophilia* are intrinsically resistant to both carbapenem and colistin. Use of either carbapenem or colistin is the major risk factor that promotes the acquisition of *S. maltophilia*. Timely and appropriate laboratory investigation and reporting are essential to avoid delays in appropriate treatment, which are associated with increased morbidity and mortality. Therefore, the implementation of comprehensive antimicrobial stewardship programmes, with emphasis on carbapenem use, is recommended for the prevention of emergence and spread of carbapenem-resistant gram negative pathogens.

Table 3.7. Location-wise susceptible percentage of *Stenotrophomonas maltophilia* isolated from all samples across OPD, Ward and ICU

AMA	Total n=766	OPD n=91	Ward n=414	ICU n=261
	(S %)	(S %)	(S %)	(S %)
Ticarcillin-clavulanic acid	34/39 (87.2)	*3/4	*12/16	*19/19
Ceftazidime	42/84 (50)	*7/11	16/33 (48.5)	19/40 (47.5)
Minocycline	717/739 (97)	86/89 (96.6)	388/397 (97.7)	243/253 (96)
Levofloxacin	694/764 (90.8)	85/91 (93.4)	375/412 (91)	234/261 (89.7)
Trimethoprim-sulfamethoxazole	674/765 (88.1)	82/91 (90.1)	368/414 (88.9)	224/260 (86.2)
Chloramphenicol	*2/2	*1/1	*0/0	*1/1

Table 3.8: Sample-wise susceptible percentage of *Stenotrophomonas maltophilia*

AMA	All Specimens (except faeces)	Blood	LRT	Superficial Infection	Deep Infection
	n=766	n=235	n=262	n=102	n=58
Ticarcillin-clavulanic acid	34/39 (87.2%)	*14/16 (-)	*12/12 (-)	*2/3 (-)	*0/0
Ceftazidime	42/84 (50%)	16/31 (51.6%)	18/31 (58.1%)	*0/5 (-)	*1/3 (-)
Minocycline	720/742 (97%)	218/222 (98.2%)	251/258 (97.3%)	96/100 (96%)	56/58 (96.6%)
Levofloxacin	697/767 (90.9%)	220/234 (94%)	241/264 (91.3%)	91/102 (89.2%)	53/58 (91.4%)
Trimethoprim-sulfamethoxazole	677/768 (88.2%)	213/234 (91%)	237/265 (89.4%)	88/102 (86.3%)	49/58 (84.5%)
Chloramphenicol	*2/2 (-)	*1/1 (-)	*0/0	*0/0	*0/0

Table 3.9: Yearly susceptible trend of *Stenotrophomonas maltophilia* isolated from all samples

AMA	Year-2017	Year-2018	Year-2019	Year-2020	Year 2021
	Total n=157	Total n=310	Total n=374	Total n=360	Total n=766
	(S%)	(S%)	(S%)	(S%)	(S%)
Ticarcillin-clavulanic acid	19/26 (73.1)	45/60 (75)	59/68 (86.8)	28/33 (84.8)	34/39 (87.2)
Ceftazidime	15/27 (55.6)	42/63 (66.7)	46/73 (63)	41/73 (56.2)	42/84 (50)
Minocycline	143/151 (94.7)	272/299 (91)	331/350 (94.6)	332/346 (96)	717/739 (97)
Levofloxacin	126/152 (82.9)	225/257 (87.5)	225/261 (86.2)	324/358 (90.5)	694/764 (90.8)
Trimethoprim-sulfamethoxazole	132/150 (88)	255/308 (82.8)	333/372 (89.5)	318/359 (88.6)	674/765 (88.1)
Chloramphenicol	*0/0	*1/2	*3/3	*8/9	*2/2

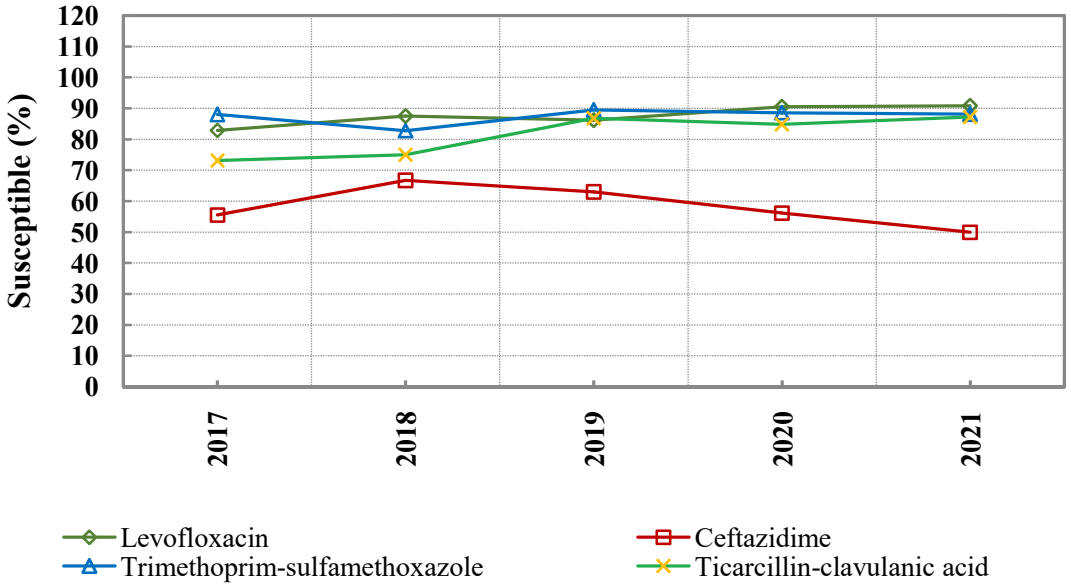


Figure 3.3: Yearly susceptible trend of *Stenotrophomonas maltophilia* isolated from all samples

Burkholderia cepacia

Burkholderia cepacia is an important opportunistic pathogen and are intrinsically resistant to multiple classes of antibiotics, including aminoglycosides and polymyxins. Among the tested antibiotics, higher rate susceptibility to ceftazidime (75.9%), meropenem (82.6%), minocycline (84.9%) and trimethoprim-sulfamethoxazole (82.5%) were seen. There is no significant difference in the susceptibility profile of *B. cepacia* in location-wise (Table 3.10) and the clinical source of isolation (Table 3.11). There is no notable change in the trend of susceptibility in *B. cepacia* during the surveillance period from 2017 to 2021 (Table 3.12 and Figure 3.4). Trimethoprim-sulfamethoxazole (TMP-SMX) and ceftazidime are considered first-line options for *B. cepacia* infections, however, in-vitro resistance to trimethoprim-sulfamethoxazole and ceftazidime seen in this surveillance, clearly demonstrates limited treatment options. Carbapenems and minocycline can be used as an alternative.

Table 3.10: Location-wise susceptible percentage of *Burkholderia cepacia* isolated from all samples across OPD, Ward and ICU

AMA	Total n=247	OPD n=27	Ward n=64	ICU n=156
	(S %)	(S %)	(S %)	(S %)
Ticarcillin-clavulanic acid	13/58 (22.4)	*2/6	6/20 (30)	5/32 (15.6)
Ceftazidime	180/237 (75.9)	24/27 (88.9)	45/60 (75)	111/150 (74)
Meropenem	199/241 (82.6)	20/26 (76.9)	49/63 (77.8)	130/152 (85.5)
Minocycline	191/225 (84.9)	22/24 (91.7)	44/54 (81.5)	125/147 (85)
Levofloxacin	49/90 (54.4)	*9/17	9/25 (36)	31/48 (64.6)
Trimethoprim-sulfamethoxazole	193/234 (82.5)	26/27 (96.3)	47/60 (78.3)	120/147 (81.6)
Chloramphenicol	*3/3	*1/1	*2/2	*0/0

Table 3.11: Sample-wise susceptible percentage of *Burkholderia cepacia*

AMA	All Specimens (except faeces)	Blood	LRT	Superficial Infection	Deep Infection	Urine
	n=247	n=147	n=61	n=*8	n=*5	n=*9
Ticarcillin-clavulanic acid	13/58 (22.4%)	10/42 (23.8%)	*1/6 (-)	*0/2 (-)	*0/1 (-)	*1/3 (-)
Ceftazidime	180/237 (75.9%)	101/140 (72.1%)	51/61 (83.6%)	*6/8 (-)	*4/4 (-)	*7/9 (-)
Meropenem	199/241 (82.6%)	123/143 (86%)	48/61 (78.7%)	*6/8 (-)	*5/5 (-)	*7/9 (-)
Minocycline	191/225 (84.9%)	108/131 (82.4%)	56/60 (93.3%)	*5/7 (-)	*4/5 (-)	*3/6 (-)
Levofloxacin	49/90 (54.4%)	35/57 (61.4%)	*6/12 (-)	*0/5 (-)	*2/3 (-)	*0/5 (-)
Trimethoprim-sulfamethoxazole	193/234 (82.5%)	117/136 (86%)	51/61 (83.6%)	*4/8 (-)	*4/5 (-)	*5/9 (-)
Chloramphenicol	*3/3 (-)	*0/0	*0/0	*1/1 (-)	*0/0	*0/0

Table 3.12: Yearly susceptible trend of *Burkholderia cepacia* isolated from all samples

AMA	Year-2017 Total n=112	Year-2018 Total n=197	Year-2019 Total n=181	Year-2020 Total n=200	Year-2021 Total n=247
	(S%)	(S%)	(S%)	(S%)	(S%)
Ticarcillin-clavulanic acid	*0/9	4/51 (7.8)	36/103 (35)	36/80 (45)	13/58 (22.4)
Ceftazidime	73/101 (72.3)	137/192 (71.4)	156/178 (87.6)	172/198 (86.9)	180/237 (75.9)
Meropenem	83/111 (74.8)	140/171 (81.9)	161/181 (89)	166/198 (83.8)	199/241 (82.6)
Minocycline	89/104 (85.6)	146/185 (78.9)	133/174 (76.4)	163/191 (85.3)	191/225 (84.9)
Levofloxacin	*4/13	34/66 (51.5)	70/124 (56.5)	81/125 (64.8)	49/90 (54.4)
Trimethoprim-sulfamethoxazole	84/109 (77.1)	179/192 (93.2)	164/177 (92.7)	174/200 (87)	193/234 (82.5)
Chloramphenicol	*0/0	*1/1	*3/3	*4/4	*3/3

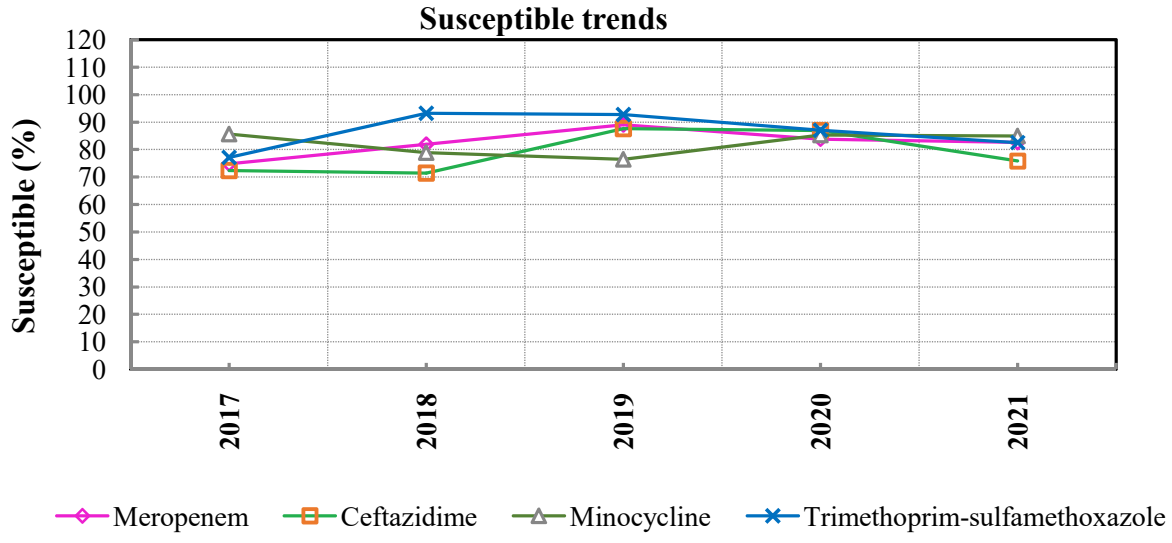


Figure 3.4: Yearly susceptible trend of *Burkholderia cepacia* isolated from all samples

Molecular mechanism

Characterization of resistance mechanism in P. aeruginosa

A total of 879 *P. aeruginosa* isolated from various clinical specimens were received at the reference laboratory. Of which, 222 were identified as carbapenem resistant and were screened for the presence of beta lactamases by molecular methods (ESBLs and carbapenemases). Of the entire beta lactamases screened, *bla*$_{VEB}$ was the most common ESBL followed by *bla*$_{TEM}$ gene; *bla*$_{SHV}$ and *bla*$_{PER}$ were absent in all the isolates as the previous year (Table 3.13). Similarly, among the carbapenemases, *bla*$_{NDM}$ was the most common metallo beta lactamase (carbapenemase) identified, followed by *bla*$_{VIM}$ and *bla*$_{IMP}$ genes. Unlike *A. baumannii*, co- producers of ESBLs and carbapenemases seems to be higher in *P. aeruginosa*. Among the co- producers, *bla*$_{NDM}$ co-carried with ESBLs such as *bla*$_{VEB}$ and *bla*$_{TEM}$ genes were predominantly seen (n = 48, 81%). Trend analysis over the last two years highlights that there has been a shift from *bla*$_{VIM}$ to *bla*$_{NDM}$ producers across different geographical location.

Characterization of resistance mechanism in A. baumannii

A total of 563 isolates received from various regional centers were subjected to PCR for characterization of antimicrobial resistance genes. All the isolates harboured the *bla*$_{OXA-51}$ like gene, which is intrinsic to *Acinetobacter baumannii*. Molecular gene profile of all the centers tested in 2021 was tabulated (Table 3.14). As expected, *bla*$_{OXA-23}$ like only was the predominant carbapenemase across all the centers contributing to 38% of the carbapenem resistance. Co- producers of various AMR genes like ESBLs with carbapenemases and dual carbapenemases were observed across all the centers. Of which, co-producers of *bla*$_{OXA-23}$ like with *bla*$_{NDM}$ like n=262 (46%) were found to be predominant followed by *bla*$_{OXA-23}$ like with *bla*$_{PER}$ like n=33 (6%), *bla*$_{TEM}$ like n=27 (5%) and *bla*$_{OXA-23}$ like with *bla*$_{NDM}$, *bla*$_{TEM}$/ *bla*$_{PER}$ n = 15 (3%). One isolates each from LTMMC and PGIMER carried *bla*$_{OXA-58}$ like gene. None of the isolates had *bla*$_{OXA-24}$ like, *bla*$_{IMP}$ like, *bla*$_{VIM}$ like, *bla*$_{SIM}$ like, *bla*$_{KPC}$ like and *bla*$_{GES}$ like carbapenemases.

The antimicrobial resistance gene profile was found to be consistent across all the centers with *bla*$_{OXA-23}$ like being the predominant carbapenemase and sporadic presence of *bla*$_{OXA-58}$ like were observed. Trend analysis shows there has been an increase in the prevalence of co- producers from 57% in 2020 to 60% in 2021 however this is not a significant raise.

Table 3.13: Molecular characterization of carbapenem resistant *P. aeruginosa* collected across India during the year 2021

CENTER	*P.aeruginosa*	ESBL				Class A Carbapenemase		Class B carbapenemase(MβLs)				Combination genes
	Total (R tested)	SHV	TEM	VEB	PER	KPC	GES	SPM	IMP	VIM	NDM	Co-producers
RC3	60(60)	-	2	-	-	-	2	-	2	3	12	TEM+IMP&NDM-3VEB+IMP&NDM-1VIM&NDM-4TEM&NDM-1VEB&NDM-6VEB&VIM&NDM-1VEB&VIM-1
RC1	137(45)	-	-	3	-	-	-	-	1	1	19	VEB&NDM-10TEM+NDM-3VEB+VIM+NDM-1
RC4	65(17)	-	-	-	-	-	-	-	-	1	5	VEB&IMP-1PER&NDM-1VIM&NDM-4VEB&NDM-4VEB&VIM-1
RC2	65(11)	-	-	-	-	-	-	-	1	1	6	VEB&NDM-1VIM&NDM-1
RC8	-	-	-	-	-	-	-	-	-	-	-	-
RC6	60(24)	-	-	2	-	-	2	-	-	-	7	VEB&NDM-4VEB&VIM-1TEM&NDM-1VEB&VIM&NDM-1VEB&TEM+VIM&NDM-1
RC 9	40(01)	-	-		-	-	-	-	-	-	1	-

Table 3.14: Molecular characterization of carbapenem resistant *A. baumannii* collected across India during the year 2021

Centres	*A.baumannii*	ESBL				Class ACarbapenemase		Class B carbapenemase(MβLs)				Class Dcarbapenemase			Combinationgenes
	Total (R tested)	SHV	TEM	VEB	PER	KPC	GES	IMP	VIM	NDM	SIM	OXA-23	OXA-24	OXA-58	Co-producers
RC3	70(63)	-	-	-	-	-	-	-	-	-	-	13	-	-	OXA23&NDM=40OXA23&PER=7OXA23,NDM,TEM=1OXA23,NDM,PER=2
RC14	46(33)	-	-	-	-	-	-	-	-	-	-	11	-	-	OXA23&NDM=18OXA23&PER=3OXA23,NDM,TEM=1
RC10	59(19)	-	-	-	-	-	-	-	-	-	-	7	-	-	OXA23&NDM=7OXA23&PER=4OXA23,NDM&PER=1
RC5	29(14)	-	-	-	-	-	-	-	-	-	-	6	-	-	OXA23&NDM=7OXA23&TEM=1
RC9	62(22)	-	-	-	-	-	-	-	-	-	-	4	-	-	OXA23&NDM=9OXA23&TEM=4OXA23,&PER=5
RC18	89(30)	-	-	-	-	-	-	-	-	-	-	7	-	-	OXA23&NDM=20OXA23&PER=2OXA23,NDM,PER=1
RC4	64(32)	-	-	-	-	-	-	-	-	-	-	10	-	-	OXA23&NDM=231OXA23&TEM=1
RC15	75(54)	-	-	-	-	-	-	-	-	-	-	35	-	-	OXA23&NDM=12OXA23&PER=2OXA23&TEM=4OXA23,OXA58,TEM=1
RC20	15(10)	-	-	-	-	-	-	-	-	-	-	4	-	-	OXA23&NDM=4OXA23&PER=2
RC7	8(6)	-	-	-	-	-	-	-	-	-	-	3	-	-	OXA23&NDM=1OXA23&PER=1OXA23,NDM,PER=1

Chapter 4 Staphylococci and Enterococci

Summary

A total of 8827 *S.aureus*, 2655 CoNS and 5647 enterococci isolates collected across India were analysed in the year 2021. The total number of isolates available for analysis in 2021 was higher than in 2020. Identification of MRSA was done by testing susceptibility to cefoxitin (6740) and/or oxacillin (3685). The overall proportion of MRSA was 42.6% and 33.8% respectively. Penicillin susceptibility was extremely low as expected (9.5% in MSSA and 6.9% among CoNS). Susceptibility to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA. The anti MRSA antibiotics such as vancomycin and tigecycline showed excellent in vitro activity (100% against MRSA isolates). Linezolid resistance was encountered in both MRSA and CoNS isolates albeit at very low rates of 0.1%. Teicoplanin resistance was much higher among CoNS isolates at 4.1% compared to 0.5% in MRSA.

Staphyloccus aureus

A total of 8827 isolates of *S. aureus* were reported from different centres across India. The overall proportion of MRSA was 42.6% which is a slight increase over the rate reported in 2020 (41.4%) (Table 4.1). Cefoxitin resistance, the surrogate marker for MRSA, was observed nearly twice as commonly among CoNS as *S.aureus* (76.8% vs 42.6%). There was a discrepancy in the MRSA rates detected by Oxacillin MIC (33.8% vs 42.6%). This discrepancy could be because of the smaller number of isolates tested against oxacillin than against cefoxitin. Moreover the same isolates may not have been tested by both the methods. Penicillin susceptibility was extremely low as expected (9.5% in MSSA and 6.9% among CoNS). Susceptibility to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA. The anti MRSA antibiotics such as vancomycin and tigecycline showed excellent in vitro activity (100% Vs 99.2% against MRSA isolates). Linezolid and teicoplanin resistance was encountered in MRSA isolates albeit at very low rates of 0.1 % respectively but in CoNS slightly increased 0.5% Vs 4.1% respectively.

Table 4.2 shows the susceptibility pattern of *S. aureus* and CoNS across different hospital locations. As expected, the overall MRSA rates among *S. aureus* were lowest in the OPD isolates (38%) while it was moderate among ICU isolates (40.7%) and higher among the ward isolates 46.2%. The susceptibility to most antibiotics was least among ICU isolates and highest among OPD isolates of *S.aureus* including MRSA and CoNS. However, among MSSA, susceptibility to co-trimoxazole was slightly higher among ward and ICU isolates than OPD although the difference was not significant. Linezolid resistance among CoNS, MRSA, and MSSA isolates showed rates of

0.5%, 0.1%, and 0.1% respectively. Teicoplanin resistance was slightly higher among CoNS and MSSA than the MRSA isolates and showed rates of 4.1%, 0.2 percent, and 0.1 percent, respectively.

Among the centre wise susceptibility rates of *S. aureus* isolates, there were significant differences observed between the various regional centres, the highest MRSA rate in the isolates from RC18 and RC20 (69.9% and 80.9%). The lowest MRSA rates were observed from the RC04 (23.6%) and RC10 (30.1%) based on cefoxitin test results. However it should be noted that in RC 7, oxacillin resistance was used to identify MRSA rather than cefoxitin (123 Vs 5 respectively) (Table 4.3). This variation in MRSA rates across centres may be indicative of the differences in the antibiotic prescription practices and usage in the different regions. It could also reflect different methodologies adopted across centres to identify MRSA. Ciprofloxacin susceptibility was extremely low across all centres. The susceptibility rate of other antibiotics varied widely between the centres for many of the antibiotics like erythromycin (5 % in RC 21 to 63.3% in RC 04), tetracycline (75.3 % in RC 16 to 96.6% in RC 09) , clindamycin (22.8% in RC 21 to 99 % in RC 08), cotrimoxazole (23.7% in RC 21 to 91.6 % in RC 03). These unexpected differences could be a reflection of the methodologies employed (DD or MIC) or the pattern of antibiotic usage in the different regions. Linezolid resistance was documented in the RC 04 (0.1%) and in RC 12 (0.5%).

Most of the *S. aureus* isolates were obtained from superficial infections followed by blood stream infections. MRSA rates differed based on the source of isolation, with blood isolates demonstrating highest rates (47.4%) while those from deep infections showed the lowest rates (38.8%), the MRSA rates were lower among OPD isolates (38%) while it was 40.7% among ICU isolates and higher among in the ward isolates 46.2%. The susceptibility to most antibiotics was least among ICU isolates and highest among OPD isolates of *S. aureus* including MRSA and CoNS. However, among MSSA, susceptibility to co-trimoxazole was slightly higher among ward and ICU isolates than OPD although the difference was not significant. Linezolid resistance among CoNS, MRSA isolates showed rates of 0.5%, 0.1% respectively. Teicoplanin resistance was slightly higher among CoNS and MSSA than the MRSA isolates showed rates of 4.1%, 0.2 percent, and 0.1 percent, respectively (Table 4.2).

Although *S.aureus*, overall, showed increasing trends of resistance to most antibiotics over the years, no such prominent trend could be observed with MSSA isolates. There was only a marginal decrease in the susceptibility rates to erythromycin. Overall susceptibility rates to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA.

Centerwise analysis

Ciprofloxacin susceptibility was extremely low across all centres. The susceptibility rate of other antibiotics varied widely between the centres for many of the antibiotics like erythromycin (5 % in RC 21 to 63.3% in RC 04), tetracycline (75.3 % in RC 16 to 96.6% in RC

09), clindamycin (22.8% in RC 21 to 99 % in RC 08), cotrimoxazole (23.7% in RC 21 to 91.6 % in RC 03. These unexpected differences could be a reflection of the methodologies employed (DD or MIC) or the pattern of antibiotic usage in the different regions. Linezolid resistance was documented in the RC 04 (0.1%) and in RC 12 (0.5%).

Most laboratories depend on cefoxitin disc diffusion to identify MRSA. It has been observed that this test tends to misidentify a small number of isolates. This feature was noticed with both our isolates as well as those received as part of EQAS from regional centres. Some of the centres identified MRSA based on VITEK results. Here a discrepancy was found between cefoxitin and oxacillin results. As per the data shared by ICMR, MRSA rate based on cefoxitin DD results is 42.6% whereas, the rate was 33.8% based on oxacillin MIC results. This discrepancy could be due to the difference in the number of isolates being tested by both methods. Moreover the same isolates may not have been tested by both the methods (Table 4.3).

The MRSA phenotype was conferred by the mecA gene as determined by PCR of randomly selected isolates from all centres. However in about 0.8% of MRSA, *mec*A PCR was negative. PCR for the mecC gene was also negative in these isolates. Recently plasmid mediated *mec*B and *mec*D genes have been reported in *S.aureus* which may complicate detection methods even further (Becker K, 2018, Lakhundi and Zhang 2018). These genes were looked for among randomly selected isolates in the 2021, none of the isolates harboured the genes. On the other hand, a few randomly selected MSSA isolates were found to carry the *mec*A gene demonstrating the occurrence of dormant MRSA.

Among the non-beta lactam antibiotics, macrolide resistance was conferred either through *erm*A/*erm*C/*msr*A/B genes. In the present study, the overall prevalence of *erm*C genes was high (38.5 %) followed by *msr*A/B (36.8%) and *erm*A (4.3%). None of the isolates harboured *erm*B genes. These genes are usually found among streptococci.

Full blown vancomycin resistance was not encountered in 2021. Of the 84 MRSA isolates from JIPMER subjected to PAP-AUC, 3 were identified as VISA (3.5%) while 4 were identified as hVISA (4.7 %) while in other regional centres; the rates of both VISA and hVISA were 6.3% (22/349). Overall, mupirocin resistance in *S.aureus* was stable at 5.7 % in 2021 and in MRSA it was slightly reduced from10.7% to 9.9%. These rates have remained almost the same for last 3 years possibly suggesting that mupirocin resistance genes exert a large fitness cost on MRSA. Resistance to tigecycline was not seen in 2016 but it appeared in a small number of isolates in 2017, 2018, 2019 and 2021. In 2020, none of the isolates exhibited tigecycline resistance.

MIC creep

MIC creep for the anti MRSA antibiotics will be presented taking 2018 as the index year. There was a slight increase in MIC level of vancomycin in a few centres like RC06 (0.25 to 0.38µg/ml), and RC09 (0.38 to 0.5 µg/ml) isolates, while in the other centres there was no change in the MIC when compared to the previous year. The median MIC for linezolid among RC06 and RC17 isolates increased slightly, but it remained unchanged in isolates from other centres from the

previous year. In the case of daptomycin, MIC level was slightly lower among RC04, RC03, RC15, RC09 and RC20 isolates, but it was twice as high among isolates from RC01, RC06 and RC17 (0.125 to 0.25ug/ml). Due to a shortage of stocks from the manufacturer, tigecycline was not tested in 2020, but the results from 2019 were comparable to those from 2021, with little difference in MIC level.

Table 4.1: Percentage susceptibility of *S. aureus*, MSSA, MRSA and CoNS isolated from all samples

AMA	All Specimens			
	S. aureus n=8827	MSSA n=5273	MRSA n=3423	CoNS n=2655
Cefoxitin	3869/6740 (57.4)	3845/3845 (100)	24/2895 (0.8)	566/2443 (23.2)
Oxacillin	2440/3685 (66.2)	2399/2399 (100)	41/1286 (3.2)	11/57 (19.3)
Penicillin	229/4293 (5.3)	203/2131 (9.5)	24/2101 (1.1)	138/1994 (6.9)
Vancomycin	6203/6204 (100)	4010/4010 (100)	2153/2154 (100)	1374/1376 (99.8)
Teicoplanin	3351/3356 (99.9)	1945/1949 (99.8)	1369/1370 (99.9)	497/518 (95.9)
Erythromycin	3617/8355 (43.3)	2665/4975 (53.6)	917/3274 (28)	455/2607 (17.4)
Tetracycline	5686/6400 (88.8)	3297/3579 (92.1)	2348/2772 (84.7)	1809/2536 (71.3)
Tigecycline	2113/2131 (99.2)	1102/1112 (99.1)	990/998 (99.2)	344/354 (96.9)
Ciprofloxacin	1455/8341 (17.4)	1112/4971 (22.4)	328/3257 (10.1)	778/2209 (35.2)
Clindamycin	6334/8579 (73.8)	4057/5137 (79)	2228/3362 (66.3)	1363/2625 (51.9)
Trimethoprim-sulfamethoxazole	4718/6954 (67.8)	2884/3927 (73.4)	1796/2961 (60.7)	1224/2609 (46.9)
Linezolid	8233/8236 (100)	4838/4839 (100)	3317/3319 (99.9)	2600/2613 (99.5)
Mupirocin High Level	2704/2866 (94.3)	1436/1460 (98.4)	1253/1391 (90.1)	*0/0 (-)

Table 4.2: Location-wise susceptibility of *S. aureus*, MSSA, MRSA and CoNS from all samples

AMA	*Staphylococcus aureus*				*MSSA*				*MRSA*				*CoNS*			
	Total n=8827	OPD n=3132	Ward n=4573	ICU n=1122	Total n=5273	OPD n=1965	Ward n=2602	ICU n=706	Total n=3423	OPD n=1125	Ward n=1916	ICU n=382	Total n=2655	OPD n=610	Ward n=1505	ICU n=540
	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)
Cefoxitin	3869/6740 (57.4)	1525/2461 (62)	1898/3527 (53.8)	446/752 (59.3)	3845/3845 (100)	1517/1517 (100)	1885/1885 (100)	443/443 (100)	24/2895 (0.8)	8/944 (0.8)	13/1642 (0.8)	3/309 (1)	566/2443 (23.2)	161/554 (29.1)	307/1410 (21.8)	98/479 (20.5)
Oxacillin	2440/3685 (66.2)	911/1373 (66.4)	1189/1858 (64)	340/454 (74.9)	2399/2399 (100)	893/893 (100)	1168/1168 (100)	338/338 (100)	41/1286 (3.2)	18/480 (3.8)	21/690 (3)	2/116 (1.7)	11/57 (19.3)	*3/13	5/32 (15.6)	*3/12
Penicillin	229/4293 (5.3)	90/1518 (5.9)	111/2293 (4.8)	28/482 (5.8)	203/2131 (9.5)	84/819 (10.3)	96/1066 (9)	23/246 (9.3)	24/2101 (1.1)	5/676 (0.7)	15/1200 (1.3)	4/225 (1.8)	138/1994 (6.9)	38/472 (8.1)	76/1135 (6.7)	24/387 (6.2)
Vancomycin	6203/6204 (100)	2328/2329 (100)	3241/3241 (100)	634/634 (100)	4010/4010 (100)	1567/1567 (100)	2010/2010 (100)	433/433 (100)	2153/2154 (100)	746/747 (99.9)	1216/1216 (100)	191/191 (100)	1373/1376 (99.8)	334/334 (100)	841/841 (100)	198/201 (98.5)
Teicoplanin	3351/3356 (99.9)	1328/1329 (99.9)	1712/1715 (99.8)	311/312 (99.7)	1945/1949 (99.8)	806/807 (99.9)	941/943 (99.8)	198/199 (99.5)	1369/1370 (99.9)	505/505 (100)	757/758 (99.9)	107/107 (100)	496/517 (95.9)	115/120 (95.8)	269/278 (96.8)	112/119 (94.1)
Erythromycin	3617/8355 (43.3)	1386/2980 (46.5)	1835/4378 (41.9)	396/997 (39.7)	2665/4975 (53.6)	1054/1881 (56)	1314/2474 (53.1)	297/620 (47.9)	917/3274 (28)	322/1067 (30.2)	502/1856 (27)	93/351 (26.5)	455/2607 (17.5)	126/595 (21.2)	263/1481 (17.8)	66/531 (12.4)
Tetracycline	5686/6400 (88.8)	2180/2424 (89.9)	3000/3404 (88.1)	506/572 (88.5)	3297/3579 (92.1)	1389/1488 (93.3)	1624/1785 (91)	284/306 (92.8)	2348/2772 (84.7)	775/916 (84.6)	1356/1596 (85)	217/260 (83.5)	1809/2536 (71.3)	442/597 (74)	1030/1448 (71.1)	337/491 (68.6)

Tigecycline	2113/2131 (99.2)	908/916 (99.1)	1072/1080 (99.3)	133/135 (98.5)	1102/1112 (99.1)	517/522 (99)	515/518 (99.4)	70/72 (97.2)	990/998 (99.2)	383/386 (99.2)	547/552 (99.1)	60/60 (100)	344/354 (97.2)	93/95 (97.9)	180/185 (97.3)	71/74 (95.9)
Ciprofloxacin	1456/8341 (17.5)	534/3028 (17.6)	750/4319 (17.4)	172/994 (17.3)	1113/4971 (22.4)	442/1904 (23.2)	544/2445 (22.2)	127/622 (20.4)	328/3257 (10.1)	87/1088 (8)	198/1829 (10.8)	43/340 (12.6)	778/2209 (35.2)	229/542 (42.3)	426/1231 (34.6)	123/436 (28.2)
Clindamycin	6334/8579 (73.8)	2384/3076 (77.5)	3247/4483 (72.4)	703/1020 (68.9)	4057/5137 (79)	1584/1935 (81.9)	1980/2556 (77.5)	493/646 (76.3)	2228/3362 (66.3)	780/1110 (70.3)	1245/1892 (65.8)	203/360 (56.4)	1363/2625 (51.9)	341/604 (56.5)	784/1489 (52.7)	238/532 (44.7)
Trimethoprim-sulfamethoxazole	4718/6954 (67.8)	1687/2571 (65.6)	2540/3639 (69.8)	491/744 (66)	2884/3927 (73.4)	1117/1573 (71)	1448/1925 (75.2)	319/429 (74.4)	1796/2961 (60.7)	556/973 (57.1)	1071/1684 (63.6)	169/304 (55.6)	1223/2609 (46.9)	305/604 (50.5)	685/1477 (46.4)	233/528 (44.1)
Linezolid	8233/8236 (99.9)	2925/2925 (100)	4342/4344 (99.9)	966/967 (99.9)	4838/4839 (99.9)	1809/1809 (100)	2433/2433 (100)	596/597 (99.8)	3317/3319 (99.9)	1088/1088 (100)	1873/1875 (99.9)	356/356 (100)	2599/2613 (99.5)	599/600 (99.8)	1485/1494 (99.4)	515/519 (99.2)
Mupircoin High Level	2704/2866 (94.3)	1026/1051 (97.6)	1400/1510 (92.7)	278/305 (91.1)	1436/1460 (98.4)	590/595 (99.2)	702/718 (97.8)	144/147 (98)	1253/1391 (90.1)	431/451 (95.6)	689/783 (88)	133/157 (84.7)	*0/0	*0/0	*0/0	*0/0

Table 4.3: Antimicrobial Susceptibility (AMS) Percentage RC wise of *Staphylococcus aureus* from all samples except faeces and urine

RC/ Antibiotics	Cefoxitin (n=6528)	Oxacillin (n=3653)	Penicillin (n=4098)	Vancomycin (n=6123)	Teicoplanin (n=3302)	Erythromycin (n=8141)	Tetracycline (n=6253)	Tigecycline (n=2104)	Ciprofloxacin (n=8128)	Clindamycin (n=8357)	Trimethoprim-sulfamethoxazole (n=6739)	Linezolid (n=8039)	Mupirocin High Level (n=2770)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
RC2	-	1254/1631 (76.9)	-	1616/1617 (99.9)	887/891 (99.6)	616/1476 (41.7)	1/1* (-)	-	88/1617 (5.4)	811/1619 (50.1)	-	1396/1396 (100)	-
RC4	1047/1370 (76.4)	0/1* (-)	0/3* (-)	1368/1368 (100)	257/257 (100)	867/1370 (63.3)	1268/1369 (92.6)	182/183 (99.5)	425/1370 (31)	1235/1370 (90.1)	920/1370 (67.2)	1368/1370 (99.9)	1246/1273 (97.9)
RC1	277/477 (58.1)	-	8/477 (1.7)	264/264 (100)	-	158/471 (33.5)	386/477 (80.9)	-	95/476 (20)	372/475 (78.3)	286/465 (61.5)	477/477 (100)	199/199 (100)
RC14	475/813 (58.4)	484/813 (59.5)	1/1* (-)	810/810 (100)	814/814 (100)	398/783 (50.8)	774/814 (95.1)	806/814 (99)	142/814 (17.4)	773/813 (95.1)	678/814 (83.3)	813/813 (100)	1/1* (-)
RC6	108/247 (43.7)	112/249 (45)	15/249 (6)	249/249 (100)	249/249 (100)	93/244 (38.1)	219/249 (88)	248/249 (99.6)	12/249 (4.8)	159/249 (63.9)	113/249 (45.4)	249/249 (100)	-
RC15	345/521 (66.2)	-	20/522 (3.8)	516/516 (100)	-	201/522 (38.5)	403/482 (83.6)	-	39/522 (7.5)	334/521 (64.1)	358/520 (68.8)	522/522 (100)	-
RC3	218/337 (64.7)	-	-	-	-	98/229 (42.8)	187/211 (88.6)	-	-	141/203 (69.5)	283/309 (91.6)	337/337 (100)	1/1* (-)
RC13	110/222 (49.5)	0/1* (-)	12/230 (5.2)	9/9* (-)	9/9* (-)	60/232 (25.9)	123/142 (86.6)	2/2* (-)	44/212 (20.8)	136/233 (58.4)	169/239 (70.7)	233/233 (100)	1/1* (-)
RC10	225/322 (69.9)	0/3* (-)	41/326 (12.6)	148/148 (100)	154/154 (100)	111/321 (34.6)	19/22 (86.4)	-	66/323 (20.4)	237/319 (74.3)	156/254 (61.4)	86/86 (100)	-
RC20	67/351 (19.1)	-	14/319 (4.4)	21/21 (100)	20/20 (100)	94/354 (26.6)	251/310 (81)	1/1* (-)	41/348 (11.8)	191/353 (54.1)	164/346 (47.4)	354/354 (100)	339/348 (97.4)
RC7	0/5* (-)	64/123 (52)	0/4* (-)	132/132 (100)	131/131 (100)	75/133 (56.4)	124/133 (93.2)	127/128 (99.2)	14/138 (10.1)	128/138 (92.8)	79/139 (56.8)	137/137 (100)	-

RC18	147/488 (30.1)	-	4/488 (0.8)	-	-	164/488 (33.6)	438/488 (89.8)	-	203/488 (41.6)	342/488 (70.1)	319/488 (65.4)	488/488 (100)	404/488 (82.8)
RC5	185/281 (65.8)	172/256 (67.2)	31/282 (11)	177/177 (100)	177/177 (100)	122/256 (47.7)	242/271 (89.3)	214/214 (100)	37/282 (13.1)	277/282 (98.2)	199/282 (70.6)	281/281 (100)	-
RC19	78/166 (47)	-	3/166 (1.8)	115/115 (100)	-	59/166 (35.5)	132/166 (79.5)	-	31/158 (19.6)	100/166 (60.2)	113/164 (68.9)	166/166 (100)	0/1* (-)
RC9	159/258 (61.6)	-	21/266 (7.9)	1/1* (-)	-	76/264 (28.8)	256/265 (96.6)	1/1* (-)	31/267 (11.6)	251/262 (95.8)	238/266 (89.5)	266/266 (100)	262/263 (99.6)
RC17	132/262 (50.4)	149/263 (56.7)	17/263 (6.5)	261/261 (100)	261/261 (100)	132/252 (52.4)	241/263 (91.6)	186/186 (100)	52/263 (19.8)	222/263 (84.4)	201/262 (76.7)	263/263 (100)	-
RC12	23/33 (69.7)	95/171 (55.6)	5/196 (2.6)	179/179 (100)	182/182 (100)	89/182 (48.9)	177/190 (93.2)	174/180 (96.7)	16/194 (8.2)	173/196 (88.3)	70/177 (39.5)	195/196 (99.5)	-
RC16	47/188 (25)	2/3* (-)	13/185 (7)	103/103 (100)	3/3* (-)	65/187 (34.8)	137/182 (75.3)	3/3* (-)	45/191 (23.6)	156/190 (82.1)	117/179 (65.4)	189/189 (100)	140/167 (83.8)
RC8	67/97 (69.1)	71/102 (69.6)	5/49 (10.2)	102/102 (100)	103/103 (100)	31/96 (32.3)	92/103 (89.3)	103/103 (100)	18/101 (17.8)	102/103 (99)	71/103 (68.9)	103/103 (100)	-
RC21	50/90 (55.6)	10/17* (-)	12/72 (16.7)	30/30 (100)	30/30 (100)	5/93 (5.4)	75/93 (80.6)	18/18* (-)	11/93 (11.8)	21/92 (22.8)	22/93 (23.7)	92/92 (100)	28/28 (100)
RC11	-	7/20 (35)	-	21/21 (100)	21/21 (100)	9/22 (40.9)	20/22 (90.9)	22/22 (100)	3/22 (13.6)	22/22 (100)	16/20 (80)	21/21 (100)	-
Total	3760/6528 (57.6)	2420/3653 (66.2)	222/4098 (5.4)	6122/6123 (100)	3298/3302 (99.9)	3523/8141 (43.3)	5565/6253 (89)	2087/2104 (99.2)	1413/8128 (17.4)	6183/8357 (74)	4572/6739 (67.8)	8036/8039 (100)	2621/2770 (94.6)

Table 4.4 and Fig 4.1 depict the comparison of the susceptibility rates of *S. aureus* in 2021 with the rates seen between the years 2016-2020. Overall MRSA rates are slightly increasing each year from 2016 to 2021(28.4% to 42.6%). Susceptibility to most antibiotics showed almost similar rates as in the previous years. However mupirocin susceptibility, which was stable between 2016 and 2018, showed a decline in 2019 and remained the same between 2020 and 2021. Resistance to tigecycline was not seen in 2016 but it appeared in a small number of isolates in2017, 2018, 2019 and 2021. In the 2020, none of the isolates exhibited tigecycline resistance. Cefoxitin resistance, the surrogate marker for MRSA, was observed nearly twice as commonly among CoNS as *S. aureus* (76.8% vs 42.6%). There was a discrepancy in the MRSA rates detected by oxacillin MIC (33.8% vs 42.6%). This discrepancy could be because of the smaller number of isolates tested with oxacillin than with cefoxitin.

Table 4.5 depicts the susceptibility rates of staphylococci from blood. MRSA rate was slightly higher among blood isolates when compared to the overall rate (47.4% vs 42.6%). CoNS were more commonly isolated from blood than *S.aureus* from the different centres across India. Cefoxitin resistance was observed more commonly among CoNS than the *S.aureus* (79.9% vs 47.4%). Only 10.7 % of MSSA isolates were susceptible to penicillin. When compared to MRSA, MSSA was more susceptible to erythromycin, clindamycin, ciprofloxacin, co-trimoxazole, tetracycline, and high-level mupirocin. The anti MRSA antibiotics such as vancomycin, linezolid, teicoplanin, and tigecycline showed excellent in vitro activity ranging from 100%. Teicoplanin resistance was found only in CoNS isolates (4.9%). As seen from **Table 4.6**, around 50% of the total *S. aureus* and 12.7% of CoNS isolates were from superficial infections. MRSA rate was 38.8% which was similar to the overall rate. Susceptibility of these isolates to different antibiotics followed the same general pattern as previously mentioned.

As seen from **Table 4.7**, the proportion of MRSA from deep seated infections increased from 38.6% to 51%. Mupirocin resistance was lower among isolates from deep infections (4.9%) when compared to those from superficial infections (9.1%).

Table 4.8 and figure 4.2 depict trends in antimicrobial susceptibility among MSSA isolates across the 6 years of study (2016-21). Although *S. aureus*, overall, showed increasing trends of resistance to most antibiotics over the years, no such prominent trend could be observed with MSSA isolates. There was only a marginal increase in the susceptibility rates to co-trimoxazole and mupirocin. The unusual occurrence of linezolid resistance rates was decreased in MSSA isolates (0.2 to 0.1 %). **Table 4.9 and figure 4.3** depict trends in antimicrobial resistance in MRSA isolates across the 6 years (2016-21). Susceptibility rates across the years were similar to most antibiotics except tetracycline which showed a significant fall in susceptibility among 2020 isolates which continued into 2021. The teicoplanin resistance rates were decreased in 2021 (0.5% and 0.1 %) when compared to 2020 rates.

Table 4.4: Year wise susceptibility trends of *Staphylococcus aureus* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=960	Total n=5708	Total n=8644	Total n=12320	Total n=6281	Total n=8827
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Cefoxitin	686/958 (71.6)	3805/5668 (67.1)	4863/7919 (61.4)	6272/10835 (57.9)	3394/5787 (58.6)	3869/6740 (57.4)
Oxacillin	*0/0	314/438 (71.7)	1218/2196 (55.5)	2280/3773 (60.4)	1140/1869 (61)	2440/3685 (66.2)
Penicillin	60/737 (8.1)	267/3519 (7.6)	246/4047 (6.1)	458/7008 (6.5)	251/3608 (7)	229/4293 (5.3)
Vancomycin	565/565 (100)	2602/2602 (100)	4640/4640 (100)	6996/6996 (100)	3846/3846 (100)	6203/6204 (100)
Teicoplanin	877/880 (99.7)	5233/5257 (99.5)	6544/6697 (97.7)	6194/6269 (98.8)	2043/2050 (99.7)	3351/3356 (99.9)
Erythromycin	492/955 (51.5)	2755/5570 (49.5)	3593/8102 (44.3)	4803/11975 (40.1)	2594/6096 (42.6)	3617/8355 (43.3)
Tetracycline	669/738 (90.7)	3492/3860 (90.5)	6255/7050 (88.7)	9269/10329 (89.7)	4734/5284 (89.6)	5686/6400 (88.8)
Tigecycline	*0/0	433/435 (99.5)	1529/1536 (99.5)	2902/2914 (99.6)	1559/1559 (100)	2113/2131 (99.2)
Ciprofloxacin	191/838 (22.8)	1224/5260 (23.3)	1497/8094 (18.5)	1990/11200 (17.8)	1101/5845 (18.8)	1455/8341 (17.4)
Clindamycin	729/921 (79.2)	4235/5475 (77.4)	6460/8456 (76.4)	9153/11984 (76.4)	4645/6084 (76.3)	6334/8579 (73.8)
Trimethoprim-sulfamethoxazole	513/852 (60.2)	3064/4306 (71.2)	4764/7565 (63)	7927/11401 (69.5)	3926/5821 (67.4)	4718/6954 (67.8)
Linezolid	860/863 (99.7)	5424/5445 (99.6)	8054/8148 (98.8)	11461/11547 (99.3)	5846/5877 (99.5)	8233/8236 (100)
Mupirocin High Level	573/584 (98.1)	2971/3012 (98.6)	3656/3742 (97.7)	4624/4892 (94.5)	2563/2719 (94.3)	2704/2866 (94.3)

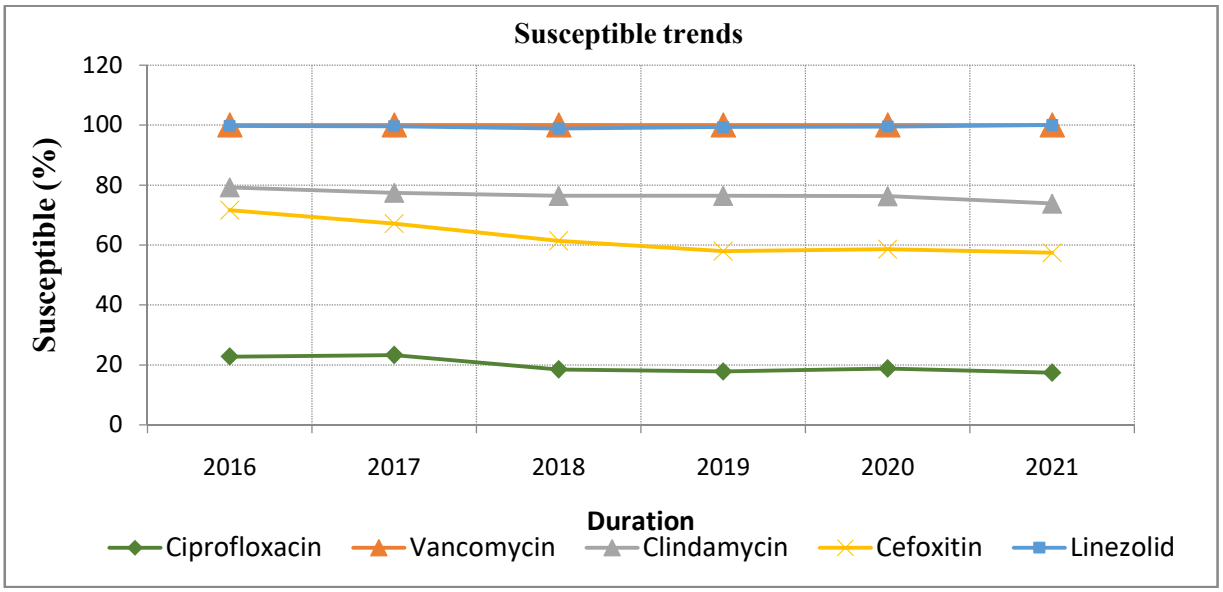


Figure 4.1: Year wise susceptibility trends of *S. aureus* from all Samples

Table 4.5 Susceptible percentages of staphylococci isolated from blood

AMA	Blood			
	S. aureus n=1663	MSSA n=944	MRSA n=698	CoNS n=1995
Cefoxitin	705/1341 (52.6)	700/700 (100)	5/641 (0.8)	371/1843 (20.1)
Oxacillin	413/573 (72.1)	402/402 (100)	11/171 (6.4)	7/35 (20)
Penicillin	44/819 (5.4)	39/363 (10.7)	5/448 (1.1)	108/1464 (7.4)
Vancomycin	962/962 (100)	606/606 (100)	347/347 (100)	1004/1007 (99.7)
Teicoplanin	592/592 (100)	364/364 (100)	222/222 (100)	366/385 (95.1)
Erythromycin	626/1598 (39.2)	459/913 (50.3)	163/671 (24.3)	317/1983 (16)
Tetracycline	1127/1266 (89)	594/648 (91.7)	527/612 (86.1)	1385/1952 (71)
Tigecycline	303/305 (99.3)	168/170 (98.8)	133/133 (100)	243/252 (96.4)
Ciprofloxacin	338/1428 (23.7)	222/807 (27.5)	114/606 (18.8)	517/1576 (32.8)
Clindamycin	1181/1636 (72.2)	734/937 (78.3)	439/685 (64.1)	988/1983 (49.8)
Trimethoprim-sulfamethoxazole	944/1354 (69.7)	551/712 (77.4)	389/634 (61.4)	903/1974 (45.7)
Linezolid	1552/1553 (99.9)	871/872 (99.9)	668/668 (100)	1956/1968 (99.4)
Mupirocin High Level	534/589 (90.7)	249/254 (98)	284/334 (85)	*0/0 (-)

Table 4.6 Susceptible percentages of staphylococci isolated from Superficial Infections

AMA	Superficial Infection			
	S. aureus **n=3719**	**MSSA n=2254**	**MRSA n=1434**	**CoNS n=339**
Cefoxitin	1994/3258 (61.2)	1977/1977 (100)	17/1281 (1.3)	115/335 (34.3)
Oxacillin	880/1468 (59.9)	859/859 (100)	21/609 (3.4)	*0/6 (-)
Penicillin	96/1970 (4.9)	84/1071 (7.8)	12/878 (1.4)	15/295 (5.1)
Vancomycin	2964/2965 (100)	1867/1867 (100)	1083/1084 (99.9)	187/187 (100)
Teicoplanin	1479/1480 (99.9)	789/789 (100)	676/677 (99.9)	42/42 (100)
Erythromycin	1668/3614 (46.2)	1260/2203 (57.2)	397/1386 (28.6)	62/332 (18.7)
Tetracycline	3010/3367 (89.4)	1862/2019 (92.2)	1123/1322 (84.9)	238/331 (71.9)
Tigecycline	1266/1279 (99)	670/677 (99)	583/589 (99)	33/34 (97.1)
Ciprofloxacin	637/3684 (17.3)	527/2235 (23.6)	103/1423 (7.2)	140/334 (41.9)
Clindamycin	3013/3705 (81.3)	1944/2246 (86.6)	1045/1431 (73)	208/339 (61.4)
Trimethoprim-sulfamethoxazole	2357/3428 (68.8)	1489/2062 (72.2)	852/1341 (63.5)	158/334 (47.3)
Linezolid	3624/3625 (100)	2184/2184 (100)	1412/1413 (99.9)	335/336 (99.7)
Mupirocin High Level	1237/1300 (95.2)	729/742 (98.2)	499/549 (90.9)	*0/0 (-)

Table 4.7 Susceptible percentages of staphylococci isolated from Deep Infections

AMA	Deep Infection			
	S. aureus n=1563	MSSA n=981	MRSA n=566	CoNS n=52
Cefoxitin	332/677 (49)	331/331 (100)	1/346 (0.3)	10/39 (25.6)
Oxacillin	740/1020 (72.5)	738/738 (100)	2/282 (0.7)	*0/3 (-)
Penicillin	43/620 (6.9)	39/287 (13.6)	4/325 (1.2)	4/47 (8.5)
Vancomycin	1142/1142 (100)	790/790 (100)	344/344 (100)	24/24 (100)
Teicoplanin	744/745 (99.9)	502/503 (99.8)	235/235 (100)	*17/18 (-)
Erythromycin	595/1453 (40.9)	425/891 (47.7)	166/548 (30.3)	9/45 (20)
Tetracycline	507/573 (88.5)	226/249 (90.8)	275/315 (87.3)	17/32 (53.1)
Tigecycline	218/219 (99.5)	114/114 (100)	100/101 (99)	*11/11 (-)
Ciprofloxacin	164/1512 (10.8)	123/945 (13)	39/554 (7)	17/47 (36.2)
Clindamycin	954/1548 (61.6)	613/970 (63.2)	332/565 (58.8)	23/50 (46)
Trimethoprim-sulfamethoxazole	417/717 (58.2)	222/336 (66.1)	187/367 (51)	27/49 (55.1)
Linezolid	1333/1333 (100)	789/789 (100)	529/529 (100)	48/48 (100)
Mupirocin High Level	256/266 (96.2)	78/79 (98.7)	176/185 (95.1)	*0/0 (-)

Table 4.8: Year wise susceptibility trends of MSSA from All samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=686 (S%)	Total n=3819 (S%)	Total n=5135 (S%)	Total n=7029 (S%)	Total n=3655 (S%)	Total n=5273 (S%)
Cefoxitin	686/686 (100)	3801/3801 (100)	4857/4857 (100)	6255/6255 (100)	3388/3388 (100)	3845/3845 (100)
Oxacillin	*0/0	306/306 (100)	1187/1187 (100)	2195/2195 (100)	1100/1100 (100)	2399/2399 (100)
Penicillin	59/557 (10.6)	248/2393 (10.4)	218/2068 (10.5)	410/3729 (11)	231/1931 (12)	203/2131 (9.5)
Vancomycin	428/428 (100)	1935/1935 (100)	3041/3041 (100)	3986/3986 (100)	2153/2153 (100)	4010/4010 (100)
Teicoplanin	636/636 (100)	3509/3517 (99.8)	3642/3682 (98.9)	3391/3419 (99.2)	1074/1075 (99.9)	1945/1949 (99.8)
Erythromycin	419/684 (61.3)	2251/3739 (60.2)	2757/4841 (57)	3527/6895 (51.2)	1962/3570 (55)	2665/4975 (53.6)
Tetracycline	528/557 (94.8)	2508/2665 (94.1)	3809/4137 (92.1)	5383/5791 (93)	2838/3047 (93.1)	3297/3579 (92.1)
Tigecycline	*0/0	300/302 (99.3)	902/902 (100)	1608/1613 (99.7)	861/861 (100)	1102/1112 (99.1)
Ciprofloxacin	168/609 (27.6)	1051/3524 (29.8)	1167/4816 (24.2)	1587/6452 (24.6)	888/3386 (26.2)	1112/4971 (22.4)
Clindamycin	561/661 (84.9)	3162/3666 (86.3)	4341/5021 (86.5)	5837/6839 (85.3)	3021/3548 (85.1)	4057/5137 (79)
Trimethoprim-sulfamethoxazole	414/629 (65.8)	2202/2959 (74.4)	3030/4499 (67.3)	4750/6475 (73.4)	2425/3344 (72.5)	2884/3927 (73.4)
Linezolid	634/634 (100)	3630/3636 (99.8)	4775/4800 (99.5)	6433/6448 (99.8)	3343/3349 (99.8)	4838/4839 (100)
Mupirocin High Level	434/440 (98.6)	2119/2139 (99.1)	2414/2441 (98.9)	2775/2820 (98.4)	1564/1600 (97.8)	1436/1460 (98.4)

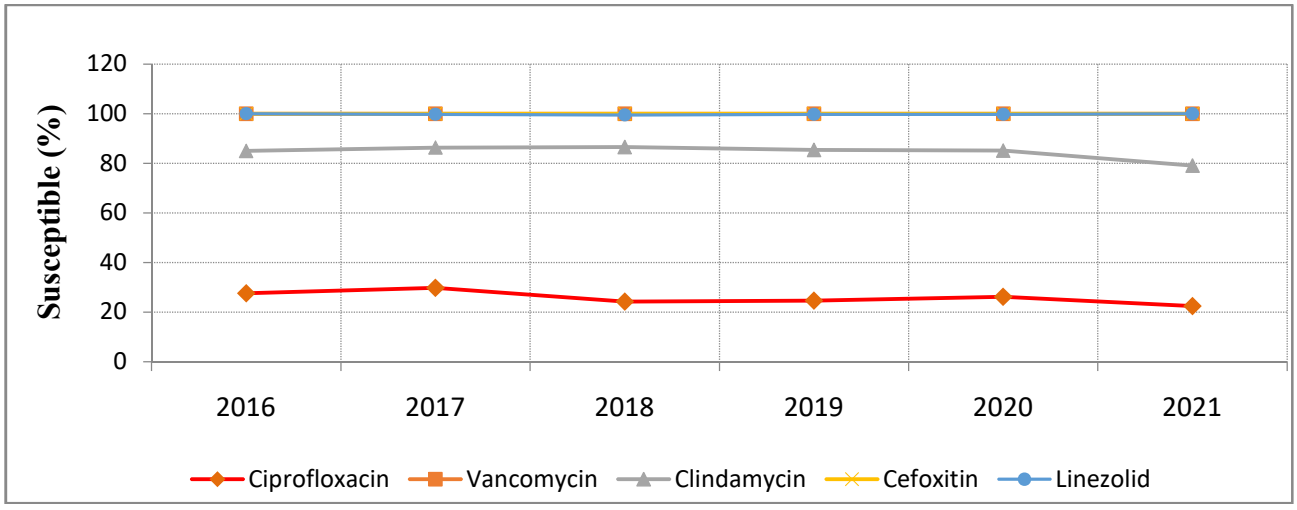


Figure 4.2: Year wise susceptibility trends of MSSA from All Samples

Table 4.9: Year wise susceptibility trends of MRSA from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=272	Total n=1870	Total n=3445	Total n=5185	Total n=2582	Total n=3423
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Cefoxitin	0/272 (0)	0/1867 (0)	0/3062 (0)	0/4578 (0)	0/2399 (0)	24/2895 (0.8)
Oxacillin	*0/0	8/132 (6.1)	31/1009 (3.1)	85/1578 (5.4)	40/769 (5.2)	41/1286 (3.2)
Penicillin	0/180 (0)	0/1111 (0)	0/1959 (0)	0/3240 (0)	0/1652 (0)	24/2101 (1.1)
Vancomycin	137/137 (100)	667/667 (100)	1581/1581 (100)	2960/2960 (100)	1676/1676 (100)	2153/2154 (100)
Teicoplanin	240/242 (99.2)	1719/1735 (99.1)	2848/2956 (96.3)	2729/2775 (98.3)	948/953 (99.5)	1369/1370 (99.9)
Erythromycin	72/270 (26.7)	494/1813 (27.2)	822/3228 (25.5)	1251/4988 (25.1)	621/2490 (24.9)	917/3274 (28)
Tetracycline	141/181 (77.9)	983/1193 (82.4)	2397/2859 (83.8)	3829/4473 (85.6)	1885/2223 (84.8)	2348/2772 (84.7)
Tigecycline	*0/0	133/133 (100)	627/634 (98.9)	1280/1286 (99.5)	694/694 (100)	990/998 (99.2)
Ciprofloxacin	23/228 (10.1)	165/1718 (9.6)	323/3222 (10)	397/4654 (8.5)	204/2417 (8.4)	328/3257 (10.1)
Clindamycin	167/259 (64.5)	1067/1802 (59.2)	2083/3373 (61.8)	3248/5044 (64.4)	1598/2497 (64)	2228/3362 (66.3)
Trimethoprim-sulfamethoxazole	99/223 (44.4)	851/1332 (63.9)	1701/3006 (56.6)	3127/4848 (64.5)	1484/2449 (60.6)	1796/2961 (60.7)
Linezolid	225/228 (98.7)	1779/1794 (99.2)	3228/3296 (97.9)	4936/5001 (98.7)	2476/2500 (99)	3317/3319 (99.9)
Mupirocin High Level	139/144 (96.5)	852/873 (97.6)	1238/1297 (95.5)	1829/2051 (89.2)	997/1117 (89.3)	1253/1391 (90.1)

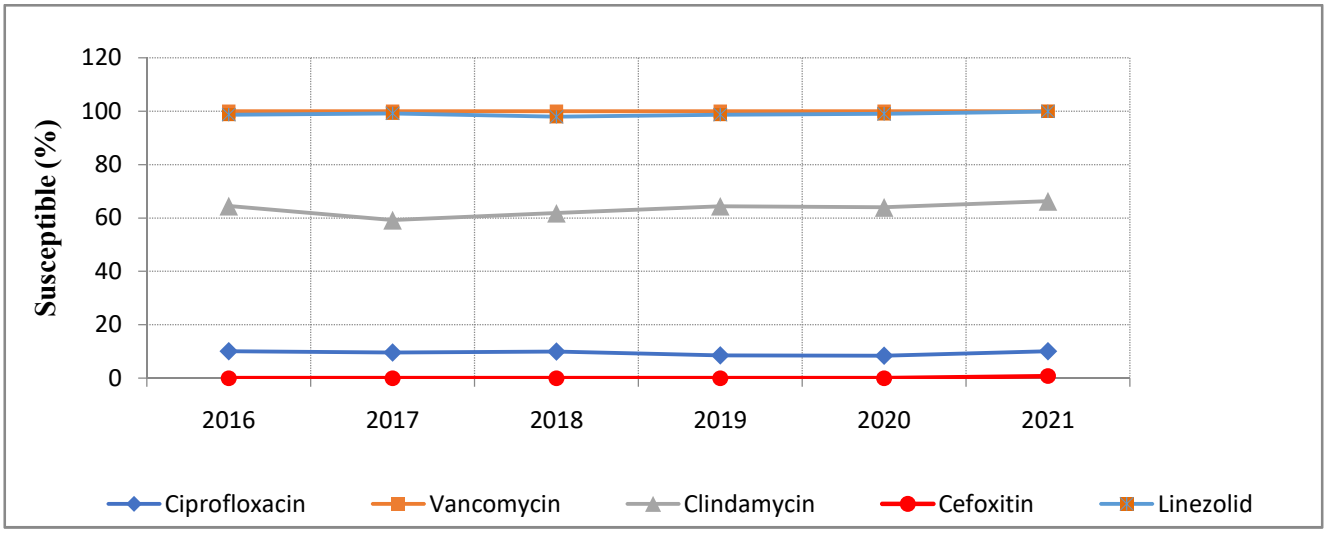


Figure 4.3: Year wise susceptibility trends of MRSA from All Samples

Coagulase negative staphylococci

The common species were *S.haemolyticus, S.epidermidis, S.hominis, S.lugdunensis* and *S.saprophyticus*. Cefoxitin resistance was highest in *S.haemolyticus* (87.8 %) followed by *S.hominis* (74.2%) and *S.epidermidis* (66.1%). With the exception of teicoplanin and tetracycline, *S.haemolyticus* exhibited much lower rates of susceptibility to all antibiotics when compared to the other species. Tigeccline resistance was increased from 2.8% to 6.3% in *S. haemolyticus* while all other species *S. hominis (*1.8%) and S. epidermidis (1.6%). Linezolid resistance increased from 2.8% to 6.3% in *S.haemolyticus* while it was low among all other species *S. hominis (*1.8%) and S. epidermidis (1.6%). encountered in CoNS isolates. (Table 4.10). It can be clearly observed that there is a decrease in the susceptibility rates for most of the antibiotics except linezolid and trimethoprim-sulfamethoxazole in 2020 and 2021. For these two antibiotics susceptibility rates slightly increased to 0.5% and 2.4 % in 2021 when compared to 2020 (Table 4.11 and Figure 4.4).

Table 4.10: Susceptibility percentages of CoNS isolated from all specimens

AMA	All Specimens					
	S. haemolyticus n=836	*Staphylococcus spp.* n=669	*S. epidermidis* n=595	*S. hominis* n=400	*S. lugdunensis* n=120	*S. saprophyticus* n=35
Cefoxitin	96/785 (12.2)	125/628 (19.9)	181/534 (33.9)	95/368 (25.8)	62/116 (53.4)	*7/12 (-)
Penicillin	34/751 (4.5)	22/248 (8.9)	26/502 (5.2)	33/366 (9)	19/115 (16.5)	*4/12 (-)
Vancomycin	563/563 (100)	113/114 (99.1)	408/410 (99.5)	249/249 (100)	*8/8 (-)	32/32 (100)
Teicoplanin	182/187 (97.3)	48/50 (96)	167/173 (96.5)	69/77 (89.6)	*6/6 (-)	24/24 (100)
Erythromycin	96/828 (11.6)	132/649 (20.3)	119/580 (20.5)	65/397 (16.4)	28/119 (23.5)	15/34 (44.1)
Tigecycline	119/126 (93.7)	23/23 (100)	122/124 (98.4)	56/57 (98.2)	*1/1 (-)	23/23 (100)
Tetracycline	573/823 (69.5)	432/595 (72.6)	420/572 (73.4)	257/395 (65.1)	95/118 (80.5)	32/33 (97)
Ciprofloxacin	150/823 (18.2)	116/263 (44.1)	268/579 (46.3)	146/391 (37.3)	66/119 (55.5)	32/34 (94.1)
Clindamycin	299/829 (36)	371/660 (56.2)	349/587 (59.5)	235/399 (58.9)	89/116 (76.7)	20/34 (58.8)
Linezolid	814/823 (98.9)	656/657 (99.8)	583/585 (99.7)	396/397 (99.7)	116/117 (99.1)	34/34 (100)
Trimethoprim sulfamethoxazole	322/823 (39.2)	344/651 (52.8)	276/587 (47)	186/395 (47.1)	63/118 (53.4)	32/35 (91.4)

Table 4.11: Year wise susceptibility trends of CoNS from all Samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=490 (S%)	Total n=2830 (S%)	Total n=4016 (S%)	Total n=3571 (S%)	Total n=2018 (S%)	Total n=2655 (S%)
Cefoxitin	173/490 (35.3)	930/2810 (33.1)	982/3574 (27.5)	921/3298 (27.9)	487/1907 (25.5)	566/2444 (23.2)
Penicillin	58/224 (25.9)	223/1227 (18.2)	185/2021 (9.2)	268/2601 (10.3)	134/1391 (9.6)	138/1995 (6.9)
Vancomycin	86/86 (100)	718/718 (100)	1619/1679 (96.4)	1681/1691 (99.4)	890/890 (100)	1374/1377 (99.8)
Teicoplanin	335/336 (99.7)	2212/2236 (98.9)	2912/3083 (94.5)	1324/1379 (96)	229/238 (96.2)	497/518 (95.9)
Erythromycin	148/488 (30.3)	742/2679 (27.7)	755/3459 (21.8)	815/3514 (23.2)	396/1999 (19.8)	455/2608 (17.4)
Tigecycline	*0/1	165/167 (98.8)	434/441 (98.4)	287/292 (98.3)	116/117 (99.1)	344/355 (96.9)
Tetracycline	176/226 (77.9)	1177/1358 (86.7)	2236/2811 (79.5)	2658/3269 (81.3)	1582/1916 (82.6)	1809/2537 (71.3)
Ciprofloxacin	159/335 (47.5)	986/2236 (44.1)	1145/3015 (38)	1178/2798 (42.1)	563/1597 (35.3)	778/2210 (35.2)
Clindamycin	297/488 (60.9)	1613/2782 (58)	2151/3952 (54.4)	2058/3509 (58.6)	1057/2005 (52.7)	1363/2626 (51.9)
Linezolid	375/381 (98.4)	2638/2680 (98.4)	3796/3900 (97.3)	3340/3429 (97.4)	1958/1978 (99)	2600/2614 (99.5)
Trimethoprim-sulfamethoxazole	199/379 (52.5)	923/1940 (47.6)	1579/3452 (45.7)	1687/3428 (49.2)	861/1935 (44.5)	1224/2610 (46.9)

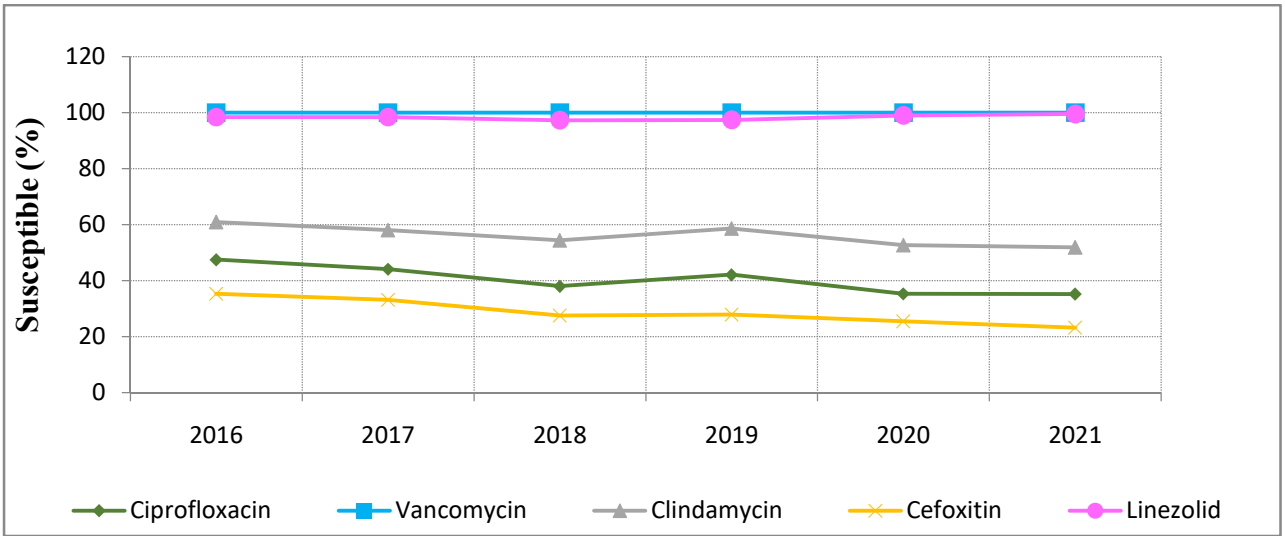


Figure 4.4: Year wise susceptibility trends of CoNS from all Samples

Enterococci

E. faecalis is usually the commonest species followed by *E. faecium*. However unlike in the previous years, *E. faecium* was found to be the predominant species among the 2021 isolates in many of the centres. The susceptibility rate in *E. faecium* was significantly lower for ampicillin, high level gentamicin and vancomycin than in *E. faecalis*. Overall vancomycin resistance in enterococci (*E. faecalis* and *E. faecium*) was 14.9%. However, the rate was 6 times higher in *E. faecium* compared *to E. faecalis* (25.4% vs 3.8%). Isolates from blood (both the species) appear to be more resistant when compared to isolates from superficial and deep infections. Although the numbers are too small for significance, vancomycin resistance among CSF isolates was much lower than the overall rate (Table 4.12).

A similar pattern as the rest of the specimens was noted for urine isolates. Ciprofloxacin appeared to be equally ineffective against both the species while nitrofurantoin susceptibility was high in. *E. faecalis*. Fosfomycin resistance increased from 3% in 2020 to 8.5% in 2021 (Table 4.13). As expected, most antibiotics showed lower rates of susceptibility among ICU isolates when compared to ward or OPD isolates. This difference was noted in *E. faecalis* species (except for high level gentamicin and linezolid in *E. faecalis*) (Table 4.14).

E. faecium

Table 4.15 and figure 4.5 depict the year wise susceptibility rates of *E. faecium.* The susceptibility rate was slightly increased for ampicillin, high-level gentamicin, nitrofurantoin antibiotics in 2021 when compared to 2020 while there was a slight reduction in susceptibility to vancomycin, nitrofurantoin and teicoplanin. The susceptibility rates to vancomycin ranged from 50% to 97.6 % across regional centres. Though the overall VRE rate is 25.7% slightly increased than the 2020 (22.9%), there were significant differences observed between the various regional centres, the highest rate in the isolates from RC08 and RC20 (47.7 and 50%). The lowest VRE rates were observed from the RC18 (2.4%) and RC14 (6.9%) (Table 4.16)

Susceptibility to linezolid was high in most of the centres ranging from 75.4% to 100%. Linezolid susceptibility was found to be the lowest (75.4 %) among RC06 isolates. Susceptibility to ampicillin and high level gentamicin was uniformly low across all centres except RC09 (ampicillin 23.8%) and RC02 (HLG 61.8%).

E. faecalis

Table 4.17 and figure 4.6 depict the trends in antibiotic susceptibility rates in *E. faecalis* from 2016-2021. Lower susceptibility trends were observed for all the antibiotics in 2021 isolates when compared to 2020 except for linezolid.

The susceptibility rates of vancomycin and teicoplanin ranged from 83.3% to 100 % from most of the regional centres. Though the overall VRE rate is 3.8%, there were significant differences

observed between the various regional centres, the highest rate in the isolates from RC20 and RC01 (16.7% and 15.6%). The lowest VRE rates were observed from the RC04 (1.2%) and RC03 (1.6%) (Table 4.18). Susceptibility to linezolid was high in most of the centres ranging from 93.5% to 100%. Linezolid susceptibility was found to be the lowest (90%) among RC16 isolates. The least effective antibiotic was high level gentamicin with only 56.55 of isolates showing susceptibility. Lowest susceptibility to ampicillin and high level gentamicin were recorded from RC20 (16.7%) and RC06 (38.8%), while highest susceptibility was observed in isolates from RC 05 (97.9%) and RC02 (76.2%).

Vancomycin variable Enterococcus

There were 3/339 VVE isolates among the phenotypically vancomycin susceptible isolates of *E. faecium* (n=3/156) and *E. faecalis* (n=0/183), two from RC04 and one from RC12. This finding is of concern because these isolates can convert to a resistant phenotype during antibiotic treatment, severely compromising the success of therapy.

Biocide resistance genes (*qac*A/B and *smr)* among MRSA and VRE isolates

453 isolates of MRSA and 220 VRE isolates were tested for the presence of *qac*A/B and *smr* genes. The overall prevalence of *qac*A/B and *smr* genes in MRSA isolates was 2.4 % (11/453) and 0.2% (1/453) respectively. In *Enterococcus*, *qac*A/B was detected in 0.45 % (1/220) isolates while none had *smr* genes. Among MRSA isolates, *qac*A/B and smr genes slightly decreased from 2.6 % in 2020 to 2.4% in 2021 while in enterococci there was a 2 fold decrease from 6.5% to 2.4%. Most disinfectant-resistance genes are plasmid borne and can spread between staphylococcal species.

Table 4.12: Susceptibility pattern of enterococci from all samples except urine

AMA	All Specimens (except urine)		Blood		Superficial Infection		Deep Infection		CSF	
	Enterococcus faecium n=1611	*Enterococcus faecalis* n=1502	*Enterococcus faecium* n=700	*Enterococcus faecalis* n=472	*Enterococcus faecium* n=402	*Enterococcus faecalis* n=546	*Enterococcus faecium* n=109	*Enterococcus faecalis* n=129	*E. faecium* n=46	*E. faecalis* n=35
Ampicillin	162/1424 (11.4)	1143/1394 (82)	46/573 (8)	303/417 (72.7)	55/378 (14.6)	440/511 (86.1)	11/103 (10.7)	120/127 (94.5)	4/44 (9.1)	23/33 (69.7)
Vancomycin	1169/1569 (74.3)	1419/1475 (96.2)	454/671 (67.7)	424/450 (94.2)	331/401 (82.5)	530/545 (97.2)	80/102 (78.4)	127/128 (99.2)	25/46 (54.3)	32/34 (94.1)
Teicoplanin	1195/1549 (76.9)	1421/1467 (96.9)	468/654 (71.6)	424/447 (94.9)	338/401 (84.3)	536/543 (98.7)	79/103 (76.7)	124/126 (98.4)	24/46 (52.2)	32/35 (91.4)
Gentamicin HL	405/1126 (35.9)	683/1208 (56.5)	161/463 (34.8)	201/351 (57.3)	97/291 (33.3)	277/486 (57)	41/87 (47.1)	50/102 (49)	*2/12 (-)	*8/17 (-)
Linezolid	1481/1546 (95.5)	1429/1437 (99.4)	624/661 (94.4)	437/440 (99.3)	389/400 (97.3)	541/545 (99.3)	90/96 (93.8)	109/109 (100)	46/46 (100)	35/35 (100)

Table 4.13: Susceptibility pattern of enterococci from Urine

AMA	Urine	
	Enterococcus faecalis n=871	*Enterococcus faecium* n=811
Ampicillin	466/733 (63.6)	107/730 (14.7)
Vancomycin	823/860 (95.7)	664/803 (82.7)
Teicoplanin	814/843 (96.6)	657/793 (82.8)
Gentamicin HL	332/617 (53.8)	208/575 (36.2)
Ciprofloxacin	121/633 (19.1)	46/630 (7.3)
Nitrofurantoin	737/856 (86.1)	340/788 (43.1)
Fosfomycin	476/520 (91.5)	-
Linezolid	778/785 (99.1)	739/774 (95.5)

Table 4.14: Susceptibility pattern of enterococci from all samples across OPD, Ward and ICU

AMA	*Enterococcus faecium*				*Enterococcus faecalis*			
	Total n=2422	OPD n=311	Ward n=1482	ICU n=629	Total n=2373	OPD n=671	Ward n=1339	ICU n=363
	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)	(S %)
Ampicillin	269/2154 (12.5)	66/273 (24.2)	158/1299 (12.2)	45/582 (7.7)	1609/2127 (75.6)	451/583 (77.4)	910/1203 (75.6)	248/341 (72.7)
Vancomycin	1830/2372 (77.2)	246/301 (81.7)	1163/1465 (79.4)	421/606 (69.5)	2242/2335 (96)	645/661 (97.6)	1273/1325 (96.1)	324/349 (92.8)
Teicoplanin	1849/2342 (78.9)	248/302 (82.1)	1168/1444 (80.9)	433/596 (72.7)	2235/2310 (96.8)	640/651 (98.3)	1268/1313 (96.6)	327/346 (94.5)
Gentamicin HL	612/1701 (36)	104/201 (51.7)	375/1074 (34.9)	133/426 (31.2)	1015/1825 (55.6)	305/492 (62)	555/1043 (53.2)	155/290 (53.4)
Ciprofloxacin	47/640 (7.3)	17/96 (17.7)	21/426 (4.9)	9/118 (7.6)	126/646 (19.5)	49/215 (22.8)	68/386 (17.6)	9/45 (20)
Nitrofurantoin	342/791 (43.2)	75/131 (57.3)	218/500 (43.6)	49/160 (30.6)	757/878 (86.2)	308/333 (92.5)	390/472 (82.6)	59/73 (80.8)
Fosfomycin	452/516 (87.6)	70/79 (88.6)	308/344 (89.5)	74/93 (79.6)	478/524 (91.2)	150/165 (90.9)	296/319 (92.8)	32/40 (80)
Linezolid	2216/2320 (95.5)	281/293 (95.9)	1408/1451 (97)	527/576 (91.5)	2207/2222 (99.3)	595/599 (99.3)	1298/1308 (99.2)	314/315 (99.7)

Table 4.15: Year wise susceptibility trends of *Enterococcus faecium* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=180	Total n=937	Total n=1476	Total n=2700	Total n=1994	Total n=2422
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Ampicillin	56/178 (31.5)	172/860 (20)	214/1213 (17.6)	414/2290 (18.1)	200/1810 (11)	269/2154 (12.5)
Vancomycin	156/178 (87.6)	697/914 (76.3)	1139/1465 (77.7)	2214/2683 (82.5)	1546/1966 (78.6)	1830/2372 (77.2)
Teicoplanin	158/179 (88.3)	740/926 (79.9)	1148/1461 (78.6)	2206/2638 (83.6)	1570/1947 (80.6)	1849/2342 (78.9)
Gentamicin HL	27/102 (26.5)	208/812 (25.6)	360/1247 (28.9)	836/2392 (34.9)	577/1696 (34)	612/1701 (36)
Ciprofloxacin	2/34 (5.9)	10/230 (4.3)	26/446 (5.8)	79/984 (8)	38/544 (7)	47/640 (7.3)
Nitrofurantoin	16/33 (48.5)	181/251 (72.1)	259/509 (50.9)	559/1221 (45.8)	319/779 (40.9)	342/791 (43.2)
Linezolid	170/179 (95)	860/910 (94.5)	1352/1411 (95.8)	2562/2644 (96.9)	1813/1896 (95.6)	2216/2320 (95.5)

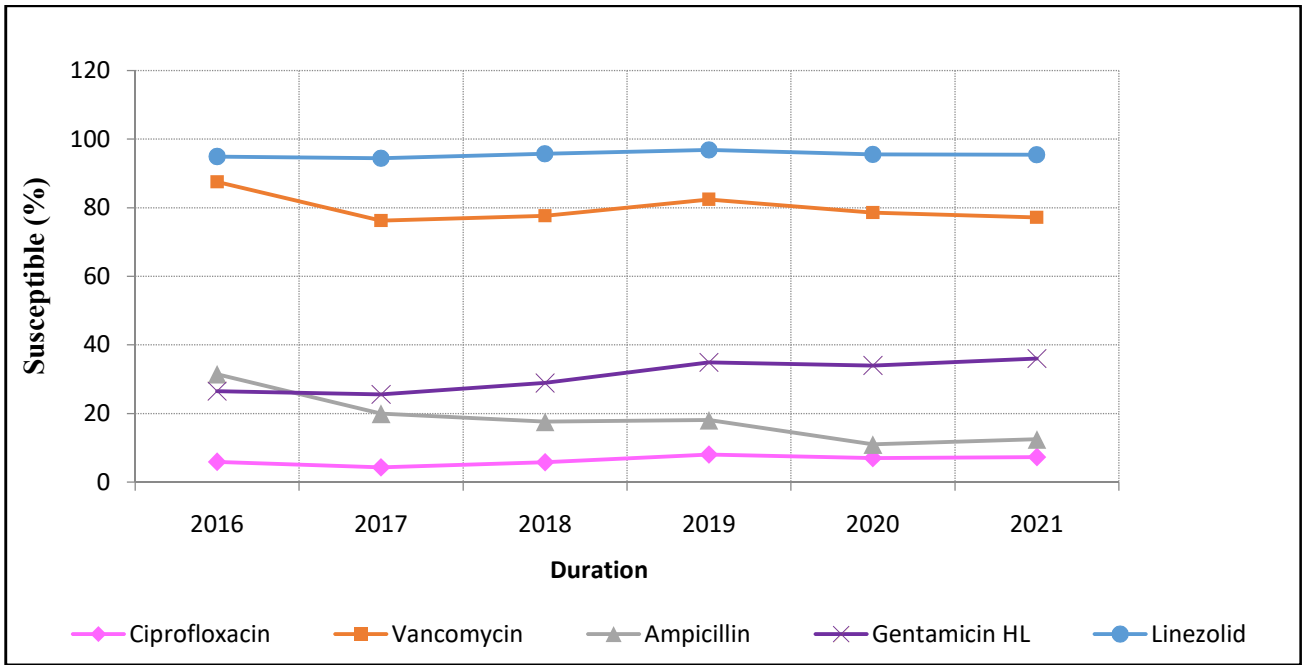


Figure 4.5: Year wise susceptibility trends of *Enterococcus faecium* from all samples

Table 4.16. Antimicrobial Susceptibilities (AMS) Percentage RC wise of *Enterococcus faecium* from Total (Except Faeces & Urine)

RC/ Antibiotics	Ampicillin (n=1424)	Vancomycin (n=1569)	Teicoplanin (n=1549)	Gentamicin HL (n=1126)	Linezolid (n=1546)
	n(%)	n(%)	n(%)	n(%)	n(%)
RC2	3/54 (5.6)	22/31 (71)	25/31 (80.6)	34/55 (61.8)	26/26 (100)
RC4	44/415 (10.6)	338/416 (81.3)	342/416 (82.2)	163/413 (39.5)	411/416 (98.8)
RC1	21/188 (11.2)	124/188 (66)	127/188 (67.6)	2/5* (-)	188/188 (100)
RC14	-	27/29 (93.1)	27/29 (93.1)	-	29/29 (100)
RC6	4/142 (2.8)	84/142 (59.2)	84/142 (59.2)	24/142 (16.9)	107/142 (75.4)
RC15	4/57 (7)	31/57 (54.4)	23/40 (57.5)	14/52 (26.9)	53/57 (93)
RC3	15/121 (12.4)	91/123 (74)	96/121 (79.3)	-	119/123 (96.7)
RC13	5/7* (-)	6/11* (-)	7/11* (-)	2/5* (-)	12/12* (-)
RC10	16/94 (17)	74/94 (78.7)	74/94 (78.7)	25/63 (39.7)	73/79 (92.4)
RC20	1/34 (2.9)	17/34 (50)	19/33 (57.6)	15/34 (44.1)	33/34 (97.1)
RC7	0/1* (-)	1/1* (-)	1/1* (-)	1/1* (-)	0/1* (-)
RC18	10/42 (23.8)	41/42 (97.6)	40/42 (95.2)	23/42 (54.8)	42/42 (100)
RC5	0/48 (0)	30/48 (62.5)	30/48 (62.5)	17/48 (35.4)	41/48 (85.4)
RC19	10/61 (16.4)	50/61 (82)	56/60 (93.3)	10/39 (25.6)	61/61 (100)
RC9	9/29 (31)	22/27 (81.5)	28/29 (96.6)	13/28 (46.4)	28/28 (100)
RC17	-	82/90 (91.1)	82/90 (91.1)	23/89 (25.8)	90/90 (100)
RC12	12/63 (19)	50/63 (79.4)	52/63 (82.5)	8/39 (20.5)	62/62 (100)
RC16	4/19* (-)	18/20 (90)	19/20 (95)	9/19* (-)	19/20 (95)
RC8	3/20 (15)	23/44 (52.3)	24/43 (55.8)	11/21 (52.4)	37/41 (90.2)
RC21	0/26 (0)	23/31 (74.2)	24/31 (77.4)	10/31 (32.3)	30/31 (96.8)
RC11	1/3* (-)	12/17* (-)	12/17* (-)	-	16/16* (-)
Total	162/1424 (11.4)	1166/1569 (74.3)	1192/1549 (77)	404/1126 (35.9)	1477/1546 (95.5)

Table 4.17: Year wise susceptibility trends of *Enterococcus faecalis* from all samples

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=126	Total n=1034	Total n=2014	Total n=2895	Total n=2101	Total n=2373
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Ampicillin	82/123 (66.7)	633/987 (64.1)	1338/1813 (73.8)	1993/2467 (80.8)	1606/1942 (82.7)	1609/2127 (75.6)
Vancomycin	123/125 (98.4)	978/1016 (96.3)	1921/2000 (96.1)	2791/2860 (97.6)	2018/2073 (97.3)	2242/2335 (96)
Teicoplanin	124/126 (98.4)	992/1030 (96.3)	1889/1970 (95.9)	2582/2633 (98.1)	2001/2039 (98.1)	2235/2310 (96.8)
Gentamicin HL	73/119 (61.3)	512/993 (51.6)	982/1890 (52)	1411/2458 (57.4)	1059/1818 (58.3)	1015/1825 (55.6)
Ciprofloxacin	3/40 (7.5)	41/358 (11.5)	87/641 (13.6)	162/982 (16.5)	127/586 (21.7)	126/646 (19.5)
Nitrofurantoin	38/40 (95)	352/375 (93.9)	710/763 (93.1)	1293/1421 (91)	812/895 (90.7)	757/878 (86.2)
Fosfomycin	*0/0	209/222 (94.1)	469/536 (87.5)	669/706 (94.8)	483/498 (97)	478/524 (91.2)
Linezolid	123/126 (97.6)	998/1011 (98.7)	1832/1863 (98.3)	2727/2753 (99.1)	1874/1897 (98.8)	2207/2222 (99.3)

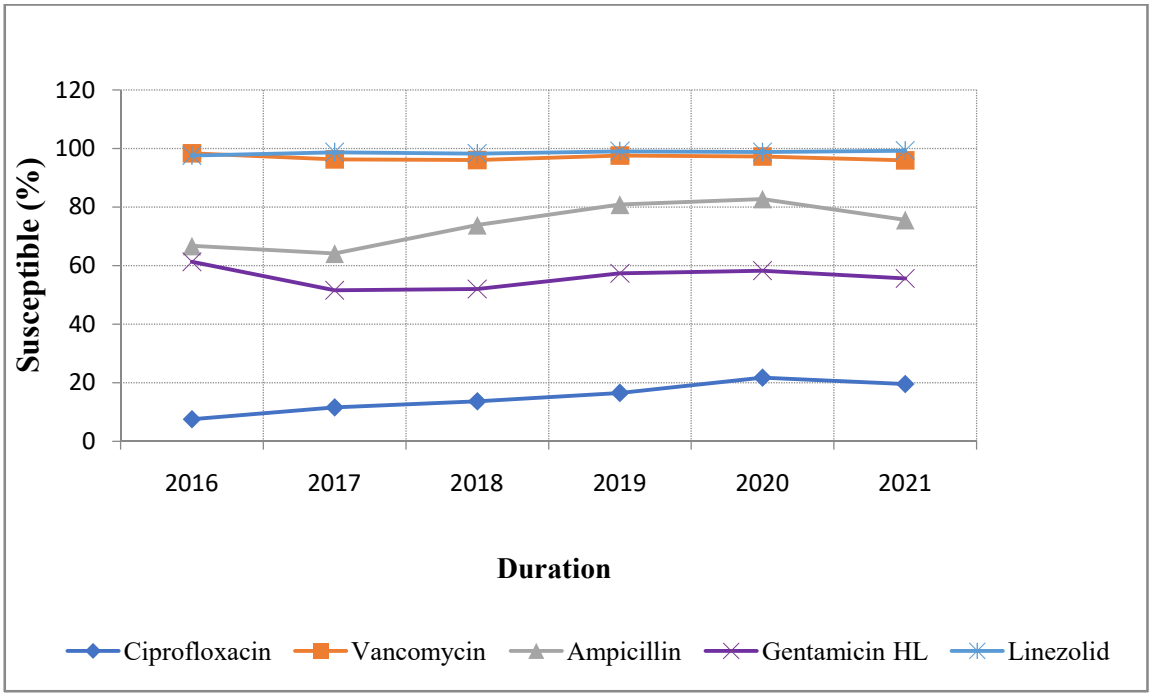


Figure 4.6: Year wise susceptibility trends of *Enterococcus faecalis* from all samples

Table 4.18 Antimicrobial Susceptibilities (AMS) Percentage RC wise of *Enterococcus faecalis* from Total (Except Faeces & Urine)

RC/ Antibiotics	Ampicillin (n=1394)	Vancomycin (n=1475)	Teicoplanin (n=1467)	Gentamicin HL (n=1208)	Linezolid (n=1437)
	n(%)	n(%)	n(%)	n(%)	n(%)
RC2	15/32 (46.9)	22/25 (88)	24/25 (96)	32/42 (76.2)	18/18* (-)
RC4	612/651 (94)	643/651 (98.8)	644/651 (98.9)	364/650 (56)	650/651 (99.8)
RC1	19/77 (24.7)	65/77 (84.4)	66/77 (85.7)	2/3* (-)	77/77 (100)
RC14	-	31/31 (100)	31/31 (100)	-	29/31 (93.5)
RC6	45/49 (91.8)	43/49 (87.8)	44/49 (89.8)	19/49 (38.8)	49/49 (100)
RC15	8/17* (-)	15/16* (-)	13/13* (-)	11/15* (-)	17/17* (-)
RC3	61/64 (95.3)	63/64 (98.4)	63/64 (98.4)	-	62/62 (100)
RC13	1/2* (-)	2/3* (-)	2/4* (-)	-	4/4* (-)
RC10	196/202 (97)	198/202 (98)	195/202 (96.5)	80/135 (59.3)	170/170 (100)
RC20	4/24 (16.7)	20/24 (83.3)	18/20 (90)	16/24 (66.7)	24/24 (100)
RC7	1/3* (-)	3/3* (-)	2/3* (-)	1/3* (-)	3/3* (-)
RC18	26/36 (72.2)	36/36 (100)	35/36 (97.2)	22/36 (61.1)	35/36 (97.2)
RC5	46/47 (97.9)	47/47 (100)	45/45 (100)	28/47 (59.6)	46/46 (100)
RC19	44/76 (57.9)	66/76 (86.8)	71/76 (93.4)	22/48 (45.8)	75/76 (98.7)
RC9	26/50 (52)	47/50 (94)	50/50 (100)	30/52 (57.7)	51/51 (100)
RC17	-	46/47 (97.9)	47/48 (97.9)	23/46 (50)	48/48 (100)
RC12	16/18* (-)	18/18* (-)	18/18* (-)	5/15* (-)	18/18* (-)
RC16	10/28 (35.7)	29/30 (96.7)	28/29 (96.6)	22/29 (75.9)	27/30 (90)
RC8	13/14* (-)	16/16* (-)	16/16* (-)	5/8* (-)	16/16* (-)
RC21	0/3* (-)	6/7* (-)	6/7* (-)	1/6* (-)	7/7* (-)
RC11	0/1* (-)	3/3* (-)	3/3* (-)	-	3/3* (-)
Total	1143/1394 (82)	1419/1475 (96.2)	1421/1467 (96.9)	683/1208 (56.5)	1429/1437 (99.4)

Clinical relevance and treatment guidelines

The proportion of MRSA and VRE was found to be higher among blood isolates than from other specimens which are a cause for concern. Although vancomycin susceptibility remains very high among MRSA isolates, the occurrence of hVISA which is not usually detected in most clinical laboratories is worrisome as it may lead to therapeutic failure. Although vancomycin may continue to be used for serious MRSA infections, it is better to use alternate drugs if the MIC value is close to the breakpoint as such isolates are likely to be hVISA. As susceptibility to daptomycin continues to be close to 100% among MRSA isolates, this antimicrobial may be considered as alternative agents besides vancomycin and linezolid for infections other than those of the respiratory tract. This may also remove some of the selection pressure on antimicrobial resistance genes exerted by these agents. The decision to start vancomycin empirically for serious *S. aureus* infections depends on the MRSA rates in that centre. In centres where MRSA rates are high, vancomycin or linezolid may be used as empirical therapy with de-escalation if required. On the other hand, in centres where MRSA rates are low, Beta lactams may be used as empirical therapy with escalation to glycopeptides/ linezolid/ daptomycin as required. For skin and soft tissue infections, the possibility of using tetracyclines and or clindamycin may be considered as susceptibility rates to these two antibiotics continue to be high.

While it is relatively easy to assign clinical significance to *S. aureus* and enterococcus species, the same is not true for CoNS. They are often dismissed as colonizers though they are being increasingly recognized as opportunistic pathogens, particularly *S. haemolyticus*. Another feature of importance is that these isolates are often multi drug resistant; the genes are carried on mobile elements which make transfer of resistance a distinct possibility. In cases where there is a strong possibility of CoNS being pathogens, it may be prudent to use either vancomycin or linezolid as the rates of resistance to beta lactams are extremely high.

The emergence of *E. faecium* as the predominant species in 2021 across most centres of India is of concern as this species is far more drug resistant when compared to *E. faecalis*. In serious infections, such as meningitis or bacteraemia, linezolid may be tried as empirical therapy, with de-escalation if indicated. The detection of *Enterococcus* species other than *faecalis* and *faecium* in high numbers is also significant as some of these species are intrinsically resistant to glycopeptides. Hence speciation of enterococci is of clinical significance and is not just an academic exercise. Antibiotic resistance genes among phenotypically resistant isolates and sensitive isolates of *S.aureus*, CoNS and enterococci from nodal and regional centres are depicted in Table 4.20 A and 4. 20 B respectively.

WHOLE GENOME SEQUENCE ANALYSIS OF hVISA ISOLATES

Molecular typing of hVISA isolates by WGS

SCC*mec* and sequence types of hVISA (n=29) was determined using centre for genomics software. The most common SCC*mec* type was IV (51.7%) followed by SCC*mec* V (27.5%) and III (13.7%). A total of thirteen different STs (ST1, ST6, ST22, ST30, ST239, ST368, ST121, ST1482, ST2689, ST291, ST88, ST672 and ST772) were identified. The most predominant were ST772 (17.2%) and ST22 (17.2%) followed by ST239, ST88 and ST1482 (10.3%) (Table 4.21). These sequence types belonged to seven distinct clonal complexes (CC1, CC5, CC8, CC22, CC30, CC121, CC672). The most representative were CC1 (20.6%), CC22 (17.2%) and both CC8, CC30 (13.7% each). In addition, two singletons (ST88, ST291) were identified.

Mutation analysis

Candidate genes in hVISA genomes were analysed for amino acid substitutions. These candidate genes include *vra*SR, *vra*T, *gra*SR, and *wal*KR, (regulates the electrical potential of cell membrane); *sae*S (virulence regulator), *mprF* (multiple peptide resistance factor) gene which is involved in the production of wall-teichoic acid (WTA). In addition, all the genomes were screened for the mutations in *rpo*B gene encoding for β subunit of bacterial RNA polymerase. Mutations were analysed for the hVISA study isolates using VSSA reference genome (MSSA476 NC 002953).

Mutations identified in hVISA isolates

The lack of universal resistance markers in hVISA/VISA strains is a major problem in understanding the genetic mechanism of glycopeptide resistance. The genes vraSR, graSR, walKR and rpoB have been frequently associated with the development of heterogeneous resistance to vancomycin. In the present study whole-genome analysis of hVISA (n = 29) revealed distinct amino acid substitutions in eight candidate genes (Table 4.21). However, none of the tested isolates showed mutations in *wal*R genes. Several novel mutations which were identified in an earlier study (Hafer *et al* 2012 and Bakthavatchalam *et al* 2019) were also detected in our study, for eg. *vra*R (T24K) and *mpr*F (T635I). Other commonly reported mutations were also found in *vra*R (E59D, K38N, M81I, R121I) , *vra*S (V15G), *gra*S (L26F, I59L, T224I, R232K), *gra*R(D148E, S207R), *rpo*B (L466S, H481N, Y737F, R594C, V536A), *sae*S (S351T, S227N, D269N) and in *mpr*F (A26V, K47N) (Table 4.22). Among hVISA, T224I (22/29, 76%) was identified as the predominant mutation in *gra*S followed by A26V (20/29, 69%) substitution in *mprF* and D148E (14/29, 48%) in *gra*R. A strong link was seen between hVISA phenotype and the mutations identified in *gra*S (T224I) and *gra*R (D148E). Of these mutations, T224I and D148E were identified in various STs. M81I substitution was most commonly seen in *vra*R genes among the hVISA strains which belonged to ST772 and D148E substitution in *gra*R genes was associated with ST22.

Table- 4.20A: Antibiotic resistance genes among phenotypically resistant isolates of *S. aureus*, CoNS and enterococci from nodal and regional centres

S.No	Phenotypic resistance	Genes detected	Nodal center (No.positive /no tested)	Regional centers (No.positive /no tested)
1	Methicillin resistant *S.aureus* (MRSA)	*mec*A	*mec*A : 101/101 (100%)	*mec*A :351/351 (100%)
2	Erythromycin resistant *S.aureus*	*erm* A, *erm* B and *erm* C	*erm* A :7/77 (9%) *erm* B :0/77 *erm* C :34/77 (44.1 %) msrA/B : 22/77 (28.6 %) ermA and ermC :11/77 (14.3%) ermC and msrA/B: 2/77 (2.6%) ermA and msrA/B: 2/77 (2.6%) Negative for *erm*A,B,C and msr A/B genes: 22/77 (28.5 %)	*erm* A :8/478 (1.7 %) *erm* B :0/478 *erm* C :213/478(44.5 %) msrA/B : 212/478 (44.3%) msrB : 2/478 (0.4 %) ermA and ermC :1/478 (0.2 %) ermC and msrA/B: 40/478 (8.4%) ermA and msrA/B: 1/478 (0.2%) ermC and msrB : 1/478 (0.2 %) Negative for *erm*A,B,C and msr A/B genes: 0/478
3	Mupirocin resistant *S.aureus*	*mup*A and *mup*B	*mup* A :9/9 (100 %) *mup* B : 0/9	*mup* A :7/7 (100 %) *mup* B : 0/7
4	Linezolid resistant MRSA	*cfr*	*cfr* : 6/6 (100%)	*cfr* : 0/0
5	Vancomycin resistant Enterococci (VRE)	*van*A, *van*B, *van*C_1/C_2	*van*A :77/77 (100%) *van*B :0/77 *van*C_1/C_2 :0/77	*van*A :138/138 (100%) *van*B :0/138 *van*C_1/C_2 :0/138

Table- 4.20B : Antibiotic resistance genes among phenotypically sensitive isolates of *S.aureus*, CoNS and enterococci from nodal and regional centres

S.No	Phenotypic resistance	Genes detected	Nodal center (No.positive /no tested)	Regional centers (No. positive /no tested)
1	Methicillin sensitive*S.aureus* (MSSA)	*mec*A	*mec*A : 0/30	*mec*A :1/296 (0.3 %)
2	Erythromycin sensitive*S.aureus*	*erm* A, *erm* B and *erm* C	*erm* A :0/30 *erm* B :0/30 *erm* C :0/30 msrA/B : 0/30 ermA and ermC :0/30 ermC and msrA/B: 0/30 ermA and msrA/B: 0/30 Negative for *erm*A,B,C and msr A/B genes: 0/30	*erm* A :0/170 *erm* B :0/170 *erm* C :0/170 msrA/B : 1/170 (0.5 %) msrB : 0/170 ermA and ermC : 0/170 ermC and msrA/B: 0/170 ermA and msrA/B: 0/170 ermC and msrB : 0/170 Negative for *erm*A,B,C and msr A/B genes : 477/478 (99.8 %)
3	Vancomycin sensitive enterococci	*van*A, *van*B, *van*C_1/C_2	*van*A :2/30 (7%) *van*B :0/30 *van*C_1/C_2 :0/30	*van*A :1/345 (0.28 %) *van*B : 0/345 *van*C_1/C_2 : 0/345

Table 4.21 : SCC*mec* and Sequence types among hVISA isolates (n=29) based on WGS sequences

S.No	Ref ID	Regional Centres Nos	Sequence Types	SCC mec types
1	EX246	RC04	ST 6	II
2	B3859	RC04	ST 121	IV
3	146158	RC07	ST 22	IV
4	137293	RC10	ST 239	III
5	154568	RC07	ST 30	IV
6	B14932	RC04	ST 239	III
7	B14994	RC04	ST 22	IV
8	B-15890	RC04	ST 291	IV
9	EX-1667	RC04	ST 368	III
10	EX-974	RC04	ST 772	V
11	EX-1771	RC04	ST 2689	IV
12	EX2298	RC04	ST 2689	IV
13	EX6069	RC04	ST 1	V
14	EX6917	RC04	ST 1482	IV
15	EX7243	RC04	ST 772	V
16	EX2706	RC04	ST 672	IV
17	198405	RC15	ST 672	II
18	168952	RC15	ST 22	IV
19	170801	RC09	ST 88	V
20	147892	PDH	ST 22	IV
21	210883	PGI	ST 772	V
22	S9733	RC04	ST 1482	IV
23	S9072	RC04	ST 772	V
24	S9132	RC04	ST 88	V
25	S7733	RC04	ST 88	IV
26	S9827	RC04	ST 239	III
27	S23384	RC04	ST 772	V
28	149774	RC06	ST 22	IV
29	AJ1032	RC13	ST1482	IV

Table 4.22. Amino acid substitutions observed in the candidate genes of hVISA isolates

Lab ID	*walK*	*graS*	*graR*	*vraR*	*vraS*	*rpoB*	*saeS*	*mprF*
RC04 EX246	-	L26F, T224I		K38N				
RC04 B3859	-	L26F, T224I		E59D				A26V
RC07 146158	-	S104L, E108D, E11D, K146E, Y156F, D218N, Y219H, F245Y, I247V, E248D, G250W, N251I, S259A, N302D, V318I, R325K	D148E	-	-	-	I9V	I9V, A26V, K47N, L53F, T635I
RC13 1032	-	A8V, R14S, M15K, M55L, Y62F, D73E, S104L, E112D, K146E, Y156F, T167A, Y182F, V212I, D218N, Y219H, T224I, F245Y, I247V, E248D, N254I, G259A, N302D,V318I, R325K	D148E , V136I, S207R	-	-	Y737F	S351T	A26V, K47N, L53F, D160N, F174L, F194Y, A223V, I371L, L406I, T409I, F413L, A426V, V446I
RC10 137293	R222K, A468T	L26F, I59L, T224I, R232K	D148E	E59D, T24K	V15G	L466S, H481N	S227N, K268E	Q692E
RC07 154568	A468T	A8V, R14S, M15K, M55L, Y62F, D73E, S104L, E112D, K146E, Y156F, T167A, Y182F, V212I, D218N, Y219H, T224I, F245Y, I247V, E248D, N254I, G259A, N302D,V318I, R325K	D148E , V136I, S207R D148H	-	-	Y737F	S351T	A26V, K47N, L53F, D160N, F174L, F194Y, A223V, I371L, L406I, T409I, F413L, A426V, V446I
RC04 B14932	R222K, A468T	L26F, I59L, T224I	D148E	E59D	-	-	S227N, K268E	Q692E
RC04 B14994	-	S104L, E108D, E11D, K146E, Y156F, D218N, Y219H, F245Y, I247V	D148E D148H	-	-	-	-	I9V, A26V, K47N, T6351
RC04 B-15890	-	D218N, Y219H, F245Y, I247V, E248D, G250S, N254I, S259A, Y261I	D148E D148H	-	-	-	-	A26V, K47N, L53F, D160N, L169F, F174L, F194Y, A223V, L293F, I371L, L406I, F413L
RC04 EX-1667	R86L, R222K, M291V, A468T	L26F, I59L, T224I	D148E	E59D	-	R594C	S227N, K268E	Q692E
RC04 EX-974	-			E59D, M81I	-	-	-	A26V
RC04 EX-1771	-	-	-	-	-	-	-	-

RC04 EX2298	-	-	-	-	-	-	-	-
RC04 EX6069	S260G	T224I	-	K38N	-	-	-	A26V, G59S
RC04 EX6917	A468T	D218N, Y219H, F245Y, I247V, E248D, G250S, N254I, S259A, Y261I	D148E V136I S207R	-	-	Y737F	S351T	A26V, K47N, D160N, F174L, F194Y, I371L, L406I, T409I, F413L, V430,A426,V446I, K522N, L575I
RC04 EX7243	-	-		E59D, M81I	-	-	-	A26V
RC04 EX2706	-	T224I	-	K38N	-	-	D269N	A26V, L53F, P267S
RC15 198405	-	T224I	D148E	-	-	-	D269N	I9V, A26V, K47N, L53F, P267S, T635I
RC15 168952	-	S104L, E108D, E11D, K146E, Y156F, D218N, Y219H, F245Y, I247V	D148E	-	-	-	-	I9V, A26V, K47N, L53F, P267S, T635I 26V, K47N, L53F
RC09 170801	-	L26F, T224I	-	R121I, K38N	-	-	-	-
RC05 147892	-	S104L, E108D, E11D, K146E, Y156F, D218N, Y219H, F245Y, I247V, E248D, G250W, N254I	-	-	-	D148E	-	I9V, A26V, K47N
RC02 210883	-	-		E59D, M81I	-	-	-	A26V
RC04 S9733	-	A8V, R14S, M15K, M55L, Y62F, D73E, S104L, E112D, K146E, Y156F, T167A, Y182F	D148E V136I S207R	-	-	Y737F	S351T	A26V, K47N, D160N, F174L, F194Y, I371L, L406I, T409I, F413L, V430,A426,V446I, K522N, L575I
RC04 S9072	-	-	-	E59D, M81I	-	-	-	A26V
RC04 S9132	-	L26F, T224I	-	R121I, K38N	-	-	-	-
RC04 S7733	-	L26F, T224I	-	R121I, K38N	-	-	-	A26V, K47N, D160N, F174L, F194Y, I371L, L406I, T409I, F413L, V430,A426,V446I, K522N, L575I
RC04 S9827	R22K, A468T	L26F, I59L, Y224I	D148E D148H	E59D		V536A	S227N, K268E	Q692E
RC04 S23384	-	-	-	M81I	-	-	-	A26V
RC06 149774	-	S104L, E108D, E11D, K146E, Y156F, D218N, Y219H, F245Y, I247V	D148E	-	-	-	-	19V, A26V, K47N, L53F, T6351

Chapter 5 Fungal pathogens

Antifungal drug susceptibility analysis

Fungal isolates accounted for 3.4% (3,452/ 95,728) of the total isolates reported in the network of which *Candida* species accounted for 2.7% (2605/95,728) isolates, isolated from all samples other than faeces. Majority of the Candida species were isolated from blood (53%), followed by urine (10%), superficial infections (5.8%), LRT samples (5.2%), deep-seated infection (2.2%), and others (19.6%). *C. tropicalis* was the leading agent (0.8%) followed by *C. albicans* (0.7%), *C. glabrata* (0.3%), *C. parapsilosis* (0.3%), *C. auris* (0.2%), and *C. utilis* (0.2%). Antifungal susceptibility profiling revealed an overwhelming fluconazole susceptibility in *C. tropicalis* (91.2%), *C. albicans* (~93%), and *C. utilis* (~94%), but declining susceptibility rates in *C. parapsilosis* (78.3%) and *C. glabrata* (80.4%) (**Table 5.1**).

C. auris and *C. krusei* were predominantly resistant to fluconazole with extremely low susceptibility percentages of 2.6% and 2.9%, respectively. On inter-species comparison, the proportions of fluconazole non-susceptible isolates was statistically non-significant between *C. tropicalis* and *C. albicans* (8.8% vs. 7.1 %, Pearson's $\chi 2$, p=0.25), *C. tropicalis* vs. *C. utilis* (8.8% vs 6.1%, Pearson's $\chi 2$ p=0.25). In contrast, *C. parapsilosis* and *C. glabrata* exhibited significantly higher proportion of fluconazole non-susceptible isolates compared to *C. tropicalis* (21.7% vs. 8.8%, 19.6% vs. 8.8%; Pearson's $\chi 2$ p<0.001). Similarly, the proportion of non-susceptible isolates in *C. glabrata* was statistically higher compared to *C. tropicalis* (19.6% *vs*. 8.8%, p<0.001). Voriconazole susceptibility was higher in *C. tropicalis* (95.6%), *C. albicans* (96.6%), *C. utilis* (98.8%), *C. krusei* (98.8%) and *C. parapsilosis* (~97%), followed by *C. glabrata* (87.6%), while *C. auris* was least susceptible among all the species (33.8%). In comparison to *C. tropicalis*, the proportion of non-susceptible isolates in *C. glabrata* (4.4% *vs*. 12.4%, P<0.001) and *C. auris* (4.4% *vs*. 66.2% P<0.001) were significantly higher. While *C. krusei* is inherently resistant to fluconazole as was also noted in the present study (~97% resistance rate), however, the majority of the isolates were susceptible to the voriconazole (1.2% resistance rate). In contrast, a large majority of *C. auris* (66.2%) were cross-resistant to voriconazole.

More than 95% of the isolates of *C. albicans*, *C. tropicalis*, and *C. utilis* were susceptible to any of the three echinocandins (**Table 5.1**). Majority of *C. parapsilosis* were susceptible to echinocandins (caspofungin, 97.8%; micafungin, 97.5%; anidulafungin, 98.8%). Acquired 'high-level' echinocandin-resistance in *C. parapsilosis* family was noted in ≤2.5% of the isolates in the present study.

Table 5.1: Susceptible pattern of *Candida* species isolated from all samples

AMA	*Candida* tropicalis n=796	*Candida albicans* n=664	*Candida glabrata* n=315	*Candida parapsilosis* n=279	*Candida auris* n=194	*Candida utilis* n=174	Candida krusei n=82
Anidulafungin	273/281 (97.2%)	161/173 (93.1%)	123/134 (91.8%)	85/86 (98.8%)	62/92 (67.4%)	159/165 (96.4%)	30/32 (93.8%)
Caspofungin	760/790 (96.2%)	634/656 (96.6%)	171/310 (55.2%)	271/277 (97.8%)	135/193 (69.9%)	159/166 (95.8%)	46/82 (56.1%)
Fluconazole	716/785 (91.2%)	614/661 (92.9%)	181/225 (80.4%)	217/277 (78.3%)	5/193 (2.6%)	155/165 (93.9%)	2/70 (2.9%)
Micafungin	672/688 (97.7%)	537/551 (97.5%)	229/234 (97.9%)	232/238 (97.5%)	145/172 (84.3%)	166/168 (98.8%)	61/73 (83.6%)
Voriconazole	736/770 (95.6%)	626/648 (96.6%)	197/225 (87.6%)	247/255 (96.9%)	48/142 (33.8%)	167/169 (98.8%)	80/81 (98.8%)

On intra-species comparison across blood and urine isolates, the frequency of fluconazole-resistance didn't vary statistically in *C. tropicalis* (9.2 vs. 14.5% p=0.05), *C. albicans* (4.2% vs 4.4%, p=0.79), *C. glabrata* (21% *vs.* 30%, p=0.38), and *C. parapsilosis* (16% vs. 11%, p=0.15) (**Table 5.2 & 5.3**).

In contrast, the frequency of voriconazole-resistance varied significantly across blood and urine isolates in *C. tropicalis* (4% vs.11.8%, p<0.01). However, higher recovery of voriconazole-resistant isolates from urine samples was not noted in *C. albicans* (3.6% vs. 2.2% p=0.56), *C. parapsilosis* (3.2% vs 5.8%, p=0.56, *C. glabrata* (9.3% vs. 17.6%, p=0.3), and *C. auris* (64.8% vs. 80%, p=0.24). The frequency of echinocandin-resistant isolates across blood and urine samples didn't vary in any of the species, though the proportion of caspofungin-resistant *C. auris* was higher in urine (44.4% vs 29%), however, the difference was statistically non-significant (p=0.17). Only a handful *of C. albicans* and *C. glabrata* were isolated from genital samples and comparison of their susceptibility profile with blood and urine isolates was not warranted due to small sample sizes **(Table 5.4).** *A. flavus* and *A. fumigatus* were among the leading moulds isolated from clinical samples. *A. flavus* was relatively less susceptible to amphotericin B compared to *A. fumigatus* (44% vs. 70%, p<0.001) (**Table 5.5**).

Table 5.2: Susceptible pattern of *Candida* species isolated from blood

AMA	*Candida* tropicalis n=444	*Candida parapsilosis* n=204	*Candida albicans* n=173	*Candida utilis* n=172	*Candida auris* n=150	*Candida glabrata* n=126	Candida krusei n=40
Anidulafungin	181/188 (96.3%)	59/60 (98.3%)	74/78 (94.9%)	157/163 (96.3%)	48/75 (64%)	48/52 (92.3%)	21/22 (95.5%)
Caspofungin	424/440 (96.4%)	199/204 (97.5%)	164/172 (95.3%)	157/164 (95.7%)	106/149 (71.1%)	61/125 (48.8%)	20/40 (50%)
Fluconazole	397/437 (90.8%)	150/203 (73.9%)	164/173 (94.8%)	154/163 (94.5%)	3/149 (2%)	64/81 (79%)	1/37 (2.7%)
Micafungin	391/399 (98%)	174/180 (96.7%)	154/161 (95.7%)	164/166 (98.8%)	114/136 (83.8%)	86/88 (97.7%)	30/39 (76.9%)
Voriconazole	411/428 (96%)	179/185 (96.8%)	162/168 (96.4%)	165/167 (98.8%)	38/108 (35.2%)	88/97 (90.7%)	39/39 (100%)

Table 5.3: Susceptible pattern of *Candida* species isolated from Urine

AMA	*Candida* tropicalis n=103	*Candida albicans* n=90	*Candida glabrata* n=25	*Candida parapsilosis* n=*19	*Candida auris* n=*18
Anidulafungin	*15/16 (-)	*5/9 (-)	*3/3 (-)	*4/4 (-)	*6/8 (-)
Caspofungin	100/103 (97.1%)	83/88 (94.3%)	12/25 (48%)	*17/17 (-)	*10/18 (-)
Fluconazole	87/103 (84.5%)	86/90 (95.6%)	14/20 (70%)	*16/18 (-)	*1/18 (-)
Micafungin	88/91 (96.7%)	81/82 (98.8%)	20/20 (100%)	*12/12 (-)	*13/16 (-)
Voriconazole	90/102 (88.2%)	87/89 (97.8%)	*14/17 (-)	*16/17 (-)	*3/15 (-)

* Less than 20 samples

Table 5.4: Susceptible pattern of *Candida* species isolated from genital samples

AMA	*Candida glabrata* n=*11	*Candida albicans* n=*8
Anidulafungin	*0/0	*1/2 (-)
Caspofungin	*11/11 (-)	*8/8 (-)
Fluconazole	*0/0	*7/8 (-)
Micafungin	*11/11 (-)	*7/7 (-)
Voriconazole	*2/2 (-)	*8/8 (-)

Table 5.5: Susceptible pattern of *Aspergillus* species isolated from all samples across different locations

AMA	*Aspergillus flavus*				*Aspergillus fumigatus*			
	Total n=243 (S %)	OPD n=55 (S %)	Ward n=162 (S %)	ICU n=26 (S %)	Total n=154 (S %)	OPD n=31 (S %)	Ward n=75 (S %)	ICU n=48 (S %)
Amphotericin B	107/243 (44)	25/55 (45.5)	72/162 (44.4)	10/26 (38.5)	107/153 (69.9)	20/30 (66.7)	57/75 (76)	30/48 (62.5)
Caspofungin	143/172 (83.1)	37/45 (82.2)	96/114 (84.2)	*10/13	82/115 (71.3)	19/24 (79.2)	40/57 (70.2)	23/34 (67.6)
Itraconazole	236/238 (99.2)	54/55 (98.2)	157/158 (99.4)	25/25 (100)	143/143 (100)	30/30 (100)	70/70 (100)	43/43 (100)
Posaconazole	227/243 (93.4)	50/55 (90.9)	154/162 (95.1)	23/26 (88.5)	148/154 (96.1)	28/31 (90.3)	74/75 (98.7)	46/48 (95.8)
Voriconazole	241/242 (99.6)	55/55 (100)	160/161 (99.4)	26/26 (100)	150/150 (100)	31/31 (100)	71/71 (100)	48/48 (100)

Invasive infections due multidrug-resistant *C. auris* continue to be reported across many centers with 10 of 16 centers of the surveillance network notifying this fungal pathogen. Interestingly, three centers reported approximately 75% of the total caseload across the network in 2021 (**Figure 5. 1**).

Susceptibility trends suggest that antifungal resistance rates in *C. albicans*, *C. tropicalis*, and *C. utilis* have remained in the ballpark of 0-10% (**Figure 5.2a-g**). In contrast, fluconazole-resistance rates in *C. parapsilosis* have almost steadily increased over the years from 9.3% to 21.7% without a concomitant increase in cross-resistance to voriconazole. Similarly, fluconazole resistance in *C. krusei* has shown an upward swing from 51.4% to 97.1%. *C. auris* continues to be the least susceptible of all the species to fluconazole with a concomitant cross-resistance to voriconazole. Fluconazole resistance in *C. glabrata* has increased in the range of 5.8% to 29.8% from 2017 to 2021.

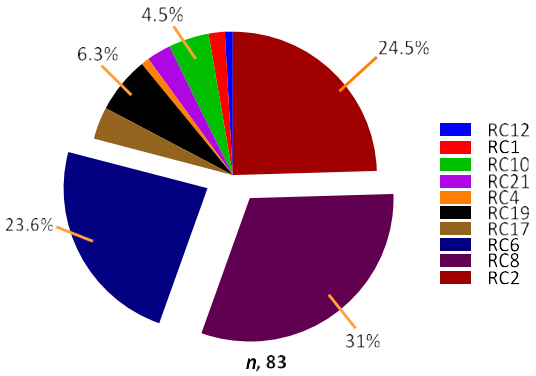


Figure 5.1: Distribution of *C. auris* in the AMRS Network noted in the study period 2021

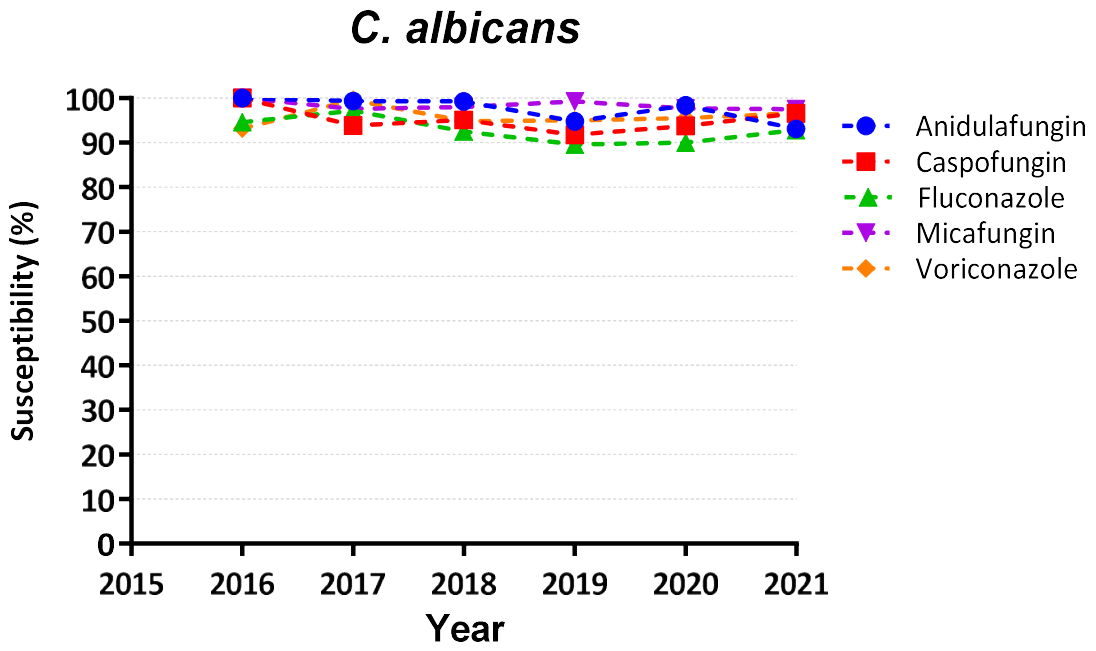


Figure 5.2a. Susceptibility trends of *C. albicans* over the years

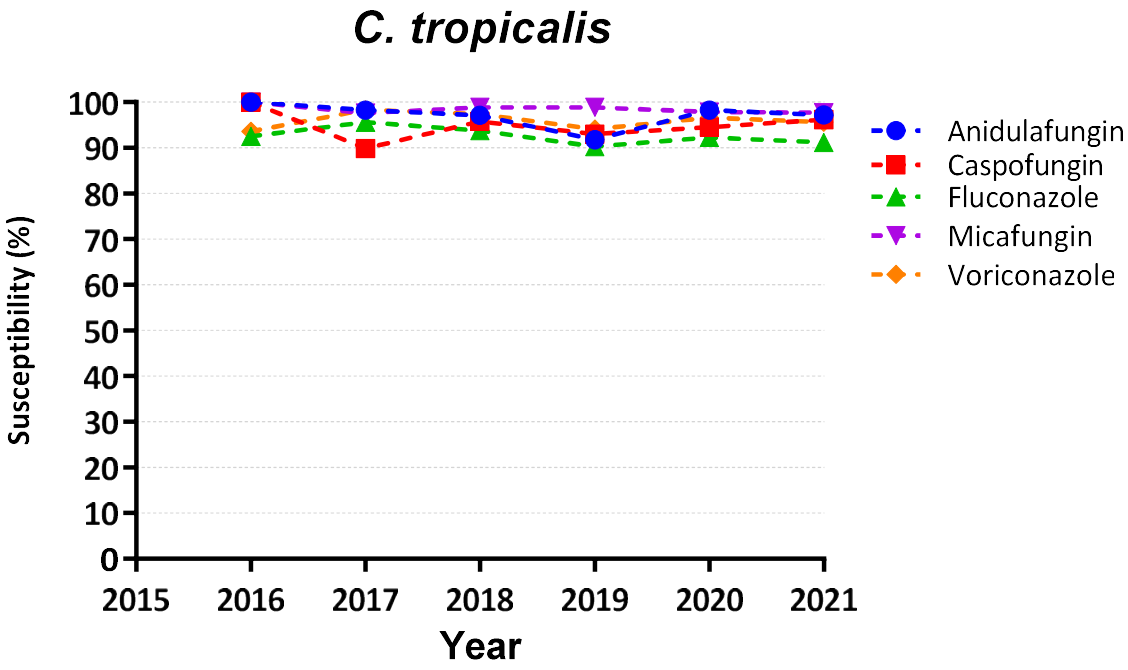


Figure 5.2b. Susceptibility trends of *C. tropicalis* over the years

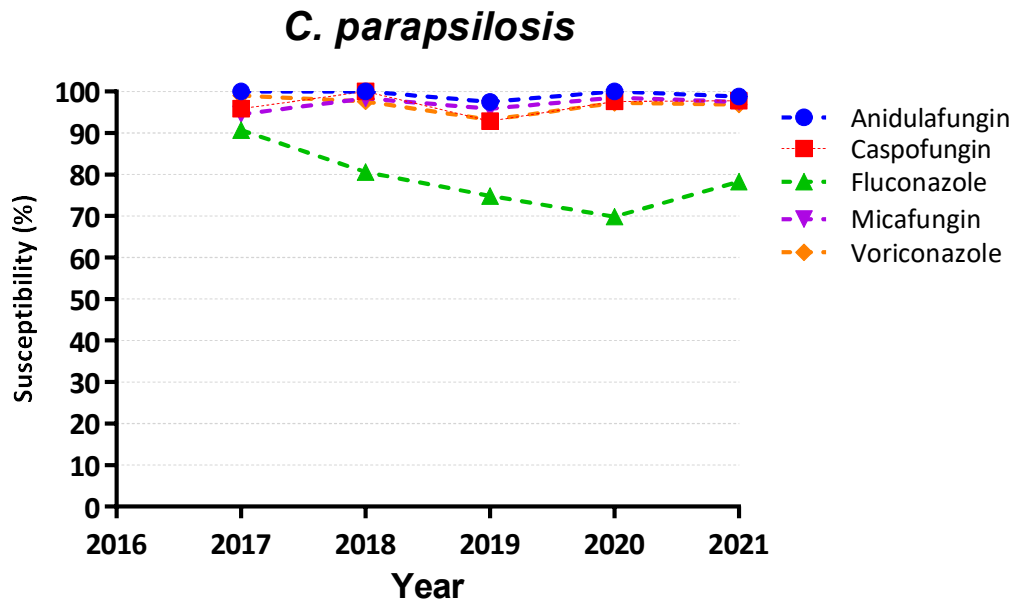


Figure 5.2c. Susceptibility trends of *C. parapsilosis* over the years

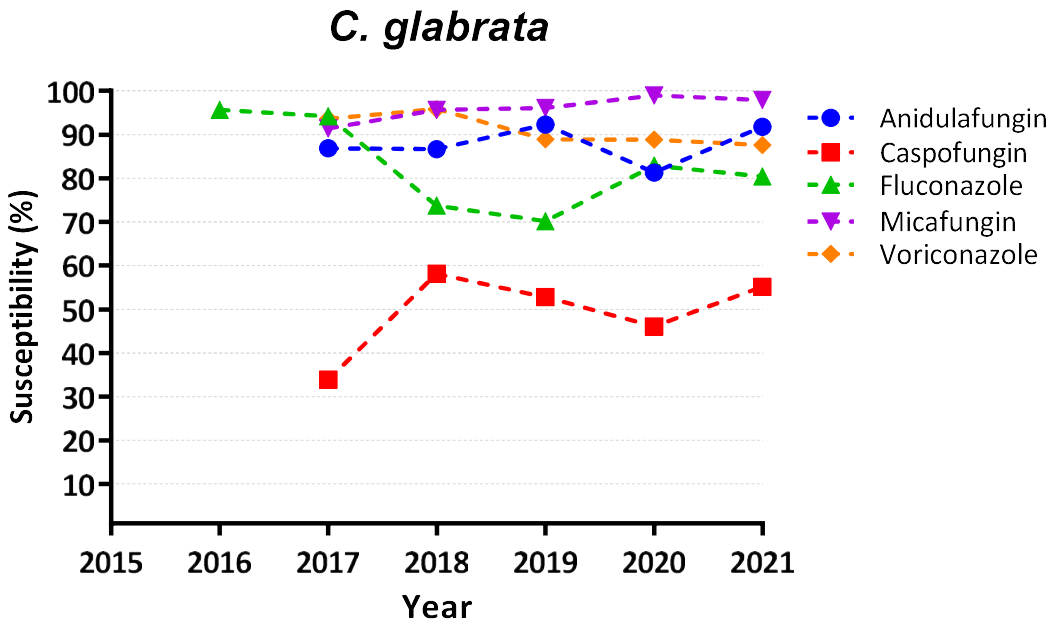


Figure 5.2d. Susceptibility trends of *C. glabrata* over the years

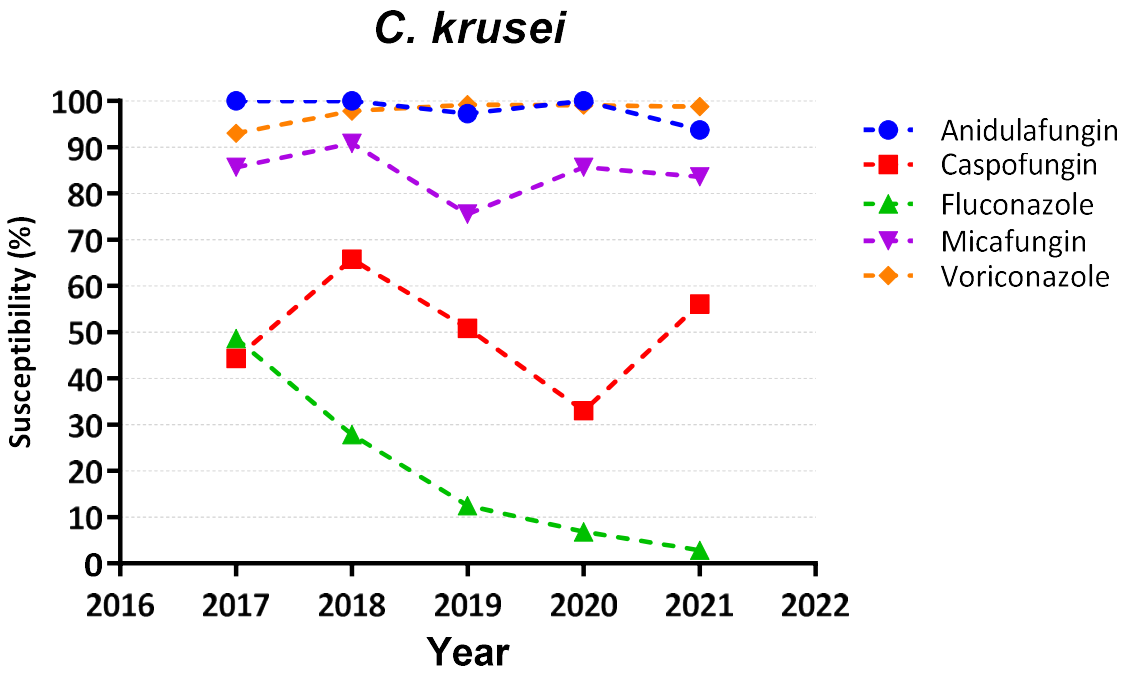


Figure 5.2e. Susceptibility trends of *C. krusei over* the years

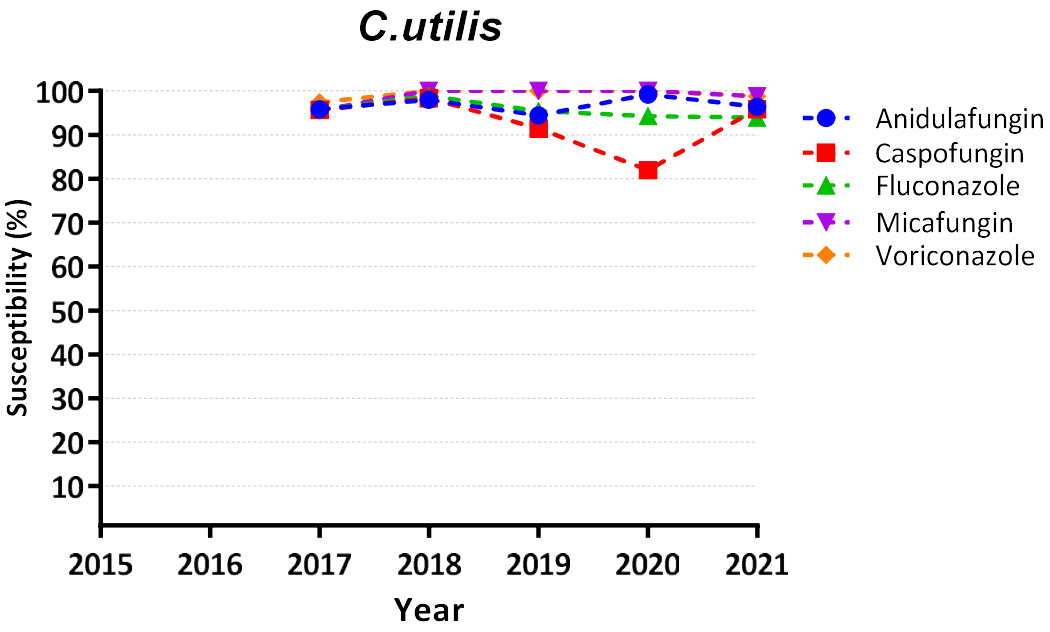


Figure 5.2f. Susceptibility trends of *C. utilis over* the years

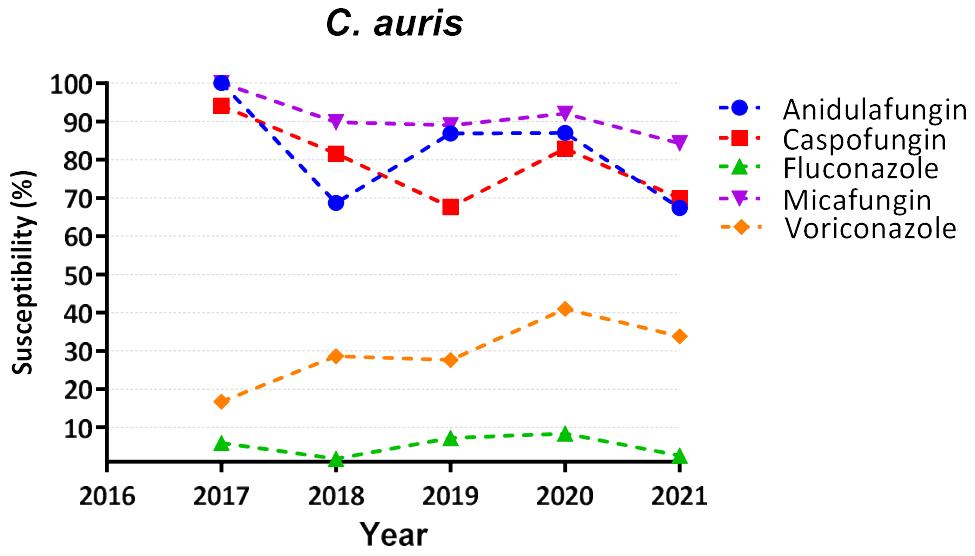


Figure 5.2g. Susceptibility trends of *C. auris over* the years

Clinical Relevance and therapeutic implications

C. albicans and *C. tropicalis,* two most common causative agents of fungemia, remain largely susceptible to azole and echinocandin antifungal drugs and year-wise trend data show a steady resistance rate below 10%. These data suggest that azoles and echinocandins remain efficient therapeutic options in the management of invasive infections due to these species. However, a declining rate of fluconazole susceptibility was noted in *C. parapsilosis* that needs to be monitored closely. *C. parapsilosis* species complex, including *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis* carry an intrinsic polymorphism P660A in Fks1p that confers reduced susceptibility to echinocandins. However, such strains still get inhibited at therapeutic levels and only sporadic clinical failures have been reported. Notwithstanding the intrinsically reduced susceptibility of *C. parapsilosis* to echinocandins, only 2.5% isolates of *C. parapsilosis* species complex exhibited 'high-level' resistance to echinocandins (MIC≥8) in this surveillance study. In view of a rising incidence of fluconazole-resistance in *C. parapsilosis*, echinocandins offer the best choice for the management of invasive infections due to this species.

A marked decline in susceptibility to caspofungin in *C. glabrata* is a cause for concern. However, these data suggest that other echinocandins, micafungin and anidulafungin effectively inhibit this species and therefore, should constitute an effective therapy against this pathogen. *C. krusei*, although, intrinsically resistant to fluconazole, remains susceptible to voriconazole. Therefore, voriconazole could be an effective and cost-effective alternative to echinocandins in the management of invasive candidiasis due to *C. krusei*. *C. auris* is intrinsically resistant to fluconazole with a concurrent reduced

susceptibility to voriconazole. Echinocandins are the only effective antifungal class against this multidrug resistant pathogen. However, a declining susceptibility to echinocandins in this pathogen is a cause for concern. Nevertheless, echinocandins are the best bet for treatment of invasive infections due to *C. auris. C. utilis* and *W. anomalus* continue to be among the leading agents of neonatal fungal sepsis. However, both species remain fairly susceptible to all the antifungal classes.

Molecular analysis showed F635Y, F635L, S639F, and R1354S mutations in FKS1p, the catalytic subunit of β-1, 3-glucan synthase were associated with echinocandin resistance in *C. auris*. Overexpression of azole-target coding gene *ERG*11, and multi-drug efflux transporter genes, *Cdr*1 and *Mdr*1 was associated with azole-resistance in this species. The azole resistance in *C. auris* was found associated with fitness cost in terms of reduced oxidative stress response and biofilm-forming capacity in fluconazole-resistant isolates. On evaluation of *FKS*1 markers in a murine model of infection, F635Y and R1354S showed most pronounced *in-vivo* resistance to caspofungin, while S639F and F635L exhibited a partial response to therapy. However, isolates with a marginal rise in MIC but carrying wild-type *FKS*1 may respond well to the treatment. Further, *C. auris* isolates with echinocandin MIC ≥1 should be evaluated for *FKS*1 mutation that can best predict the response to therapy in *C. auris* infected patients. In *C. parapsilosis*, Y132F and K143R mutations in ERG11p were found associated with fluconazole resistance. Susceptible isolates had an upregulated expression of *HOG*1 and peroxisomal catalase, *CTA*1 gene conferring them a robust ant-oxidant response. This trade-off in the fitness and resistance in *C. auris* and attenuated biofilm forming capacity could impinge upon the virulence of the organism and may lead to differential outcomes in patients infected with fluconazole-resistant and-susceptible *C. auris*.

Dermatophytosis due to the *Trichophyton mentagrophytes-Trichophyton interdigitale* complex is being increasingly reported across India. Reports of therapeutic failure have surfaced recently, but there are no clinical break points (CBP) or epidemiological cutoffs (ECVs) available to guide the treatment of dermatophytosis. The F397L mutation in the squalene epoxidase (SE) gene was observed in 77.1% of isolates with a terbinafine MIC of ≥1 mg/liter, but no mutation was detected in isolates with a terbinafine MIC of <1 mg/liter. In the absence of CBPs, evaluation of the UL-WT may be beneficial for managing dermatophytosis and monitoring the emergence of isolates with reduced susceptibility

This report also brings out a rising incidence of 'non-candida' fungemia due to once-rare, *C. utilis* and *W. anomalus* in neonates at a few centers. The reason for such high incidence is not clear and warrants a systematic epidemiological investigation. Reduced susceptibility to amphotericin B in *A. flavus* is believed to be intrinsic in nature due to high cellular ergosterol content and increased activity of peroxidase and superoxide dismutase in this mould. There has been a growing worldwide concern on triazole resistance in *A.*

fumigatus. Triazoles constitute the mainstay of therapy in aspergillosis. Scores of studies have reported triazole-resistance in *A. fumigatus* leading to higher mortality in invasive aspergillosis (IA), while complicating the clinical course of chronic pulmonary aspergillosis and ABPA patients harboring these resistant strains. In the present study, resistance to voriconazole and itraconazole was not observed in *A. fumigatus*. However, 4% and 6.6% isolates of *A. fumigatus* and *A. flavus*, respectively exhibited resistance to posaconazole while none of these isolates were cross-resistant to either itraconazole or voriconazole. This singular azole-resistance has been reported rarely and could be confounded by MICs that are just two-fold higher than the cut-off value MIC. A substantial proportion of both *A. flavus* and *A. fumigatus* were non-susceptible to caspofungin that is increasingly being used as a salvage therapy for IA. However, the clinical relevance of this moderately reduced susceptibility needs to be ascertained.

At the regional center, RC02, *R. arrhizus* was the predominant mycotic agent, mostly isolated from sino-nasal samples and accounted for 18.3% of all the fungal culture-positive samples, while *C. tropicalis* (14.8%) was the prevalent yeast species isolated from blood and other samples. This frequent isolation of mucoralean fungi could reliably be attributed to the surge in mucormycosis cases during the second wave of the COVID-19 pandemic from April, 2021 through July 2021. *R. arrhizus* was predominantly susceptible to amphotericin B with just 0.7% of the isolates exhibiting non-wild type MIC. Therefore, amphotericin B remains as the best choice for treatment of this fatal fungal infection. The report also delves into a case-control study on mucormycosis due to *Rhizopus homothalicus* which shows that this Mucoralean species is associated with a distinct clinical presentation, can cause infections even in patients with controlled diabetes and has higher mortality compared to *R. arrhizus* mucormycosis.

Treatment guidelines based on phenotypic and molecular data

1. Fluconazole, voriconazole and echinocandins remain efficient therapeutic options in the management of invasive infections due to two leading yeast pathogens, *C. albicans* and *C.tropicalis*
2. In view of rising incidence of fluconazole-resistance in *C. parapsilosis* spp. complex, either an echinocandin or voriconazole is recommended for invasive infections due to this species
3. Declining susceptibility of *C. glabrata* to caspofungin and reduced susceptibility to fluconazole warrants micafungin, anidulafungin and voriconazole as better therapeutic options for invasive infections due to *C. glabrata*
4. The data suggests that echinocandins and voriconazole are the best treatment choices for invasive infections due to *C. krusei*
5. Echinocandins constitute an effective therapy against invasive candidiasis due to *C. auris*. The genotypic and pharmacodynamic data suggests echinocandin-resistant isolates with

FKS1p mutations R1354Sand F635Y are recalcitrant to echinocandin therapy, while S639P and F635L may respond at higher than standard doses.

6. *C. utilis* and *W. anomalus*, two emerging yeast pathogens frequently isolated from neonates in some centers, are effectively inhibited by both azoles and echinocandins

7. *A. fumigatus* and *A. flavus* remain susceptible to triazoles and echinocandins, while *A. flavus* exhibits a profound resistance to amphotericin B. These data suggest triazoles as effective therapy for *Aspergillus* infections and contraindicate amphotericin B, especially for IA due to *A. flavus*.

8. *Rhizopus arrhizus*, the most common mucorales, was predominantly susceptible to amphotericin B with just 0.7% of the isolates exhibiting non-wild type MIC. Therefore, **amphotericin B remains as the best choice for treatment of mucormycosis.**

9. The data suggest a high terbinafine resistance rate (11.4%) and therapeutic failure in *Trichophyton mentagrophytes-Trichophyton interdigitale* complex primarily due to F397L mutation in allylamine target, Sequalene epoxidase (SE) enzyme and just 0.2% for itraconazole. These findings warrant itraconazole as primary therapy for dermatophytosis.

Epidemiology of fungal diseases outbreak

1. Analysis of COVID-19 associated mucormycosis (CAM) outbreak due to R. homothallicus

This was a case-control study conducted from January through October 2021 (10 months). The clinical data was collected from patient records, and they were followed up for 3 months after diagnosis. Out of 631 patients with mucormycosis, a total of 60 (9.5%) consecutive patients with infection due to *R. homothallicus* were enrolled in this study. Of these, 54 cases were rhino-orbital mucormycosis (ROM) and six were cases of pulmonary mucormycosis (PM). We also included 55 randomly selected, age-and gender- matched ROM cases due to *R. arrhizus* obtained during the same time period for analysis of clinical and demographic parameters. Thirty-four randomly selected *R. homothallicus* isolates from ROM cases and 6 from PM cases were subjected to molecular identification.

Molecular fingerprint analysis demonstrated that *R. homothallicus* isolates related to COVID-19 associated mucormycosis were distinct from R. *arrhizus* and *R. microsporus,* but were non-clonal and epidemiologically unrelated to each other. *R. homothallicus* mucormycosis had a distinct clinical presentation compared to that due to *R. arrhizus* and was associated with higher mortality rates compared to *R. arrhizus*.

The presence of *CAM* and uncontrolled diabetes was significantly higher among patients with *R. arrhizus* infection (p: 0.000, each). Out of the 49 patients with *R. homothallicus* infection who had diabetes, 18 (36.7%) were controlled diabetics compared to 1/42 (2.21%) in case of *R. arrhizus* (P value <0.001). *R. homothallicus* infection was significantly

associated with the presence of fever (20.4% vs. 5.5%, p: 0.024) and visual disturbances (40.7% vs. 12.7%; P value< 0.01). All patients in both groups underwent surgical debridement and amphotericin-B therapy was instituted to all patients with *R. arrhizus* and 81.5% patients with *R. homothallicus* infection. The mortality was significantly higher among cases infected with *R. homothallicus* vs. *R. arrhizus* cases (22.2% vs. 1.8%, P value< 0.01).

2. Fungaemia due to rare yeasts in paediatric intensive care units: A prospective study

We have been witnessing an increasing incidence of *C. utilis* and *W. anomalus* fungemia in neonates from the last few years. We undertook a prospective observational study to explore the epidemiological features and clinical characteristics of fungaemia due to rare yeasts in paediatric ICUs (PICUs) at our centre. The successive yeasts isolated from blood culture (BACTEC 9240) from patients admitted at our PICUs during December 2017 through March 2019 were identified by molecular method. Fungaemia due to yeasts other than *C. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. parapsilosis* was categorized as rare yeast fungaemia.

The rare-yeast fungemia comprised *C. utilis* and *W. anomalus* as majority. Among the risk factors, surgical intervention and gastrointestinal disease were significantly associated; overall, azole, echinocandin and amphotericin B resistance was at 9.1%, 1.02% and 1.02%, respectively; overall mortality was 65.3%.

A total of 212 yeast isolates from 159 patients in paediatric ICUs of our hospital were obtained during the study period; 127 isolates from 98 patients were considered rare yeasts and 85 isolates from 61 patients as common yeasts.. The overall rate of fungaemia due to rare yeast was 36.9 cases per 1000 ICU admission. The surgical intervention (86% vs. 58.8%; p=0.000) and gastrointestinal disease (75% vs. 55.9%, p=0.012) were significantly associated with fungaemia due to rare yeasts, while neutropenia and sepsis syndrome were significantly higher in common yeast species. Though the overall mortality was high at 65.3% with rare yeast fungaemia, there was no significant difference in mortality between fungaemia cases due to common (62.3%) and rare yeasts (65.3%).

Chapter 6 Typhoidal Salmonella

Summary of the results

Typhoid fever continues to remain an important cause of morbidity and mortality in developing countries and compounded by the emerging resistance in the two common causative agents *Salmonella* Typhi (S. Typhi) and *Salmonella* Paratyphi (S. Paratyphi A). It accounts for an estimated 10.9 million infections and total 116,800 deaths per year globally and South Asian region has the maximum disease burden, with a pooled estimate of 377 cases per one lakh people in India.

Accurate diagnosis followed by appropriate antibiotic treatment is the mainstay of treatment. But diagnosis is also complicated as all the symptoms are non specific and overlap with other febrile illnesses. Commonly the presentation is also modified by some antibiotics given by the primary healthcare physicians in the community. Antimicrobial choice for the treatment is empirical. Culture and susceptibility results are key to decide the treatment. The epidemiology of antibiotic resistance in *S*. Typhi shows how introduction of antibiotics induce stepwise acquisition of resistance with use of antibiotics. Initial reports of MDR *S.* Typhi (strain resistant to chloramphenicol, ampicillin, co-trimoxazole) resulted in fluoroquinolones as first line drugs with subsequentl emergence of ciprofloxacin (FQ) non susceptible *S.* Typhi. Currently, the third-generation cephalosporin and azithromycin are the available treatment option for MDR and FQ resistant typhoid fever, but the recent outbreaks of XDR (extensively drug resistant) strains in Asian countries is alarming as the spread of XDR is a possibility.The emergence of extended drug resistant typhoidal *Salmonella*e is becoming global threat and need continuous surveillance and attention to prevent their spread as the geographical boundaries are no longer a limiting factor in travelling and dissemination of drug resistant isolates.

S. Typhi is the most common etiological agent for typhoid fever followed by *Salmonella* Paratyphi A (S. Paratyphi A) in India. The antimicrobial resistance surveillance study in typhoid fever conducted by ICMR-AMR Network to carry out the national surveillance of yearly isolation of *S.* Typhi from five geographical regions of India i.e. North, Central, East, West and South India respectively. In 2017 maximum isolation of *S.* Typhi was from West India (4.8%) followed by South (4%) and North India (3.2%). There was no isolation of S. Typhi from East and Central India. While in 2018, isolation was maximum from Central India (10.9%) followed by West (5.7%) and North India (4.7%). Total isolation from South was (3.4%) followed by only 0.3% isolation from East India. Total isolation of *S.* Typhi in 2019 was maximum from west (5.9%) from Central (5.4%) and from South (4.3%) followed by isolation from East (0.3%) only. In 2020 -21 due to COVID-19 pandemic the lock downs were responsible for almost no visit of patients from

community to the hospitals and therefore there was minimal documentation of any other infectious or non infectious diseases in the country. This also affected the typhoid fever diagnosis. This time the isolation of *S. Typhi* from west was 6.2 % and south 3.6% followed by central and North India. In 2021, overall isolation of *S.* Typhi was only 1.6%, with central India being 2.5% followed by North India 2%. Nationally, isolation was 3.6% in 2017 which increased to 4.1% in 2018 and 4.2% in 2019 and 4.3% in 2020 but it decreased to 1.6% in 2021.

Clinical relevance of the study

To summarize, total 351 typhoidal *Salmonella*e were reported online. Out of the total culture positive cases, 293 were *S.* Typhi and 58 were *S.*Paratyphi A. In case of *S*. Typhi, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole sensitivity was 96% while it was observed 97%, 95% and 98% respectively in *S*. Paratyphi A. Cephalosporins and azithromycin were 100% sensitive in *S.* Typhi. Ciprofloxacin sensitivity was 20% as compared to pefloxacin which was noted 35% in *S.* Typhi while only 9% sensitivity was observed in *S*. Paratyphi A (Table 6.1). This discordance between ciprofloxacin and pefloxacin was not observed when we tested the isolates sent by regional centers to our Nodal Center. The reason could be due to not all the isolates being transported to our center and secondly could be due to disk variation when comparing oxoid versus Himedia disks for pefloxacin.

S. Typhi

The antimicrobial susceptibility data of *S*. Typhi from blood has been presented in table 6.2. The data shows that sensitivity for ampicillin in *S.* Typhi from South region was 95.58% (107/112), 96% (120/125) from North, 100% (40/40) from West and 95.9% (278/290) were susceptible from all over India. Trimethoprim-sulfamethoxazole susceptibility was 100% (40/40) from West, 96.3% (103/107) from South, 93.3% (111/119) from North and 95.7% (266/278) across India. Antimicrobial susceptibility for Chloramphenicol was 100% (35/35) from West region while it was 97.9% (94/96) from South and 94.1% (111/118) from North region. Overall, 4% MDR were reported nationally. Geographically in different region of India, cephalosporin's have different sensitivity pattern. Resistance to these drugs has been started to appear in India as well. Ceftriaxone was 99.6% (280/281) across the India except one resistant strain was reported from south. All S. Typhi isolated from North and west region were 100% susceptible while only one strain was cephalosporin resistant from pan India. Azithromycin susceptibility was 100% from North region, West region, central region, and east region while from South; one azithromycin resistant has been reported. Ciprofloxacin susceptibility was 8.5% (8/94) from South, 5.7% (2/35) from West was reported. Ciprofloxacin susceptibility from pan India was 19.7% (40/204). Pefloxacin

susceptibility was 40.4% (23/57) from South and 34.5% (29/84) from all over India was observed.

Table 6.1: Susceptibility pattern of *Salmonella* species from blood

AMA	*S. Typhi* n=293	*Salmonella Paratyphi A* n=58
Ampicillin	278/290 (95.9%)	55/57 (96.5%)
Azithromycin	212/213 (99.5%)	*0/0
Cefixime	209/212 (98.6%)	45/45 (100%)
Ceftriaxone	280/281 (99.6%)	57/57 (100%)
Chloramphenicol	246/257 (95.7%)	54/57 (94.7%)
Ciprofloxacin	40/204 (19.6%)	4/46 (8.7%)
Levofloxacin	9/30 (30%)	*0/8 (-)
Ofloxacin	*0/4 (-)	*0/2 (-)
Pefloxacin	29/84 (34.5%)	0/22 (0%)
Trimethoprim-sulfamethoxazole	266/278 (95.7%)	54/55 (98.2%)

*Azithromycin sensitivity cutoff values are not given in CLSI for *Salmonella Paratyphi A*

Table 6.2: Susceptibility pattern of *S.* Typhi from Blood across different regions of India

	National (n=293)	North (n=126)	South (n=113)	West (n=41)	Central (n=12)	East (n=1)
Ceftriaxone	280/281 (99.6)	126/126 (100)	109/110 (99.1)	34/34 (100)	10/10 (-)	1/1 (-)
Azithromycin	212/213 (99.5)	89/89 (100)	85/86 (98.8)	30/30 (100)	7/7 (-)	1/1 (-)
Cefixime	209/212 (98.6)	121/121 (100)	69/71 (97.2)	9/9 (-)	9/10 (-)	1/1 (-)
Ampicillin	278/290 (95.9)	120/125 (96)	107/112 (95.5)	40/40 (100)	11/12 (-)	0/1 (-)
Chloramphenicol	246/257 (95.7)	111/118 (94.1)	94/96 (97.9)	35/35 (100)	5/7 (-)	1/1 (-)
Trimethoprim-sulfamethoxazole	266/278 (95.7)	111/119 (93.3)	103/107 (96.3)	40/40 (100)	11/11 (-)	1/1 (-)
Pefloxacin	29/84 (34.5)	3/21 (14.3)	23/57 (40.4)	3/5 (-)	0/1 (-)	0/0 (-)
Levofloxacin	9/30 (30)	8/24 (33.3)	0/0 (-)	0/0 (-)	1/6 (-)	0/0 (-)
Ciprofloxacin	40/204 (19.6)	30/66 (45.5)	8/94 (8.5)	2/35 (5.7)	0/8 (-)	0/1 (-)

Table 6.3: Yearly susceptibility trends of *S.* Typhi from Blood

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=37	Total n=345	Total n=580	Total n=728	Total n=206	Total n=293
	(S%)	(S%)	(S%)	(S%)	(S%)	(S%)
Ampicillin	34/37 (91.9)	305/332 (91.9)	551/576 (95.7)	658/703 (93.6)	192/197 (97.5)	278/290 (95.9%)
Ceftriaxone	37/37 (100)	329/334 (98.5)	531/541 (98.2)	645/658 (98)	192/193 (99.5)	280/281 (99.6)
Cefixime	*15/15	221/223 (99.1)	344/349 (98.6)	434/448 (96.9)	157/158 (99.4)	209/212 (98.6)
Azithromycin	24/24 (100)	266/278 (95.7)	497/506 (98.2)	547/568 (96.3)	163/166 (98.2)	212/213 (99.5)
Ciprofloxacin	6/33 (18.2)	35/302 (11.6)	29/440 (6.6)	35/501 (7)	8/162 (4.9)	40/204 (19.6)
Levofloxacin	*0/0	*0/3	*5/18	3/35 (8.6)	*4/12	9/30 (30)
Trimethoprim-sulfamethoxazole	34/37 (91.9)	322/341 (94.4)	552/575 (96)	693/718 (96.5)	194/202 (96)	266/278 (95.7)
Chloramphenicol	31/34 (91.2)	267/278 (96)	541/560 (96.6)	582/611 (95.3)	180/185 (97.3)	246/257 (95.7)

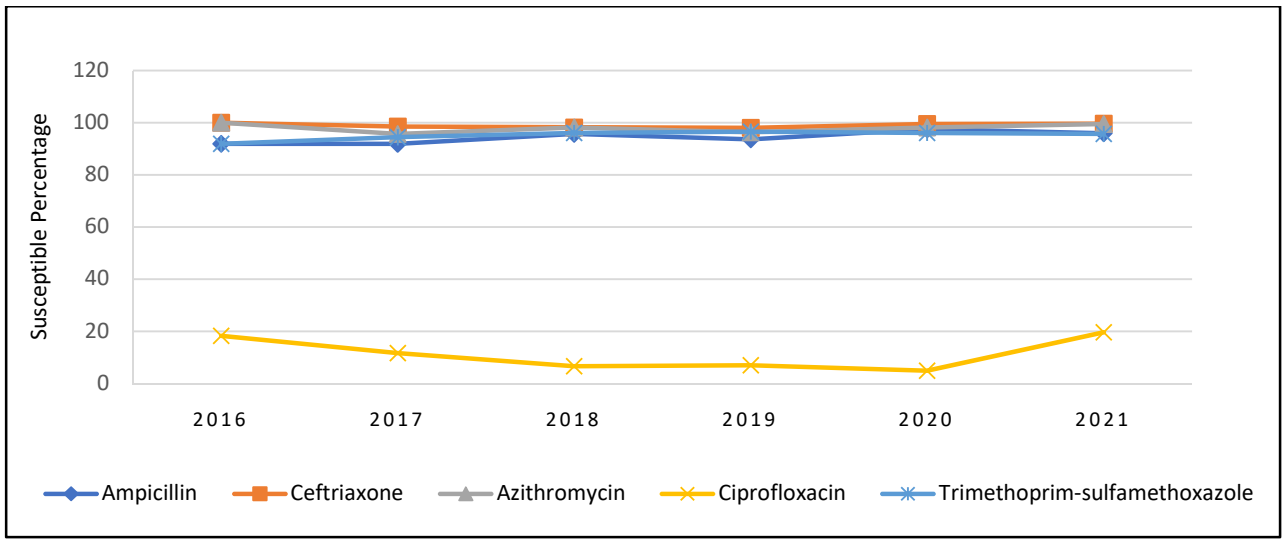


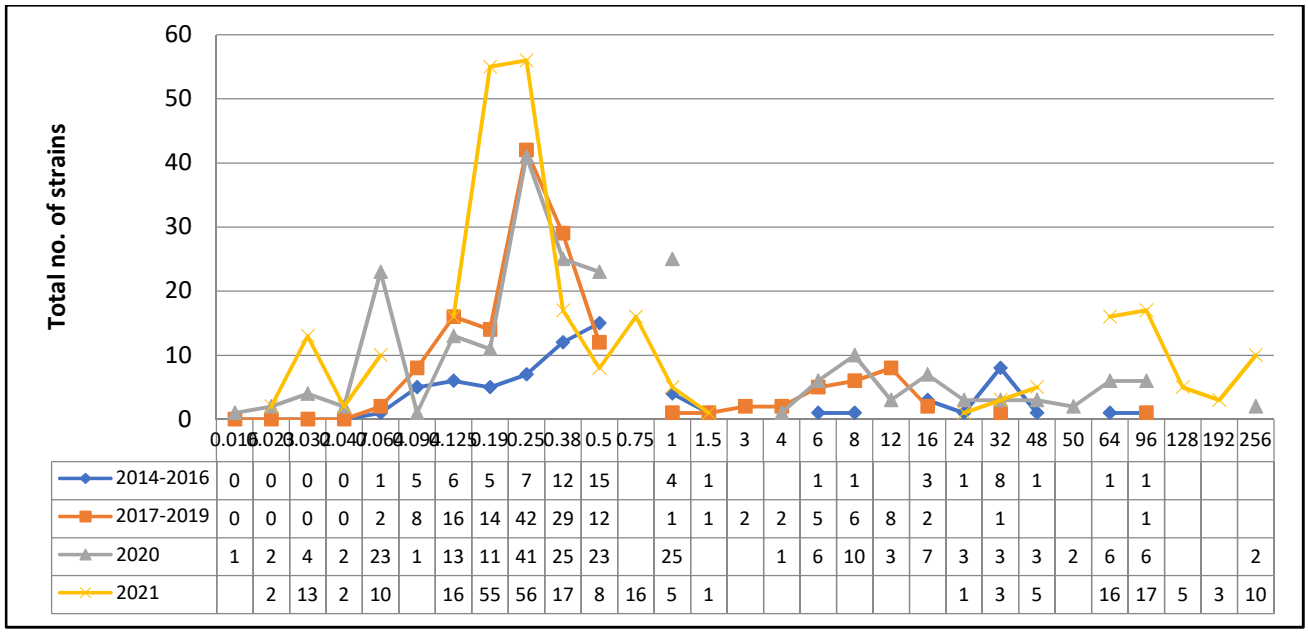
Figure 6.1: Yearly susceptibility trends of *S.* Typhi from Blood

Antimicrobial susceptibility for ampicillin in *S.* Typhi has increased from 91.9% (34/37) (305/332) in 2016 and 2017 respectively to 95.7% (551/576) in 2018 and decreases to 93.6% (658/703) in 2019 followed by an increase up to 97.5% (192/197) in 2020 while it was reported 95.9% (278/290) in 2021 (Table 6.3 and Figure 6.1). Chloramphenicol susceptibility has increased during the studied period. It was noted as 91% (31/34) in

2016 with an increase of 5% it reaches up-to 96% in 2017 and 97% in 2020 followed by a slight decrease in 2021, where susceptibility was reported 95.7% (246/257). Trimethoprim-sulfamethoxazole susceptibility was 91.9% (34/37) in 2016 and 94.4% (322/341) in 2017 and reached up to 96% (552/575) in 2018 followed by 96.5% (693/718) in 2019 and 96% (194/202), 95.7% (266/278) in 2020 and 2021 respectively. Ceftriaxone and cefixime susceptibility were also almost equal during the studied period. It was 100% in 2016 followed by 98.5% (329/334) in 2017 and 98.1% (531/541) in 2018 followed by 98% (645/658) in 2019 and 99%, 99.5% in 2020 and 2021 respectively. Ciprofloxacin sensitivity has decreased from 18.2% (6/33) in 2016 to 11.6% (35/302) in 2017 to 6.6% (29/440) in 2018 and again increased in 2019 to 7.2% (35/501) followed by 5% (8/162) in 2020. Ciprofloxacin sensitivity has been increased in 2021 from 5 to 19.7% (40/204). Levofloxacin sensitivity was 9% (3/35) in 2019 followed by 6% in 2020 while 30% susceptibility was observed in 2021. Azithromycin susceptibility was reported 100 in 2016 followed by 95.7% (266/278) in 2017, 98.4% (497/506) in 2018, 96.3% (547/568) in 2019 and 98% (163/166) in 2020 followed by 95.5% in 2021.

To study ciprofloxacin MIC trend, 6-year time has been grouped into two groups of three year each (2014-2016 and 2017-2019) while 2020 and 2021 has been added as single year (Fig 6.2). The minimum MIC value (0.016 µg/ml to 0.047 µg/ml) was not reported from 2014 to 2019 but reported in the strains isolated in 2020. The maximum MIC range (256 µg/ml) was also reported in 2020 and 2021. Total no. of strains showing higher MIC has increased in 2021.

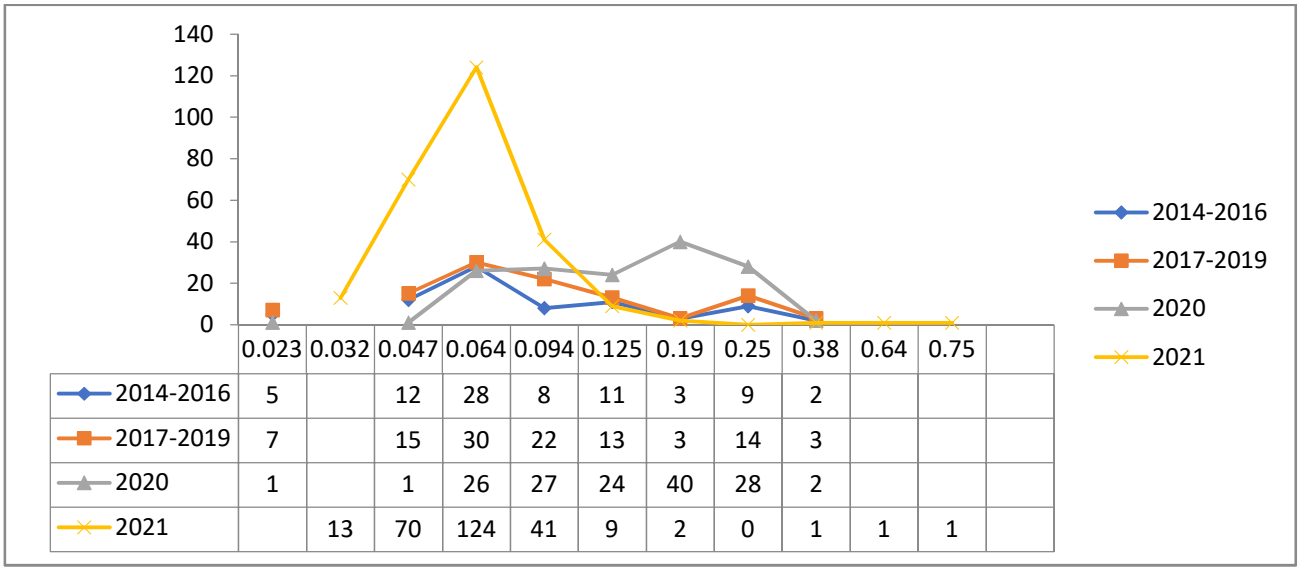
Although maximum number of *S.* Typhi 45/77 (58%) show intermediate sensitivity against ciprofloxacin in 2014-2016 and 113/160 (71%) in 2017-2019 these were considered as resistant which makes total ciprofloxacin resistance 92% (71/77) in 2014-2016 and 93% (149/160) in 2017-2019 and 98.4% (191/194) in 2020 in typhoidal *Salmonella*. In 2021, 168/263 (63.8%) isolates were intermediate and 66/263 (25%) were resistant. Total ciprofloxacin resistance was 234/263 (88.9%) in 2021. During this period, ciprofloxacin susceptibility has increased up to 10%.



	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	3	4	6	8	12	16	24	32	48	50	64	96	128	192	256
2014-2016	0	0	0	0	1	5	6	5	7	12	15		4	1			1	1		3	1	8	1		1	1			
2017-2019	0	0	0	0	2	8	16	14	42	29	12		1	1	2	2	5	6	8	2		1				1			
2020	1	2	4	2	23	1	13	11	41	25	23		25			1	6	10	3	7	3	3	3	2	6	6			2
2021		2	13	2	10		16	55	56	17	8	16	5	1							1	3	5		16	17	5	3	10

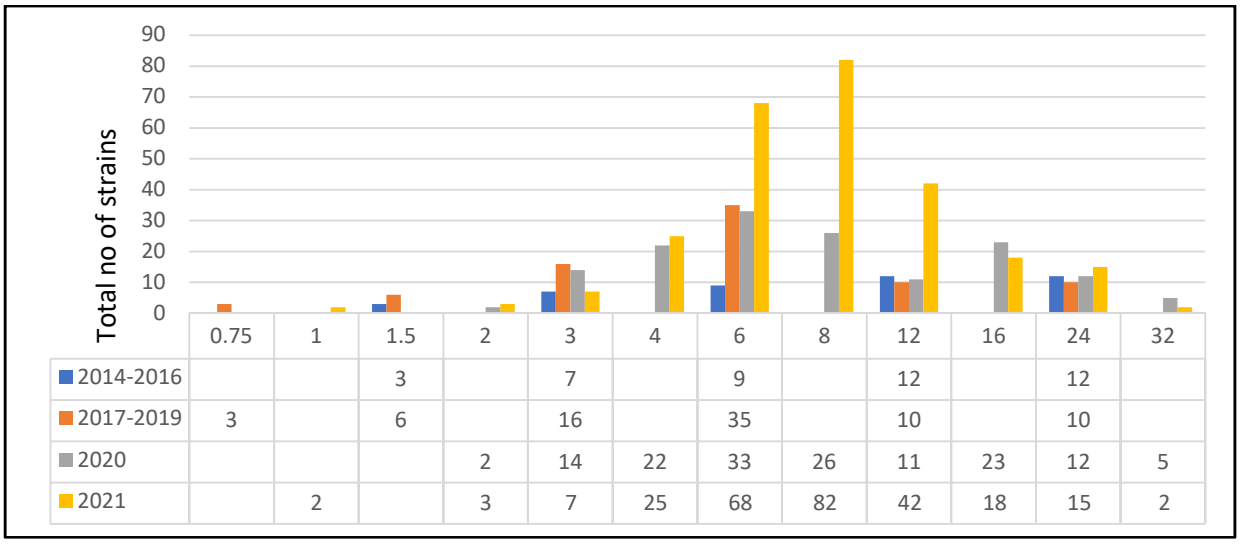
Fig 6.2: Ciprofloxacin MIC trends at AIIMS, New Delhi over a period of eight years

Ceftriaxone MIC shows creeping trend over the year. Maximum range of MIC was 0.38% during 2014-2016, 2017-2019 and 2020 but during 2021 0.64 µg/ml and 0.75 µg/ml has been reported in 1-1 isolate (Fig 6.3). Although maximum numbers of strains have MIC range from 3 µg/ ml to 16 µg/ ml, strains with higher MIC also have started to appear (Fig 6.4).



	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.64	0.75	
2014-2016	5		12	28	8	11	3	9	2			
2017-2019	7		15	30	22	13	3	14	3			
2020	1		1	26	27	24	40	28	2			
2021		13	70	124	41	9	2	0	1	1	1	

Fig 6.3: Comparison of creeping MIC for Ceftriaxone in *S.* Typhi over a period of eight years at AIIMS, New Delhi



	0.75	1	1.5	2	3	4	6	8	12	16	24	32
2014-2016			3		7		9		12		12	
2017-2019	3		6		16		35		10		10	
2020				2	14	22	33	26	11	23	12	5
2021		2		3	7	25	68	82	42	18	15	2

Fig 6.4: Comparison of Azithromycin MIC in *S.* Typhi over a period of six years at AIIMS

Salmonella Paratyphi A

S. Paratyphi A antibiotic susceptibility pattern from 2017 to 2021 shows that ampicillin was 95% (38/40) sensitive in 2017 and 97.6% (122/125) in 2018. There was an increase in ampicillin resistance in 2019 to 2021 as total sensitivity was 90.6% (125/138) less than previous years in 2019 and 91.3% in 2020 followed by 96.5% (55/57) in 2021 (Table 6.4 and Figure 6.5). Chloramphenicol and trimethoprim - sulfamethoxazole was 100% sensitive in 2017 and 2018 but decreased to 99.3% susceptibility in 2019 followed by 95.8% in 2020 and 98.1% in 2021 for trimethoprim - sulfamethoxazole. Ciprofloxacin sensitivity has decreased from 2017 to 2021 as it was 10% (4/40) in 2017 and only 1% in 2018 and 2019 but due to the smaller number of isolates it increased to 3.2% (1/31) in 2020 as only one isolate was sensitive to ciprofloxacin followed by 8.7% (4/46) in 2021. Ceftriaxone antimicrobial susceptibility has increased from 95% (38/40) in 2017 to 97.6% (122/125) in 2018 and 97.9% (139/142) in 2019 and reached up to 100% by 2020 and 2021. Cefixime was 96.3% (26/27) susceptible in 2017 followed by 100% (105/105) in 2018, 98.1% (105/107) in 2019 and again 100% (31/31) in 2020 and 2021 respectively. Azithromycin was not analysed as azithromycin susceptibility cutoff for *S.*Paratyphi A are not given in CLSI. Ciprofloxacin susceptibility has decreased up-to 0.9% during 2018 and 2019, while it has increased up-to 8.7% in 2021.

Among *S.*Paratyphi A, maximum no. of isolates has intermediate MIC for ciprofloxacin (Figure 6.6). But isolates with higher MIC have also been reported in 2021. Only one isolate was ciprofloxacin sensitive reported from Vellore. In comparison to 2020, strains with lower range of MIC have increased in 2021 followed by a decrease in maximum range of MIC which was 0.19 µg/ ml in 2021 as compare to 0.5 µg/ ml reported in 2020.

Table 6.4: Yearly susceptibility trends of *S.*Paratyphi A from Blood

AMA	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=41 (S%)	Total n=125 (S%)	Total n=147 (S%)	Total n=52 (S%)	Total n=58 (S%)
Ampicillin	38/40 (95)	122/125 (97.6)	125/138 (90.6)	42/46 (91.3)	55/57 (96.5%)
Ceftriaxone	38/40 (95)	121/124 (97.6)	139/142 (97.9)	47/47 (100)	57/57 (100%)
Cefixime	26/27 (96.3)	105/105 (100)	105/107 (98.1)	32/32 (100)	45/45 (100%)
Ciprofloxacin	4/40 (10)	1/111 (0.9)	1/86 (1.2)	1/31 (3.2)	4/46 (8.7%)
Levofloxacin	*0/2	*0/5	0/25 (0)	*0/9	*0/8
Trimethoprim-sulfamethoxazole	41/41 (100)	123/123 (100)	144/145 (99.3)	47/49 (95.9)	54/55 (98.2%)
Chloramphenicol	30/30 (100)	121/121 (100)	128/128 (100)	48/49 (98)	54/57 (94.7%)

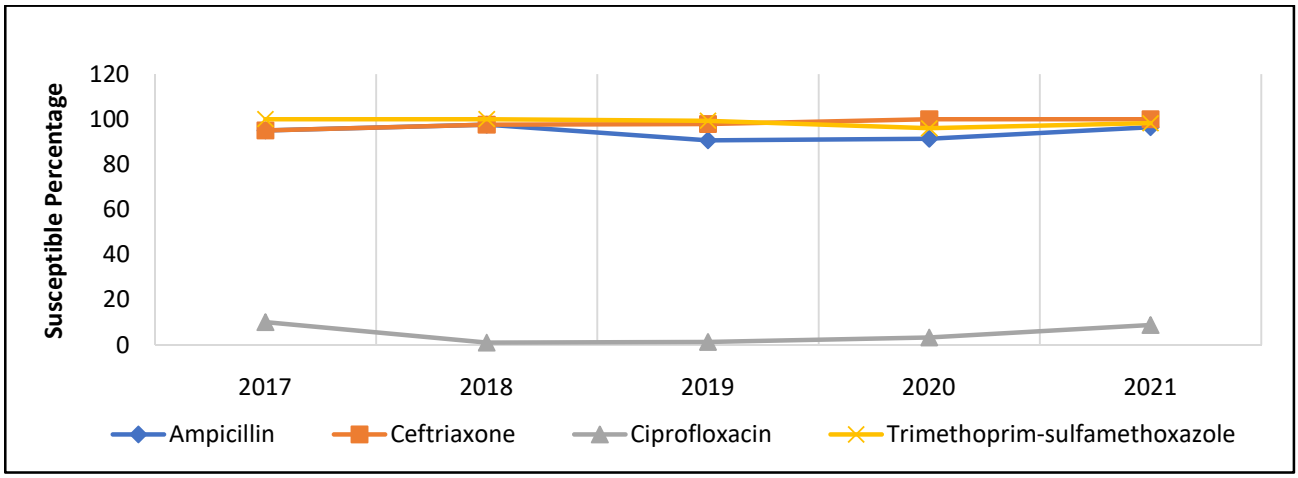


Figure 6.5: Yearly susceptibility trends of *S.* Paratyphi A from Blood

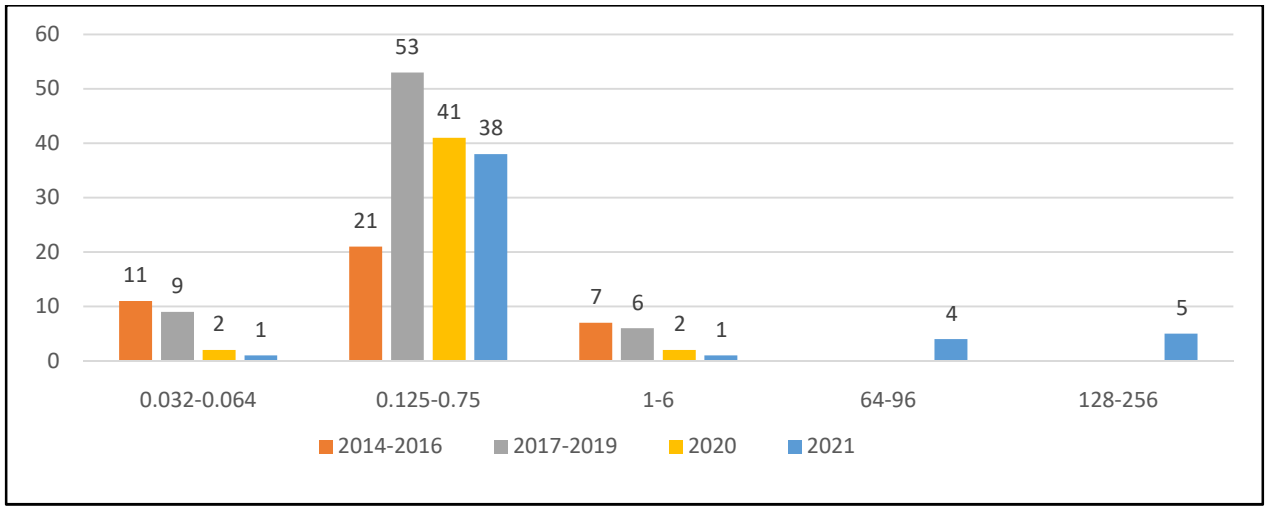


Fig 6.6: Ciprofloxacin MIC trends in *S.* Paratyphi A at AIIMS, New Delhi over a period of eight years

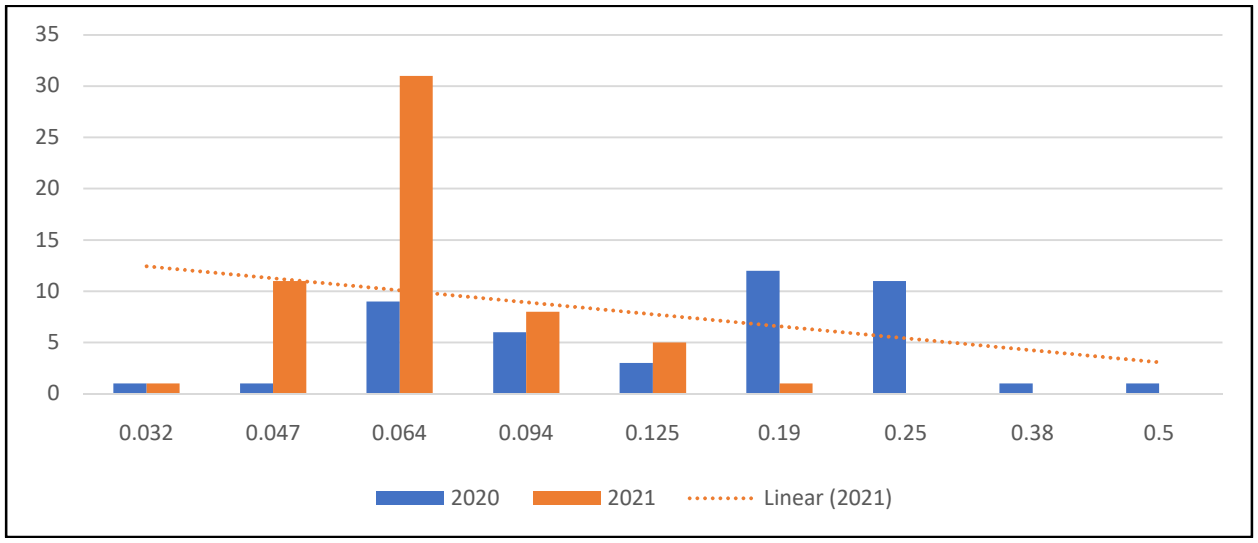


Fig 6.7: Comparison of creeping MIC for Ceftriaxone in *S.* Paratyphi A during 2020-2021 at AIIMS, New Delhi

Molecular data and its relevance

We looked for resistance genes and their phenotypic correlation for typhoidal isolates to understand the resistance mechanism at molecular level. For this study 25% of all representative samples from all centers were selected.Total 125 isolates were finalised for WGS (whole genome sequencing) which includes 89 *S.* Typhi, 27 *S.*Paratyphi A and 6 *Salmonella* Species. First strains were selected on the basis of AMR pattern with following antibiotic resistance: Azithromycin, Ceftriaxone, first line drug (Chloramphenicol,

Ampicillin and Co-trimoxazole) and Ciprofloxacin. Of the remaining strains were selected to complete 25% by selecting month-wise and batch wise.

Ampicillin Resistance

In *S.* Typhi the ampicillin resistance is associated with the presence of beta-lactam genes which were observed in 16% strains (14/88) by WGS. In all the strains blaTEM-1D beta-lactam resistance gene was observed. The resistance genes encode for the predominant plasmid-mediated β-lactamases of Enterobacteriaceae. Earlier reports from pan India for ampicillin resistance was 2%.

In case of *S.*Paratyphi A, phenotypically two strains were intermediate and one was resistant but none of them was positive for blaTEM-1D, though TEM-185, TEM-229 was detected in one sensitive isolate. Other genes responsible for resistance rsmA, sdi A and marA were present.

Chloramphenicol resistance

Chloramphenicol resistance determinants were observed in 17% (15/88) *S.* Typhi strains by WGS. Out of all resistant strains 5 strains harbored catA1 gene which encodes chloramphenicol acetyltransferase enzyme causing chloramphenicol resistance by chemical modification of the drug molecule, whereas ten isolates harboured the catI genes. Chloramphenicol resistance was exhibited by two strains of S. Paratyphi A. Both strains harboured catA1 gene.

Co-trimoxazole resistance

Out of 88 strains, trimethoprim resistance determining genes were found in 16% isolates (15/88). Likewise, gene sul1 and sul2, encoding dihydropteroate synthases known to disseminate sulfamethoxazole resistance, were also detected in 16% isolates (15/88).

Fluoroquinolones resistance

Molecular determinants of resistance to fluoroquinolone including ciprofloxacin, levofloxacin and ofloxacin antibiotics encoded by gyrA and parC genes were detected in 97% of *S.* Typhi strains (85/88) by WGS. Out of these 85 S. Typhi strains, double mutations in gyrA and parC genes, were observed in 9 strains with MIC range of 0.25-96 mg/L followed by only gyrA mutation at gyr A S83F in 28strains with MIC range between 0.125-0.38 mg/L. While triple mutations were observed in 48 strains with MIC range between 6-256 mg/L **Table 6.5**. All the strains with triple mutations were ciprofloxacin resistant with higher MIC and had double mutation in gyrA gene and single mutation in parC gene. Out of these, one had mutations at gyrA S83F, D87N and parC D420N (MIC 0.75mg/L) while rest of the 47 strains had mutation at gyrA S83F, D87N and parCat S80I (MIC 6-256mg/L). The

identified genes were associated with mutations in Quinolone Resistance Determining Region (QRDR) of DNA gyrase enzyme, the binding site for fluoroquinolone. Antimicrobial resistance to fluoroquinolones was 90% (79/88) by both disc diffusion and E-test method. Single mutation at S83F has been detected in six sensitive isolates (MIC 0.064 and 0.047mg/L). MIC distribution ranged between 2–256 mg/L and peaked at 12 mg/L. DNA Gyrase A mutations at position 83 (Ser-83→Phe, Ser-83→Tyr and Asp 87→ Phe) are the most prevalent resistance mechanisms for fluoroquinolone in India, followed by Ser-80→Ile substitution in parC gene. Ciprofloxacin resistant strains (with ciprofloxacin MIC >6 mg/L) were found to be double or triple mutants with mutations in gyrA83, gyrA87 and parC80. Strains with intermediate resistance to ciprofloxacin possessed single or double mutations in DNA gyrA gene and parC gene at Ser83 and Ser80 position. Presence of single mutation in six sensitive isolates without expression is of concern as mutation at Ser83 is an important site for conferring partial fluoroquinolone resistance in *S*. Typhi and *S.* Paratyphi A while complete ciprofloxacin resistance requires double mutations in the QRDR of GyrA gene. Additional mutation in the parC gene at S80I and D420N is responsible for higher MIC.

In case of *S.*Paratyphi A, phenotypically 29 strains were intermediate and 2 were resistant to fluoroquinolone. Mutations in gyrA genes were detected in 84% (26/31) of the strains. Out of these 26 strains, 3 strains had double mutations and 23 had single mutations in gyrA gene at S83F. Double mutation in gyrAS83F and D87N with MIC 0.75mg/L were observed in one strain while double mutation in gyrA S83F and parC S80I gene with MIC 0.75 mg/L and 0.5mg/L were detected in 2 isolates (Table 6.6). Center-wise data has been presented in table 6.7. No mutations were detected in gyrA and parC genes in 5 strains. Other fluoroquinolone resistance mechanisms CRP, acrR, marR, soxR, acrB, emrA, emrB, mdtk and rsmA were also present.

Cephalosporins resistance

Although all the strains were cephalosporin sensitive but the presence of CTX-M-117 and CTX-M-37 was detected in two strains which could only be the presence of the gene.Mutations in PBP3 gene at D350N, S357N, Escherichia coli ampC1 beta-lactamase, and Escherichia coli ampH beta-lactamase gene was present in all tested isolates. This clearly raises an alarm towards the judicial use of these antibiotics. Antimicrobial susceptibility to antibiotics, cefixime and ceftriaxone, observed for all strains is consistent with other studies from India. Though all the strains were susceptible, however, a gradual increase in median MIC values was perceived over a time period.

Azithromycin resistance

Although the majority of the isolates were azithromycin susceptible and only 1% resistance was observed by phenotypic methods. ErmC gene was present in 7 *S. Typhi* isolates.

Azithromycin cutoff value is not provided in CLSI for *S.*Paratyphi A but mutation in acrB R717Q was observed in one strain. Other genes responsible for macrolide resistance nalD, KpnE, CRP were also observed by WGS.

Genotypic resistance to other antimicrobial agents

S. Typhi can demonstrate resistance to multiple antibiotics by acquiring new resistance genes through horizontal gene transfer (HGT). The acquired antimicrobial resistance genes including aac(6')-Iaa, AAC(6')-Iy, aadA1, aph(3")-Ib, aph(6)-Id, strA, and strB that provided resistance to aminoglycosides were observed in 100% (89/89) isolates (Table 11). In addition, *S.* Typhi isolates harboured the genes baeR, emrb, H-NS, marA, mdfA, mdtK, msbA, acrA, emrR, kpnE, kpnF, marR, sdiA, crp, soxR, and soxS that could confer multidrug resistance and were detected in all 133 strains. The mds ABC complex, a multidrug transporter of *Salmonella*, comprising mdsA, mdsB, and mdsC units was also observed in all isolates. The mdsABC complex is recognized to contribute resistance against a diverse set of drugs and toxins. The identified multi-efflux pump mdtK gene, conferring resistance against the drugs, acriflavine, doxorubicin and norfloxacin, was observed in 100% (88/88) of the isolates. The gene, sdiA, a multi-drug resistance pump regulator for AcrB, was also present in 100% (88/88) of the isolates. The significance of the presence of these genes is still not very clear and needs to be monitored.

MLST

On the basis of MLST all *S.* Typhi strains subjected to MLST showed monophyletic lineage and clustered into 2 Sequence Types—ST1 and ST2. Out of a total 87 *S.* Typhi, 90% were grouped into ST1.*S.*Paratyphi A was grouped in ST85 and ST129.

Summary

Gene sequencing for understanding the antimicrobial resistance mechanisms and epidemiology was done in a selected strain collection from different regional areas. Overall, there was concordance of the presence of resistance imparting genes or their mutations and the phenotypic antimicrobial susceptibility pattern. In a small percentage the susceptible strains did carry genes for resistance but were not expressed especially for chloramphenicol and cotrimoxazole. There was no ceftriaxone resistance and also no $CTXM_{-15}$ gene detected in any strain. Azithromycin resistance genes were also not detected as all the studied isolates were susceptible.

Fluorquinolone resistance mechanisms and genes showed a varied distribution. Predominantly, mutation in gyrA at S83F was the most common resistance mechanism and accounted for more than 90% of all mutations. T strains with intermediate ciprofloxacin susceptibility had single mutation at gyrA S83F or double mutation at gyrA S83F and parC S80I of the QRDR. While strains with higher ciprofloxacin MIC had triple mutation at gyrA

at S83F, D87N and parC S80I or parE D420N. Mutation in gyrB at S464F in one ciprofloxacin sensitive (MIC 0.064µg/ml) isolate was detected. One isolate was ciprofloxacin susceptible phenotypically but had a mutation in gyrA S83F or gyrB S464F. Mutation in parE gene at L416F, D420N also was found in one isolate each with ciprofloxacin intermediate and resistant MIC. Therefore not all showed an association of genetic mutations and phenotypic resistance - w supporting the fact that mere presence of gene may not be sufficient to impart clinical resistance and many factors may come into play including expression of gene and antibiotic selection pressure while the patient is on treatment. Region wise there was no significant difference in the distribution of mutation and antibiotic susceptibility pattern. However, the presence of resistance mutation in susceptible strains is a cause of concern because it can lead to their expression on exposure to fluoroquinolones and subsequent emergence of ciprofloxacin resistance. Therefore the genotypic studies and continuous surveillance of antimicrobial resistance is necessary to understand the mechanism and epidemiology of resistance emergence

Table 6.5. Mutations imparting resistance to ciprofloxacin in *S.* Typhi

S. Typhi Ciprofloxacin resistance													
			gyrA			gyrB	ParC		ParE		CIP* MIC	CIPDisk Diffusionzone (mm)	
S.No.	Lab ID / Centre Name	Mutation	S83 F	S83 Y	D87N	S464F	S80I	D420N	L416 F	D420N	µg/ml		
1	206032/ST/H	D*	NP*	P*	Np	Np	Np	NP	Np	P	0.25	27	I*
2	181361/ST/H	T*	P	Np	P	Np	P	Np	Np	Np	32	11	R*
3	216696/ST/H	T	P	Np	P	Np	P	Np	Np	Np	32	12	R
4	216697/ST/H	T	P	Np	P	Np	P	Np	Np	Np	48	12	R
5	224885/ST/H	T	P	Np	P	Np	P	Np	Np	Np	16	10	R
6	273726/ST/H	T	P	Np	P	Np	P	Np	Np	Np	8	16	R
7	275719/ST/H	T	P	Np	P	Np	P	Np	Np	Np	8	15	R
8	303034/ST/H	T	P	Np	P	Np	P	Np	Np	Np	64	14	R
9	306958/ST/H	S*	P	Np	Np	Np	Np	Np	Np	Np	0.125	28	I
10	331671/ST/H	S	P	Np	Np	Np	Np	Np	Np	Np	0.25	29	I
11	339337/ST/H	S	P	Np	Np	Np	Np	Np	Np	Np	0.75	27	I
12	136401/ST/H	T	P	Np	P	Np	P	Np	Np	Np	48	13	R
13	145476/ST/H	S	P	Np	Np	Np	Np	Np	Np	Np	0.125	28	I
14	113595/ST/H	S	P	Np	Np	Np	Np	Np	Np	Np	0.75	26	I
15	148587/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	32	15	R
16	148589/ST/SGR	T	P	Np	P	Np	Np	NP	p	Np	24	13	R
17	201709/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	12	6	R
18	201713/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	12	14	R
19	201720/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	12	6	R
20	243087/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	48	6	R
21	243095/ST/SGR	NP	Np	Np	Np	Np	Np	Np	Np	Np	0.016	40	S**
22	269039/ST/SGR	S	P	Np	Np	Np	Np	Np	Np	Np	0.064	35	S
23	269043/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	256	13	R
24	287544/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	12	15	R
25	18449/ST/SGR	S	P	Np	NP	Np	NP	Np	Np	Np	0.19	28	I
26	19272/ST/SGR	S	NP	P	NP	Np	NP	Np	Np	Np	0.19	28	I
27	287556/ST/SGR	S	NP	Np	Np	P	Np	Np	Np	Np	0.064	31	S
28	9406/ST/SGR	D	P	Np	Np	Np	P	Np	Np	Np	0.25	28	I
29	201663/ST/SGR	T	P	Np	p	Np	P	Np	Np	Np	16	14	R

30	215321/ST/AP	S	P	Np	Np	Np	NP	Np	Np	Np	0.064	33	S
31	212656/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	128	14	R
32	206471/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	192	15	R
33	215575/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	128	18	R
34	217743/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	128	16	R
35	220566/ST/AP	S	P	Np	Np	Np	NP	Np	Np	Np	0.064	31	S
36	227754/ST/AP	D	P	Np	Np	Np	P	Np	Np	Np	96	22	R
37	256728/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	256	16	R
38	274274/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	192	17	R
39	196286/ST/AP	T	P	Np	P	Np	P	Np	Np	Np	12	10	R
40	41399/ST/AP	D	P	Np	Np	Np	P	Np	Np	Np	0.25	30	I
41	267700/ST/JP	D	P	Np	Np	Np	P	Np	Np	Np	0.5	27	I
42	270468/ST/JP	S	P	Np	Np	Np	Np	Np	Np	Np	0.25	26	I
43	204247/ST/JP	S	P	Np	Np	Np	Np	Np	Np	Np	0.19	30	I
44	268986/ST/JP	T	P	Np	P	Np	P	Np	Np	Np	6	15	R
45	208362/ST/JP	T	P	Np	P	Np	P	Np	Np	Np	8	11	R
46	208465/ST/JP	D	P	Np	Np	Np	P	Np	Np	Np	0.5	25	I
47	208676/ST/JP	T	P	Np	P	Np	P	Np	Np	Np	8	15	R
48	208797/ST/JP	T	P	Np	P	Np	P	Np	Np	Np	12	14	R
49	209897/STJP	T	P	Np	P	Np	P	Np	Np	Np	12	10	R
50	211607/ST/JP	T	P	Np	P	Np	P	Np	Np	Np	12	10	R
51	192554/ST/NIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.38	27	I
52	202697/ST/NIMS	T	P	Np	P	Np	P	Np	Np	Np	12	10	R
53	202699/ST/NIMS	T	P	Np	P	Np	P	Np	Np	Np	16	12	R
54	202701/ST/NIMS	D	P	Np	Np	Np	P	Np	Np	Np	0.5	24	I
55	202709/ST/NIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.25	25	I
56	202713/ST/NIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.25	25	I
57	132937/ST/NIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.125	27	I
58	202706/ST/NIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.38	27	I
59	202691/ST/NIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.064	31	S
60	182562/ST/MGIMS	T	P	Np	P	Np	P	Np	Np	Np	0.75	27	I
61	206906/ST/MGIMS	T	P	Np	P	Np	P	Np	Np	Np	16	16	R
62	206931/ST/MGIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.047	31	S
63	206946/ST/MGIMS	T	P	Np	P	Np	P	Np	Np	Np	12	17	R
64	216018/ST/MGIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.25	28	I
65	243138/ST/MGIMS	T	P	Np	P	Np	P	Np	Np	Np	6	14	R
66	190972/ST/MGIMS	T	P	Np	P	Np	P	Np	Np	Np	16	17	R

67	184024/ST/MGIMS	NP	Np	Np	Np	Np	Np	Np	Np	Np	0.016	37	S
68	224739/ST/MGIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.25	29	I
69	237574/ST/MGIMS	T	P	Np	P	Np	P	Np	Np	Np	6	14	R
70	235644/ST/KMC	T	p	Np	p	Np	P	Np	Np	Np	12	15	R
71	241578/ST/KMC	T	p	Np	p	Np	P	Np	Np	Np	32	15	R
72	241756/ST/KMC	S	p	Np	Np	Np	NP	Np	Np	Np	0.75	28	I
73	299245/ST/KMC	T	p	Np	p	Np	P	Np	Np	Np	96	11	R
74	124/ST/KMC	NP	NP	Np	Np	Np	NP	Np	Np	Np	0.032	34	S
75	4973/STA/SKIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.75	26	I
76	4795/STA/SKIMS	T	P	Np	P	Np	P	Np	Np	Np	16	10	R
77	106158/ST/SKIMS	S	Np	Np	P	Np	Np	Np	Np	Np	0.19	30	I
78	106871/STA/SKIMS	S	P	Np	Np	Np	Np	Np	Np	Np	0.19	29	I
79	174640/ST/AIIMS J	D	P	Np	Np	Np	P	Np	Np	Np	0.38	30	I
80	175081/ST/AIIMS J	T	P	Np	P	Np	P	Np	Np	Np	12	6	R
81	240974/ST/AIIMS J	T	P	Np	P	Np	P	Np	Np	Np	96	10	R
82	190042/ST/AIIMS J	S	P	Np	Np	Np	Np	NP	Np	P	0.38	26	I
83	202673/ST/TMC	S	p	Np	Np	Np	Np	Np	Np	Np	0.75	29	I
84	9893/ST/AIIMS ND	T	P	Np	P	Np	P	Np	Np	Np	96	14	R
85	9927/ST/AIIMS ND	D	P	Np	Np	Np	Np	P	Np	Np	0.5	28	I
86	124002/ST/AIIMS BH	T	P	Np	P	Np	P	Np	Np	Np	96	17	R
87	158960/ST/RIMS	T	P	Np	P	Np	P	Np	Np	Np	12	9	R
88	10874/ST/SGR	T	P	Np	P	Np	P	Np	Np	Np	16	14	R

H-Hinduja, Mumbai; **SGR-**Sir Gangaram, New Delhi; **AP**-Apollo Chennai; **JP-** JIPMER, Puducherry; **NIMS-**Nizam's Medical College, Manipal; **MGIMS-** Regional Center for antimicrobial resistance surveillance network, Wardha, Sevagram; **KMC-**Kasturba Medical college, Manipal, Karnataka; **SKIMS-** Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar; **AIIMS J**- AIIMS Jodhpur; **TMC**- TMC, Kolkata; **AIIMS ND**- AIIMS New Delhi; **AIIMS Bh**- AIIMS Bhopal; **RIMS-**Regional Institute of Medical Science, Manipur

CIP- Ciprofloxacin;**S*-** Single Mutation; **D*-** double Mutation; **T*-**Triple Mutation; **P*-** Present; **NP*-** Not present; **I*-** Intermediate; **R*-** Resistant; **S**-** Sensitive

Table 6.6: - Mutations imparting resistance to ciprofloxacin in *S.* Paratyphi A

			gyrA		ParC		CIP* MIC	CIP Disk Diffusion zone (mm)	
	Lab ID/ Centre Name	Mutation	S83 F	D87N	S80 I	D420N	µg/ml		
1	237503/SPA/H	D*	P*	Np	P	Np	0.75	25	I*
2	237502/SPA/H	S*	P	Np	Np	Np	0.75	24	I
3	256431/SPA/H	S	P	Np	Np	Np	0.5	29	I
4	306979/SPA/H	S	P	Np	Np	Np	0.75	26	I
5	137237/SPA/H	S	P	Np	Np	Np	0.5	22	I
6	137238/SPA/H	S	P	Np	Np	Np	0.5	23	I
7	148582/SPA/SGR	S	P	Np	Np	Np	3	25	I
8	201695/SPA/SGR	S	P	Np	Np	Np	0.38	25	I
9	201697/SPA/SGR	S	P	Np	Np	Np	0.5	26	I
10	287543/SPA/SGR	D	P	Np	P	Np	0.5	27	I
11	125/SPA/KMC	NP*	NP	Np	NP	Np	1	20	R*
12	11221/SPA/SGR	NP	Np	Np	Np	Np	0.5	24	I
13	201715/SPA/SGR	NP	Np	Np	Np	Np	0.38	26	I
14	245576/SPA/AP	S	P	Np	NP	Np	2	20	R
15	274298/SPA/AP	S	P	Np	NP	Np	0.75	27	I
16	154452/SPA/AP	S	P	Np	NP	Np	0.5	28	I
17	157993/SPA/AP	S	P	Np	NP	Np	0.5	27	I
18	158887/SPA/AP	S	P	Np	NP	Np	0.125	28	I
19	265922/SPA/JP	NP	Np	Np	Np	Np	0.38	26	I
20	202705/SPA/NIMS	S	P	Np	Np	Np	0.5	24	I
21	202708/SPA/NIMS	S	P	Np	Np	Np	0.75	25	I
22	146/SPA/KMC	S	p	Np	NP	Np	0.75	16	I
23	268/SPA/KMC	S	p	Np	NP	Np	0.75	22	I
24	3481/SPA/SKIMS	S	P	Np	Np	Np	0.38	24	I
25	5819/SPA/SKIMS	D	P	P	Np	Np	0.75	25	i
26	6556/SPA/SKIMS	S	P	Np	Np	Np	0.75	23	I
27	106824/SPA/SKIMS	S	P	Np	Np	Np	0.25	29	I
28	174678/SPA/AIIMS J	S	P	Np	Np	Np	0.75	30	I
29	184945/SPA/TMC	S	p	Np	Np	Np	0.38	26	I
30	170556/SPA/LTMMC	S	P	NP	Np	Np	0.5	27	I
31	124588/SPA/AFMC	NP	Np	Np	Np	Np	0.5	29	I

Table 6.7. a. Genotypic and phenotypic comparison of antibiotic resistance

RC 1

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	drA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_L416F																
9893/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
9927/ST	P	Np	Np	P	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2

RC 12

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_L416F																
124002/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1

RC 13

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_D420N																
174640/ST	P	Np	P	Np	R	Np	S	Np	S	P	R	P	P	R	P	R	P	P	R	ST1
174678/SPA	P	Np	Np	Np	I	Np	S	acrB_R717Q	R	Np	S	P	Np	R	Np	S	Np	Np	S	ST85
175081/ST	P	P	P	Np	R	Np	S	Np	R	Np	S	Np	Np	Np	Np	S	Np	Np	S	ST1
240974/ST	P	P	P	Np	R	Np	S	Np	S	P	R	Np	Np	Np	Np	S	Np	Np	S	ST1
190042/ST	P	Np	Np	P	I	Np	S	Np	S	P	R	Np	P	R	P	R	P	P	R	ST1

RC 7

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol antibiotic		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_L416F																
124588/SPA	Np	Np	Np	Np	S	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85

RC 10

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST
	gyrA		ParC		Phenotypic Sensitivity	CTX-M	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	D420N																
215321/ST	P	Np	NP	Np	I	NP	S	NP	S	P	R	NP	P	R	P	R	P	P	R	ST1
212656/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	P	P	R	ST1
206471/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
215575/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
217743/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
220566/ST	P	Np	NP	Np	S	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
227754/ST	P	Np	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
256728/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
274274/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
245576/SPA	P	Np	NP	Np	R	CTX M-37	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	
274298/SPA	P	Np	NP	Np	I	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST129
196286/ST	P	P	P	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST1
154452/SPA	P	Np	NP	Np	R	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST85
157993/SPA	P	Np	NP	Np	I	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST85
158887/SPA	P	Np	NP	Np	I	NP	S	NP	S	Np	S	NP	NP	S	NP	S	NP	NP	S	ST129
41399/ST	P	Np	P	Np	I	NP	S	Np	S	P	R	NP	P	R	P	R	P	P	R	ST1

RC 4

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		Diaminopyrimidine			phenicol (Chloramphenicol)		Sulfonamide			MLST Type
	gyrA		ParC	ParE	Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	L416F																
265922/SPA	Np	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85
267700/ST	P	Np	P	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
270468/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
204247/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
268986/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
208362/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
208465/ST	P	Np	P	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
208676/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
208797/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
209897/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
211607/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
266029/S.enteritidis diarizonae IIIb 61:z52:z53	P	Np	P	Np	R	Np	S	Np	S	P	R	Np	NP	S	P	S	Np	Np	S	ST1848
269611/S. group B	P	Np	P	Np	R	Np	S	Np	S	P	R	Np	Np	S	P	S	P	Np	S	ST313

RC 16

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_L416F																
158960/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1

RC 14

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	E84G																
146/SPA	p	Np	NP	Np	I	Np	S	P	R	Np	I	Np	Np	S	Np	S	Np	Np	S	ST129
235644/ST	p	p	P	Np	R	Np	S	NP	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
241578/ST	p	p	P	Np	R	Np	S	NP	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
241756/ST	p	Np	NP	Np	I	Np	S	NP	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
299245/ST	p	p	P	Np	I	Np	S	NP	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
124/ST	NP	Np	NP	Np	S	Np	S	NP	S	P	S	Np	P	S	P	S	P	P	R	ST1
125/SPA	NP	Np	NP	Np	I	Np	S	NP	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85
268/SPA	p	Np	NP	Np	I	Np	S	NP	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129

RC 15

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol antibiotic		Sulfonamide			MLST
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_L416F																
170556/SPA	P	NP	Np	Np	I	CTX-M-117	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129

RC 23

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	E84G																
182562/ST	P	Np	P	P	I	Np	S	NP	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
206906/ST	P	P	P	Np	R	Np	S	NP	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
206926/STM	P	Np	P	Np	S	Np	S	P	R	Np	S	Np	Np	S	Np	S	Np	Np	S	ST313
206931/ST	P	Np	Np	Np	R	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
206946//ST	P	P	P	Np	R	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
216018//ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
243138//ST	P	P	P	Np	R	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
190972/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
184024/ST	Np	Np	Np	Np	S	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST2
224739/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1
237574/ST	P	P	P	Np	R	Np	S	NP	S	Np	S	NP	NP	S	NP	S	Np	Np	S	ST1

RC 17

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST Type
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	E84G																
192554/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
202697/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
202699/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
202701/ST	P	Np	P	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
202705/SPA	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129
202708/SPA	P	Np	Np	Np	I	Np	S	Np	S	Np	I	Np	Np	S	Np	S	Np	Np	S	ST129
202709/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	I	Np	Np	S	Np	S	Np	Np	S	ST1
202713/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
132937/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
202706/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
202691/ST	P	Np	Np	Np	I	Np	S	Np	S	P	R	Np	P	R	P	R	P	P	R	ST1

RC 5

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	D420N																
206032/ST	P	Np	Np	P	R	Np	S	Np	S	P	R	Np	P	R	P	R	P	P	R	ST1
181361/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
216696/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
216697/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
224885/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
237503/SPA	P	Np	P	Np	I	Np	S	P	R	Np	S	P	P	R	P	R	Np	Np	S	ST85
237502/SPA	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129
256431/SPA	P	Np	Np	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129
273726/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
275719/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
303034/ST	P	P	P	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
306979/SPA	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85
306958/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
331671/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
339337/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	P	R	P	R	P	Np	R	ST1
137237/SPA	P	Np	Np	Np	I	Np	S	Np	S	TEM-185,TEM-229	S	Np	Np	S	Np	S	Np	Np	S	ST129
137238/SPA	P	Np	Np	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	P	R	ST129
136401/ST	P	P	P	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
145476/ST	P	Np	Np	Np	I	Np	S	P	R	P	R	P	P	R	P	R	P	P	R	ST1
113595/ST	P	Np	Np	Np	I	Np	S	Np	R	P	R	Np	P	R	P	R	P	P	R	ST1

RC 8

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	parE_L416F																
202673/ST	p	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
184945/SPA	p	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129

RC 20

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST TYPE
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	E84G																
3481/SPA	P	Np	Np	Np	S	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85
4973/STA	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
4795/STA	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
5819/SPA	P	P	Np	Np	i	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85
6556SPA	P	Np	Np	Np	i	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST129
106158/ST	Np	P	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
106824SPA	P	Np	Np	Np	S	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST85
106871/STA	Np	Np	Np	Np	S	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2

RC 6

	Fluoroquinolone					3rd generation Cephalosporin		Macrolide		Ampicillin		diaminopyrimidine antibiotic			phenicol		Sulfonamide			MLST Type
	gyrA		ParC		Phenotypic Sensitivity	CTX-M-15	Phenotypic Sensitivity	ErmC	Phenotypic Sensitivity	blaTEM-1D	Phenotypic Sensitivity	dfrA15	dfrA7	Phenotypic Sensitivity	catI	Phenotypic Sensitivity	Sul1	Sul2	Phenotypic Sensitivity	
	S83F	D87N	S80I	ParEL416F																
148582/SPA	P	Np	Np	Np	R	Np	S	Np	S	Np	S	P	Np	R	Np	S	Np	Np	S	
148587/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	
148589//ST	P	P	Np	P	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
201709/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
201713//ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
201720//ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
201695/SPA	P	Np	Np	Np	I	Np	S	Np	S	Np	R	Np	Np	S	Np	S	Np	Np	S	ST85
201697SPA	P	Np	Np	Np	I	Np	S	Np	S	Np	R	Np	Np	S	Np	S	Np	Np	S	ST85
243087/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	P	Np	R	Np	S	P	Np	S	ST1
243095/ST	Np	Np	Np	Np	S	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
269039/ST	P	Np	Np	Np	I	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST2
269043/ST	Np	Np	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
287544/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
287543/ST	P	Np	P	Np	I	Np	s	Np	R	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
18449/ST	P	P	P	Np	R	Np	S	Np	S	P	R	Np	P	R	P	R	P	P	R	ST1
19272/ST	P	P	P	Np	R	Np	S	Np	S	P	R	Np	P	R	P	R	P	P	R	ST1
10874/ST	P	P	P	Np	R	Np	S	Np	S	Np	S	Np	Np	S	Np	S	Np	Np	S	ST1
11221/SPA	Np	Np	Np	Np	R	Np	S	Np	S	Np	R	Np	Np	S	Np	S	Np	Np	S	ST129
287556/ST	P	Np	Np	Np	S	Np	S	P	S	P	R	P	P	R	P	R	P	P	R	ST1
9406/ST	P	Np	P	Np	R	Np	S	P	S	P	R	P	P	R	P	R	P	P	R	ST1
201663/ST	P	Np	P	Np	R	Np	S	P	S	P	R	P	P	S	P	S	Np	Np	S	ST1
201715/SPA	Np	Np	Np	Np	I		S	P	S	P	R	P	P	S	P	R	P	P	R	ST129

Conclusions

The data from the current year shows no significant change in the overall antimicrobial susceptibility pattern of *Salmonella* Typhi or *S*. Paratyhi A from India – the pattern remaining uniform across all the participating centers in the AMR network. As fluoroquinolone resistance remains highest among all the antiyphoidal drugs, further characterization of the genetic mechanisms of resistance imparting resistance to this class was carried out in representative strains from across the east, west, north and south zones. Overall the mutation in gyrA at S83F was the most common resistance mechanism and accounted for more than 90% of all mutations. Single mutation at gyrA S83F or double mutation at gyrA S83F and parC S80I of the QRDR were responsible for reduced susceptibility to fluoroquinolones. A small proportion of ciprofloxacin susceptible strains also showed the presence of mutations in gyr A gene which is a cause of concern because they may be expressed while on treatment due to selective pressure. The MDR phenotype, as in the last few years, has continued to remain around 10% across the country. In some of the susceptible isolates, CAT gene determining resistance to chloramphenicol was found though not expressed, implying that a continuous monitoring is needed to ensure that there is no reemergence. All the strains remain susceptible to ceftriaxone and azithromycin and none carried $CTX_{M\text{-}15}$ gene or genes imparting resistance to macrolides.

The present data reiterates the fact that ceftriaxone and cefixime remain the first line of drug to treat severe infections of enteric fever in the country. Azithromycin continues to be used as a drug of choice in outpatients without any associated complications and empirically. Fluoroquinolones, though an ideal drug to treat enteric fever, can only be given in the culture positive cases showing fluoroquinolone susceptibility.

Chapter 7 Diarrheal pathogens

Summary of results

There is no significant change in the pathogen isolation trend and overall antimicrobial susceptibility among these pathogens. Considering the common pathogens causing bacterial gastroenteritis, such as *Aeromonas*, *Salmonella, Shigella, E. coli* or *Vibrio* species, third generation cephalosporins or azithromycin can still be the drug of choice for severe gastroenteritis except for *Aeromonas* and *Vibrio* for which tetracycline shows good susceptibility. Since gastroenteritis is usually self-limited, antimicrobial therapy is not routinely recommended. Therefore, the drug of choices should be tailored according to local prevalence of drug-resistance.

Notably, the number tested for antibiotics are not uniform in all the years and thus increasing or decreasing trend could not be identified. This needs to be streamlined in all the centres including nodal centres for better interpretation.

Aeromonas spp

The susceptibility profile of *Aeromonas spp* in the year 2021 showed more than 70% susceptibility to meropenem and norfloxacin and 80% to tetracycline. They are highly resistant to ciprofloxacin (>85%) (Table 7.1). The five-year susceptibility trend showed that susceptibility to all the antibiotics is consistent, and no significant change observed. However, carbapbenem (imipenem and meropenem) and third generation cephalosporins (cefixime) antibiotics are inconsistently tested. The year wise antibiotic susceptibility percentage was given in Table 7.2 and year-wise trend was shown in Figure 7.1. Aeromonas-associated gastroenteritis in immunocompetent persons is usually self-limited and antibiotics are not routinely recommended.The antimicrobial therapy may differ depending on the site of infection since *Aeromonas spp* is ubiquitous in nature.

Shigella spp

S. flexneri and *S. sonnei* was the predominant serogroup for the last five years isolated with varying susceptibility profiles. As known, *S. flexneri* was highly resistant to fluoroquinolones such as nalidixic acid and norfloxacin (≤10%), ampicillin (19%) and trimethoprim-sulfamethoxazole (39%) respectively. However, susceptibility to third generation cephalosporins such as cefixime was 79% in *S. sonnei* and 68% in *S. flexneri* (Table 7.3). The trend analysis of *S. flexneri* showed steady decrease in the ampicillin susceptibility. Whereas susceptibility to trimethoprim-sulfamethoxazole is slightly

increased from 10% in 2017 to 38% in 2021, this regaining of susceptibility could be due to the limited use of this antibiotic recently. The antibiotic nalidixic acid and norfloxacin were tested only for few isolates and thus cannot be commented. Cefixime susceptibility decreased from 88% in 2020 to 68% in 2021 (Table 7.4 and Figure 7.2). Similar susceptibility profile was observed for *S. sonnei* except for ampicillin susceptibility which is higher (>50%) compared to *S. flexneri*. Alike *S. flexneri*, cefixime susceptibility is decreasing and susceptibility to trimethoprim-sulfamethoxazole is increasing (Table 7.5 and Figure 7.3).

A total of 37 *Shigella* isolates received from CMC and other centres were characterized for the presence of AMR genes such as *dhfr*A, *sul*II, bla_{OXA}, bla_{TEM}, $bla_{\text{CTX-M-1}}$, AmpCs and *qnr*A/B/S by PCR in the year 2021. As expected, majority of the isolates carried *dhfr*A and *sul*II genes which confer resistant to trimethoprim/sulfamethoxazole. Among beta-lactamases, bla_{OXA}, $bla_{\text{CTX-M}}$ followed by bla_{TEM} gene was predominantly seen. While AmpC genes were identified only in three isolates. Further, plasmid mediated quinolone resistance (PMQR) gene *qnr*S and *qnr*B was identified in four and two isolates respectively.

Vibrio spp

V. cholerae showed >90% susceptibility to norfloxacin and tetracycline, while showed 86% susceptibility to ampicillin. However, only 17% susceptibility was observed for trimethoprim-sulfamethoxazole (Table 7). Nalidixic acid was not tested this year. The year-wise susceptibility of *V. cholerae* was shown in Table 7.7 and Figure 7.4. Susceptibility of trimethoprim-sulfamethoxazole decreased from 42% in 2017 to 17% in 2021 which needs to be monitored. Otherwise, no significant change was observed for other antibiotics tested. Very few other *Vibrio spp* has been isolated this year. This data shows that tetracycline can still be the effective drug of choice for cholera since other antibiotics are widely used for other infections and may develop resistance.

Diarrheagenic *E. coli* (DEC)

The susceptibility of DEC showed that all isolates were resistant to ampicillin and showed decreased susceptibility to other antibiotics such as nalidixic acid (8%), norfloxacin (17%) and cefixime (14%), whereas 36% susceptibility was observed for trimethoprim-sulfamethoxazole (Table 7.8). The analysis of yearly susceptibility trends shows that the susceptibility of all antibiotics appears to be slightly decreased compared to the last year (Table 7.9 and Figure 7.5). The trend analysis suggests that DEC isolates are highly resistant to the currently tested antibiotics and higher antibiotic class needs to be tested in future for alternative treatment. Further, molecular analysis of 20 DEC isolates received from other centres showed the presence of AMR genes such as *dhfr*A, bla_{OXA}, bla_{TEM}, ampC,

and *qnr*S. Antibiotic treatment is generally not recommended for DEC infections but in certain cases, treatment with ciprofloxacin and azithromycin are indicated.

Table 7.1: Susceptible pattern of *Aeromonas spp*

AMA	All Specimens n=236	Faeces n=179
Cefixime	*3/8 (-)	*0/0
Ciprofloxacin	27/215 (12.6)	22/177 (12.4%)
Imipenem	102/205 (49.8)	77/154 (50%)
Meropenem	157/205 (76.6)	118/153 (77.1%)
Norfloxacin	17/23 (73.9)	*9/11 (-)
Tetracycline	168/205 (82)	145/178 (81.5%)

Table 7.2: Yearly susceptible trends of *Aeromonas spp*from Faeces

AMA	Year-2016 Total n=21 (S%)	Year-2017 Total n=131 (S%)	Year-2018 Total n=114 (S%)	Year-2019 Total n=170 (S%)	Year-2020 Total n=77 (S%)	Year-2021 Total n=179 (S%)
Cefixime	*0/0	*0/0	23/36 (63.9)	*0/0	*0/0	*0/0
Imipenem	*0/0	20/46 (43.5)	53/109 (48.6)	*1/2	*0/0	77/154 (50)
Meropenem	*0/0	26/48 (54.2)	71/109 (65.1)	*1/2	*0/0	118/153 (77.1)
Tetracycline	18/21 (85.7)	104/126 (82.5)	97/113 (85.8)	134/169 (79.3)	58/77 (75.3)	145/178 (81.5)
Ciprofloxacin	*0/0	8/78 (10.3)	11/112 (9.8)	20/169 (11.8)	4/74 (5.4)	22/177 (12.4)
Norfloxacin	19/21 (90.5)	28/29 (96.6)	*1/1	156/169 (92.3)	38/54 (70.4)	*9/11 (-)

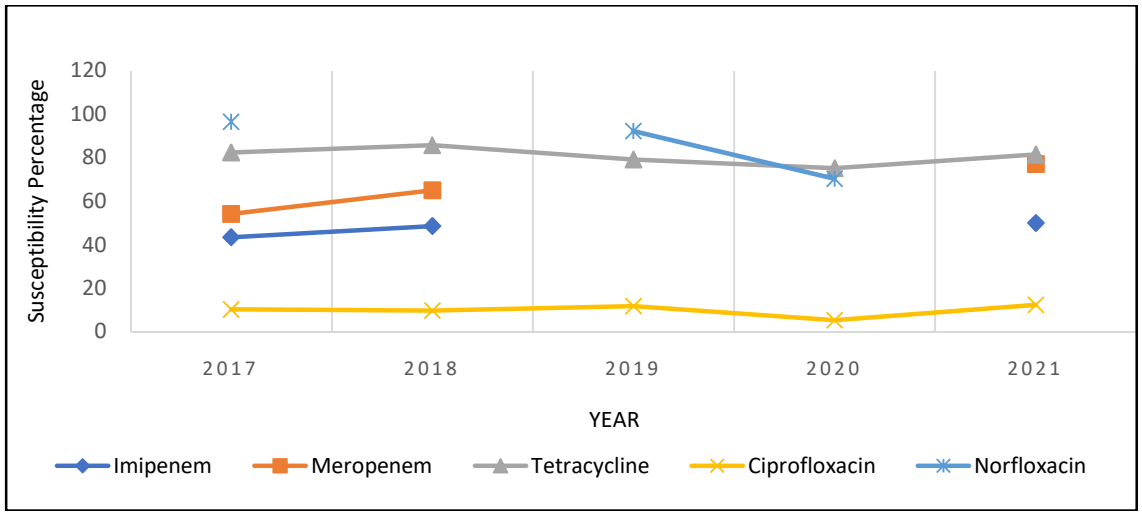


Figure 7.1: Yearly susceptible trends of *Aeromonas spp*

Table 7.3: Susceptible pattern of *Shigella* species

AMA	*S. sonnei* n=41	*S. flexneri* n=37	*Shigella spp.* n=*4
Ampicillin	22/40 (55%)	7/37 (18.9%)	*1/3 (-)
Cefixime	31/39 (79.5%)	25/37 (67.6%)	*1/1 (-)
Nalidixic acid	*0/7 (-)	*0/8 (-)	*0/2 (-)
Norfloxacin	3/32 (9.4%)	2/20 (10%)	*1/2 (-)
Trimethoprim-sulfamethoxazole	9/41 (22%)	14/37 (37.8%)	*3/4 (-)

Table 7.4: Yearly susceptible trends of *Shigella flexneri*

AMA	Year-2017 Total n=89 (S%)	Year-2018 Total n=47 (S%)	Year-2019 Total n=95 (S%)	Year-2020 Total n=55 (S%)	Year-2021 Total n=37 (S%)
Ampicillin	40/89 (44.9)	12/47 (25.5)	24/94 (25.5)	9/54 (16.7)	7/37 (18.9)
Cefixime	56/69 (81.2)	38/46 (82.6)	73/92 (79.3)	45/51 (88.2)	25/37 (67.6)
Nalidixic acid	0/24 (0)	*0/15	2/35 (5.7)	*2/13	*0/8
Norfloxacin	12/24 (50)	*1/16	8/36 (22.2)	*3/13	2/20 (10)
Trimethoprim-sulfamethoxazole	7/72 (9.7)	14/47 (29.8)	22/95 (23.2)	9/55 (16.4)	14/37 (37.8)

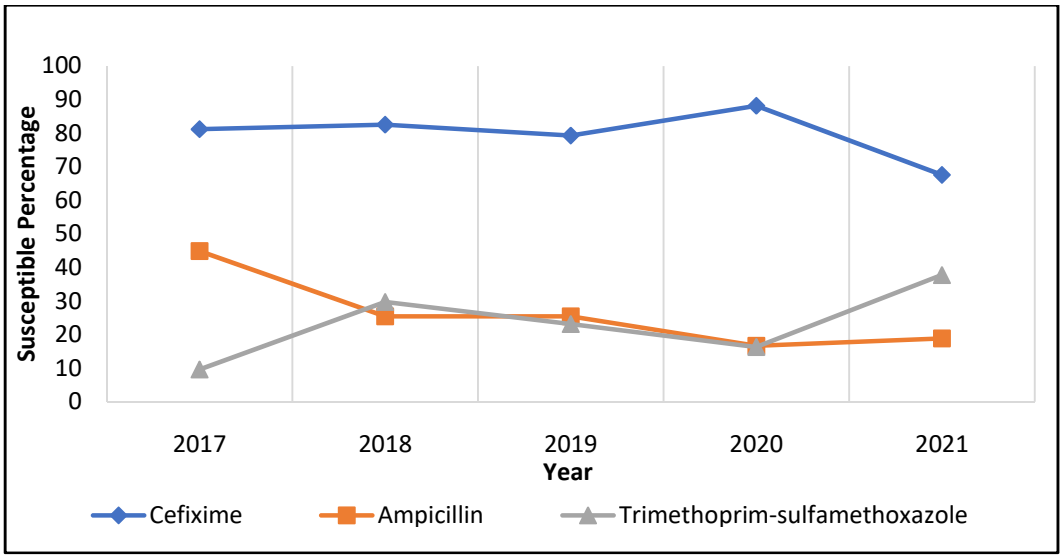


Figure 7.2: Yearly susceptible trends of *Shigella flexneri*

Table 7.5: Yearly susceptible trends of *Shigella sonnei*

AMA	Year-2017	Year-2018	Year-2019	Year-2020	Year-2021
	Total n=52 (S%)	Total n=26 (S%)	Total n=57 (S%)	Total n=*14 (S%)	Total n= 41 (S%)
Ampicillin	35/52 (67.3)	18/24 (75)	42/57 (73.7)	*10/14	22/40 (55)
Cefixime	47/50 (94)	25/26 (96.2)	52/57 (91.2)	*12/13	31/39 (79.5)
Nalidixic acid	*0/8	*0/1	*0/8	*0/0	*0/7 (-)
Norfloxacin	*2/8	*0/1	*3/9	*1/2	3/32 (9.4)
Trimethoprim-sulfamethoxazole	4/52 (7.7)	0/25 (0)	5/57 (8.8)	*1/13	9/41 (22)

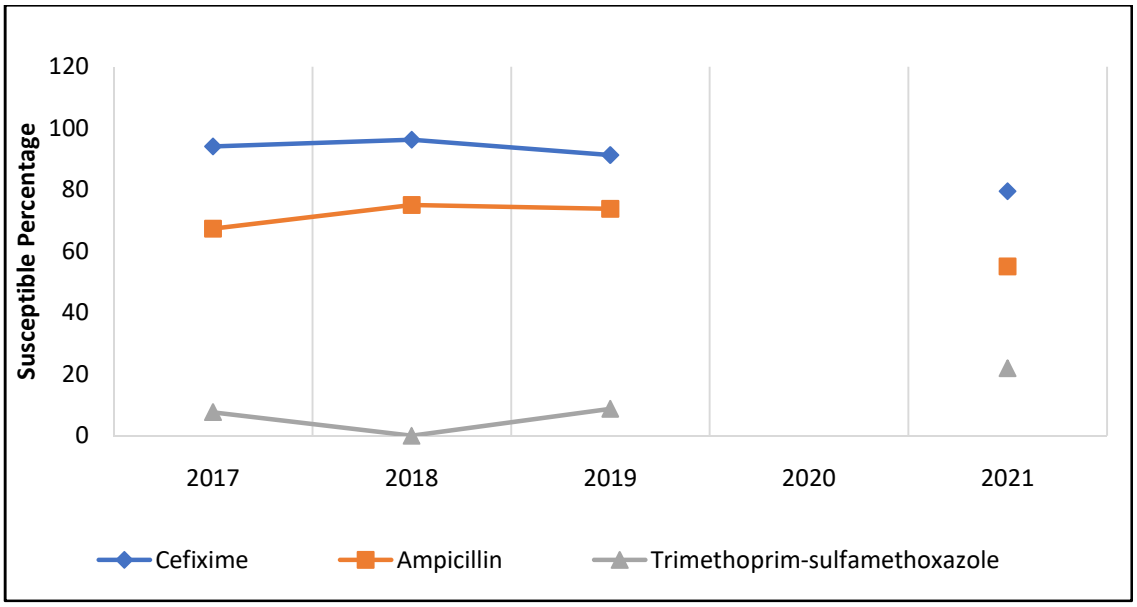


Figure 7.3: Yearly susceptible trends of *Shigella sonnei*

Table 7.6: Susceptible pattern of *Vibrio cholerae* and *Vibrio spp*

AMA	*Vibrio cholerae* n=58	*Vibrio spp.* n=*16
Ampicillin	44/51 (86.3%)	*7/16 (-)
Nalidixic acid	*0/0	*0/0
Norfloxacin	50/55 (90.9%)	*13/15 (-)
Tetracycline	55/58 (94.8%)	*16/16 (-)
Trimethoprim-sulfamethoxazole	10/58 (17.2%)	*12/16 (-)

Table 7.7: Yearly susceptible trends of *Vibrio cholerae*

AMA	Year-2017 Total n=24 (S%)	Year-2018 Total n=25 (S%)	Year-2019 Total n=39 (S%)	Year-2020 Total n=31 (S%)	Year-2021 Total n=58 (S%)
Ampicillin	17/24 (70.8)	17/24 (70.8)	22/39 (56.4)	11/28 (39.3)	44/51 (86.3)
Tetracycline	19/21 (90.5)	*7/10	36/38 (94.7)	31/31 (100)	55/58 (94.8)
Nalidixic acid	*1/8	*0/4	*0/5	*1/1	*0/0
Norfloxacin	*9/14	*4/4	29/39 (74.4)	22/29 (75.9)	50/55 (90.9)
Trimethoprim-sulfamethoxazole	10/24 (41.7)	6/24 (25)	18/38 (47.4)	13/31 (41.9)	10/58 (17.2)

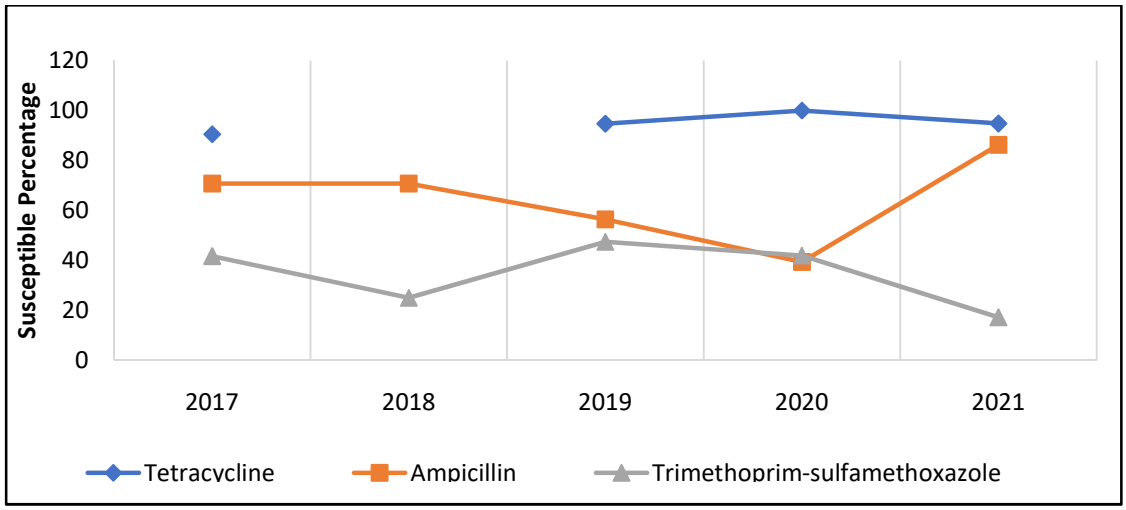


Figure 7.4: Yearly susceptible trends of *Vibrio cholera*

Table 7.8. Susceptible pattern of DEC

AMA	Faeces
	DEC n=88
Ampicillin	0/87 (0%)
Cefixime	12/87 (13.8%)
Nalidixic acid	7/87 (8%)
Norfloxacin	14/82 (17.1%)
Trimethoprim-sulfamethoxazole	32/88 (36.4%)

Table 7.9 Yearly susceptible trend of DEC

AMA	Year-2019	Year-2020	Year-2021
	Total n=134	Total n=102	Total n=88
	(S%)	(S%)	(S%)
Ampicillin	6/132 (4.5)	1/102 (1)	0/87 (0)
Cefixime	17/129 (13.2)	11/100 (11)	12/87 (13.8)
Nalidixic acid	14/122 (11.5)	11/98 (11.2)	7/87 (8)
Norfloxacin	33/127 (26)	20/100 (20)	14/82 (17.1)
Trimethoprim-sulfamethoxazole	45/133 (33.8)	32/102 (31.4)	32/88 (36.4)

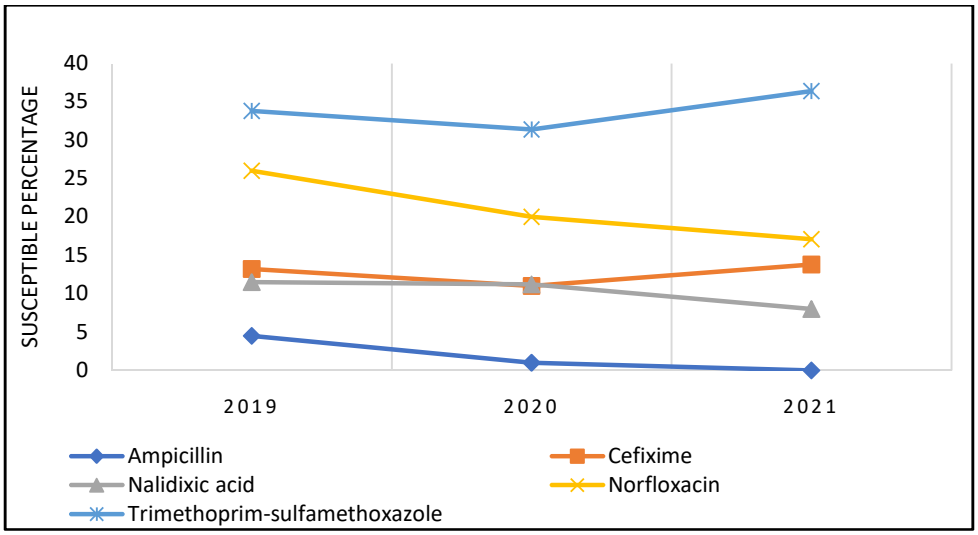


Figure 7.5 Yearly susceptible trend of DEC

Clinical relevance

The surveillance data indicates the prevalence of different pathogens associated in diarrhea cases in the country and the need for differential diagnoses for better treatment outcomes. Further, antimicrobial susceptibility profile varies over time and in different geographical regions between different pathogens. Therefore, the definite therapy should be decided based on the local susceptibility pattern.

For *Aeromonas spp*, third generation cephalosporins, fluoroquinolones and aminoglycosides remain an effective treatment option. The antimicrobial therapy of *Aeromonas spp* may differ depending on the site of infection. The susceptibility data of *Aeromonas spp* from stool specimen showed >85% susceptibility for ciprofloxacin and >80% for tetracycline. No significant change in the susceptibility trend over the five years was observed.Among *Shigella spp*, increased resistance was observed to trimethoprim/sulfamethoxazole, ciprofloxacin and ampicillin thus should not be recommended unless susceptibility is known. Decreasing susceptibility to third generation cephalosporins and azithromycin was also noted, however, needs continuous monitoring. This emerging resistance warrants the development of new antibiotics or re-purposing of existing antibiotics as these are among the few therapeutic options left for moderate to severe *Shigella* infections. Further the genotypic data correlated with the phenotypic AST profile. No change in the AMR gene profile was identified.

Susceptibility of *Vibrio spp* to trimethoprim-sulfamethoxazole decreased over the last five years thus should not be recommended for treatment. However, >90% susceptibility was seen for norfloxacin and tetracycline. Generally, tetracycline/ doxycycline is being used for

cholera infections. As expected, tetracyclines class of drugs appears to be effective for *Vibrio spp* till today. All DEC isolates were resistant to ampicillin. Decreased susceptibility to other tested antibiotics was also observed. The trend analysis suggests the decreasing susceptibility to the currently tested antibiotics and thus the antibiotics tested should be revisited.

Chapter 8 Streptococcus pneumoniae

Serotype distribution and antimicrobial susceptibility profile of invasive and non-invasive *Streptococcus pneumoniae* in India for the year 2021

As part of the national reference laboratory, *S. pneumoniae* isolates were received from various hospitals within India. The invasive isolates included, *S .pneumoniae* isolated from sterile specimens such as CSF, blood and body fluids in children less than 5 years of age. The non-invasive isolates included, *S. pneumoniae* isolated from respiratory specimens (sputum).

Serotype Distribution

A total of 60 invasive (Child n=29, adult n=31) and 91 non-invasive (child n=15, adult n= 76) *S. pneumoniae isolated* in the year 2021 were included in the analysis. The serotype distribution among the invasive and non-invasive isolates of *S. pneumonia* is depicted in Figure 8.1 and Table 8.1. PCV13 serotypes were the predominant ones, with serotype 6B, 19F and 19A the major ones among the invasive isolates. Among the non-invasive, serotypes 19F, 6A and 18C were the major types. The other non-invasive serotypes were highly diverseThe PCV13 serotype percentage coverage was 72 and 57 for the invasive and non-invasive *S. pneumoniae,* respectively. Among the serotypes not included in the Pneumosil (PCV10Sii), the serotype 18C and 4, though constitute 7-9 %, serotype 18C alone holds 8% of the non-invasive serotype. One serotype each of 9L, 10F, 23B, 28F, 33A, 35A, 35C, and 48 was isolated from non-invasive specimens

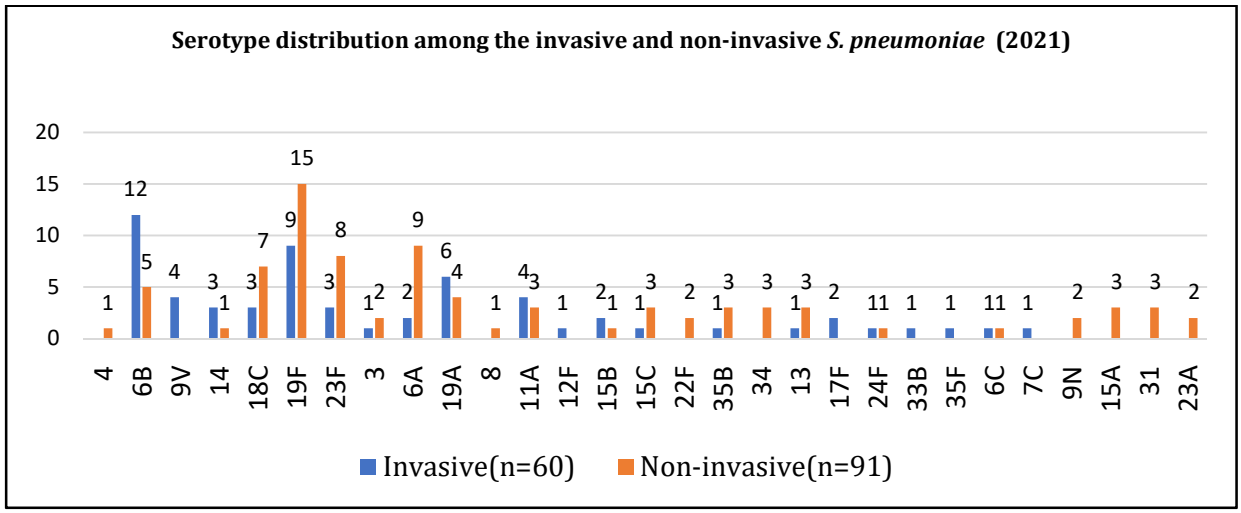


Figure 8.1: The serotype distribution of invasive (n=60) and non-invasive (n=91) isolates of *S. pneumoniae*

Table 8.1: The number of serotypes among the invasive and non-invasive isolates of *Streptococcus pneumoniae*

Serotype	Invasive(n=60)	Non-invasive(n=91)
4	0	1
6B	12	5
9V	4	0
14	3	1
18C	3	7
19F	9	15
23F	3	8
3	1	2
6A	2	9
19A	6	4
8	0	1
11A	4	3
12F	1	0
15B	2	1
15C	1	3
22F	0	2
35B	1	3
34	0	3
13	1	3
17F	2	0
24F	1	1
33B	1	0
35F	1	0
6C	1	1
7C	1	0
9N	0	2
15A	0	3
31	0	3
23A	0	2

Antimicrobial Susceptibility Profile

The penicillin and cefotaxime antimicrobial susceptibility percentage of invasive *S. pneumoniae* isolates were calculated based on meningeal or non-meningeal isolates (Figure 8.2 and Table 8.2). This is due to the different breakpoints of penicillin and cefotaxime of meningeal and non-meningeal isolates. The penicillin and cefotaxime non susceptibility was higher in meningeal isolates than the non-meningeal isolates.

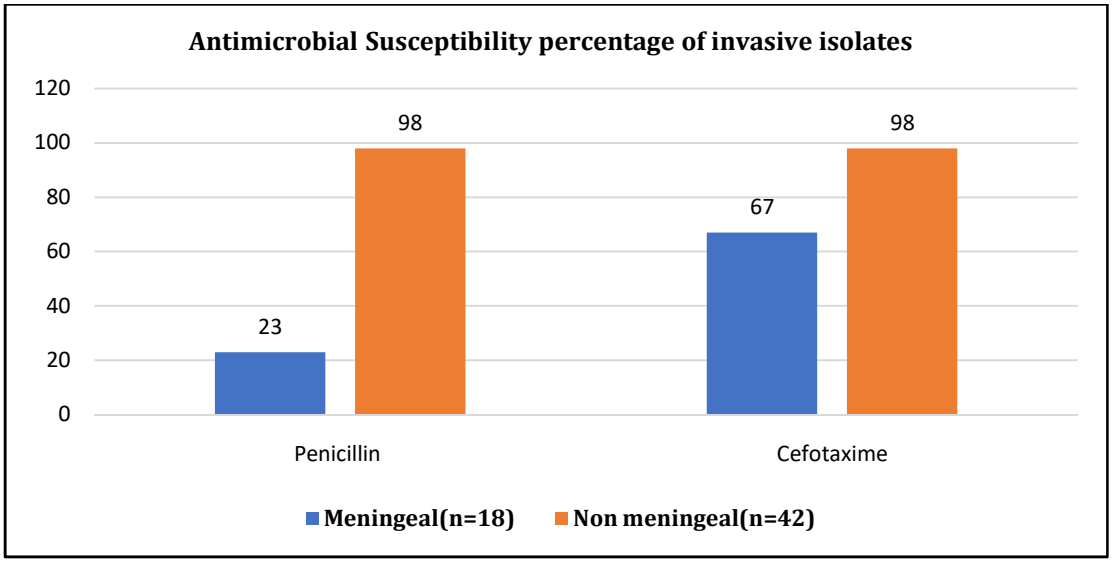


Figure 8.2: Penicillin and cefotaxime antimicrobial susceptibility of invasive isolates of S. pneumoniae (n=60)

Table 8.2: Number of *S. pneumoniae* invasive isolates susceptible to penicillin and cefotaxime

Antibiotics	Total no of invasive isolates (n=60)	
	No of susceptible Meningeal isolates=18 (%)	No of susceptible Non-meningeal isolates (n=42)
Penicillin	4(23)	41(98)
Cefotaxime	12(67)	41(98)

The antimicrobial susceptibility profile for antibiotics other than penicillin and cefotaxime is given below in Figure 8.3 and Table 8.3. The antimicrobial susceptibility profile of non invasive isolates is depicted in figure 8.4 and Table 8.4.

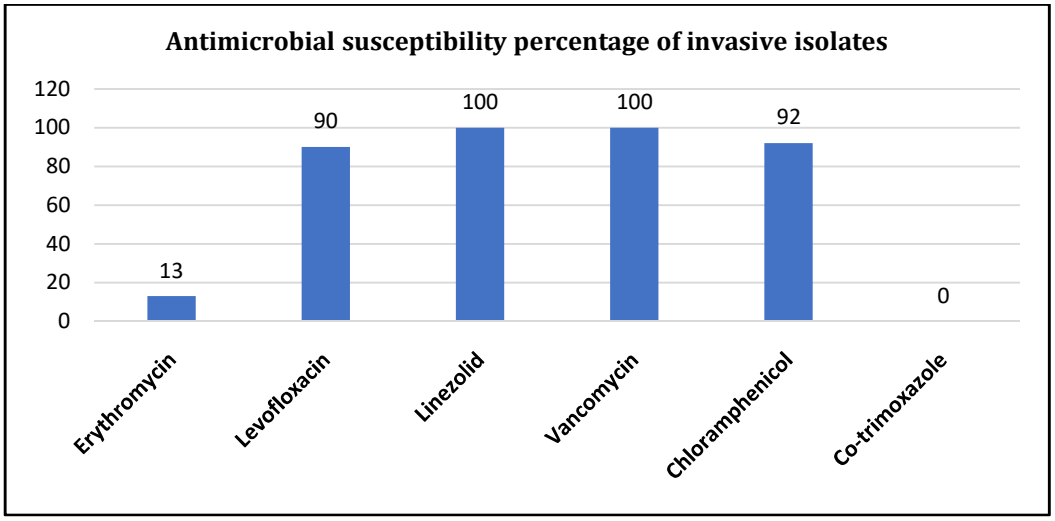


Figure 8.3: Antimicrobial susceptibility profile of invasive isolates of *S. pneumoniae* for antibiotics other than Penicillin and cefotaxime (n=60)

Table 8.3: Number of invasive isolates susceptible to Erythromycin, Levofloxacin, Linezolid, Vancomycin, Chloramphenicol, Cotrimoxazole

Antibiotics	Number of isolates susceptible, n=60(%)
Erythromycin	8 (13)
Levofloxacin	54(90)
Linezolid	60(100)
Vancomycin	60(100)
Chloramphenicol	55(92)
Co-trimoxazole	0(0)

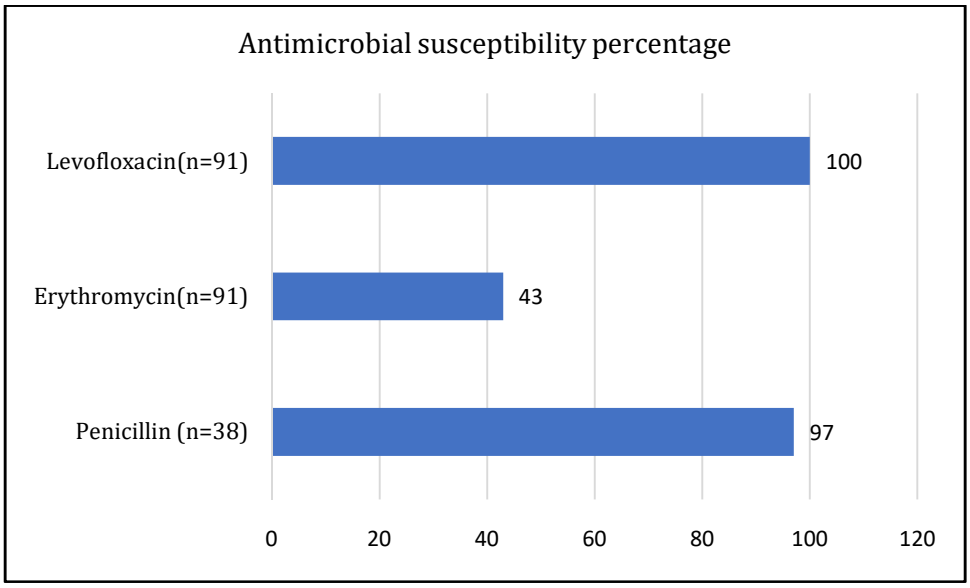


Figure 8.4: Antimicrobial Susceptibility profile of non-invasive isolates (n=91)

Table 8.4: Number of non-invasive isolates susceptible to levofloxacin, Erythromycin and Penicillin

Antibiotics	No of susceptible isolates (%)
Penicillin (n=38)	37(97)
Erythromycin (n=91)	39 (43)
Levofloxacin(n=91)	91 (100)

Summary

PCV 13 serotypes continue to be the predominant serotypes prevalent in India. The impact of replacement of PCV13 vaccine by Peumosil (Sii) has to be monitored since serotype 18C is predominant in the non invasive group. The persistence of PCV13 serotypes could be due to the less PCV vaccine coverage in India. The antimicrobial non susceptibility to penicillin and cefotaxime is decreasing gradually. Hence, monotherapy with either of these antibiotics is not recommended in the meningeal infections. Current ICMR guidelines of combination therapy (cephalosporins with vancomycin) are recommended. While for non-invasive infections, penicillin and cephalosporins are observed to be effective.

Chapter 9 Health Care Associated Infections

Summary

This chapter provides comprehensive details of bloodstream infections (BSIs) and Urinary tract infections (UTIs), reported from January, 2021 to December, 2021 from a network of 39 hospitals across India. The Methodology, SOPs and training modules for HAI surveillance are provided on our website www.haisindia.com. The regional distribution of the participating centers is shown in the executive summary. During the period from January, 2021 to December, 2021, a total of 120 ICUs from the 39 Centers reported HAI rates to our centralized database. Medical and Neonatal ICUs accounted for 21.7 and 15.8 % of the total ICUs in our network. Twelve (10%) ICUs in the network during this period were dedicated COVID ICUs. The cumulative patient days for the network for this period was 5,47,507. A total of 1,50,744 Central line days and 2,64,344 urinary catheter days were reported during this period. A total of 3,080 cases of blood stream infections and 792 cases of urinary tract infections were reported, accounting for the total BSI rate to be 5.63 per 1,000 patient days and total UTI rate to be 2.03 per 1,000 patient days. A fatal outcome (14-day outcome) was reported in 38.1% of BSIs and 27.9% UTI cases. However, this is not the attributable BSI or UTI mortality, since other predisposing factors, underlying critical illness and other infections also contribute to patient's mortality in the ICUs.

Gram Negative bacteria (GNB) accounted for 73.3% of all BSI cases; 8.6% were due to *Candida* sp. For UTI, GNB accounted for 53.1% cases. *Klebsiella* sp (32.8%) was the most common GNB and *Enterococcus spp* (54.4%) was the most common GPC causing BSIs. 50% of *Klebsiella pneumoniae* and 67% of *Acinetobacter baumannii* causing BSIs were imipenem resistant. 84% of *Staphylococcus aureus* and 37.5% of *Enterococcus faecium* causing BSIs were respectively cefoxitin and vancomycin resistant.

The focus of this network has been on generation of quality assured HAI data and to assess the impact of infection prevention and control on the rates of HAIs. This HAI Surveillance work is primarily ICU based, considering the high rate of device utilization in the ICUs. The most common ICUs represented in this network are Medical, Neonatal, Pediatric Medical and Surgical ICUs. Twelve ICUs during the reporting year were converted to Covid ICUs. The distribution of ICUs is shown in table 9.1.

This surveillance focused on BSIs (Primary and secondary BSIs) and UTIs (Catheter associated and non-catheter associated). Blood and Urine cultures were taken into consideration for fulfilling the surveillance definitions (www.haisindia.com). The distribution of organisms from blood and urine cultures is shown in table 9.2. Enterobacterales were the most common, followed by NF-GNBs.

Table 9.1: Distribution of ICUs in the network

Name of ICU	Number (Percentage)
Medical ICU	26 (21.7)
Neonatal ICU	19 (15.8)
Pediatric Medical ICU	17 (14.2)
Surgical ICU	13 (10.8)
COVID ICU	12 (10.0)
Medical/Surgical ICU	9 (7.5)
Trauma Surgical ICU	5 (4.2)
Cardiothoracic Surgical ICU	3 (2.5)
High Dependency Unit	3(2.5)
Respiratory ICU	3(2.5)
Cardiac ICU	2(1.7)
Gastrointestinal ICU	2(1.7)
Burn ICU	1(0.8)
Neurological ICU	1(0.8)
Oncologic Medical ICU	1(0.8)
Oncologic Medical/Surgical ICU	1(0.8)
Oncologic Surgical ICU	1(0.8)
Pediatric Medical/Surgical ICU	1(0.8)
Total	**120**

Table 9. 2: Specimen wise distribution of major groups of organisms isolated from BSIs and UTIs

Isolate	Culture Positive					
	Total n = 4,357		Blood n = 3474		Urine n = 883	
	n	%	n	%	n	%
Enterobacterales	1691(38.8)	100	1349(38.8)	79.8	342(38.7)	20.2
NF-GNB	1330(30.5)	100	1208(34.8)	90.8	122(13.8)	9.2
Enterococci	516(11.8)	100	353(10.2)	68.3	163(18.5)	31.6
Candida sp.	545(12.5)	100	297(8.6)	54.5	248(28.1)	45.5
Staphylococci	273(6.3)	100	265(7.6)	97.1	8(0.9)	2.9
Typhoidal Salmonella	2(0.0)	100	2(0.1)	100	0(0.0)	0

Table 9.3: Denominator Data

Indicator	Number
Patient days	**4,72,959**
Central line days	**1,50,744**
Urinary catheter days	**2,64,344**

HAI network: BSI data

A total of 3,080 cases of BSIs were reported by the network. The distribution (types) of BSI cases is shown in table 9.4. The total BSI rate in our network was 6.51/1,000 patient days, with the CLABSI rate being 3.1/ 1,000 central line days. The rates of BSIs, Primary BSIs, CLABSIs and Secondary BSIs are shown in Table 9.5. The rates of total BSIs were compared against different types of ICUs, since the morbidity of patients varies with the different types of ICUs. Table 9.6 compares the rates of BSIs across the different ICU types in our network. Of the 3,080 cases of BSIs, males accounted for 66%, as shown in table 9.7. However, no interpretation can be made from this data. It may reflect a higher admission rate in the ICUs.

Table 9.8 shows the duration of stay in the ICUs and the duration between ICU admission and the development of BSI. The duration of ICU stay is a risk factor for development of HAIs. Some patients had a very prolonged ICU stay and invariably, the BSI cases were found more in patients who had a longer ICU stay, across all ICU types. The 14-day mortality in cases of BSIs was 38.1%. This may not be the actual attributable mortality, since severe primary illness or other underlying co-morbidities may be contributing to the fatal outcome. Only 10% of BSI cases were discharged at 14-day. Table 9.9 shows the short-term outcomes of BSI cases. A total of 3,474 pathogens were isolated from the BSI cases.

Gram negative organisms predominated as the cause of BSIs in our network, as shown in Table 9.10.

The genus level distribution in Gram negative & Gram positive organisms and species distribution of Candida causing overall BSIs is shown in table 9.11 to 9.13. *Enterococcus* sp. was the most common Gram positive organism; *Klebsiella* spp was the most common Gram negative organism and *Candida tropicalis* was the most common fungal pathogen.

Table 9.4: Types of BSI cases

Type of BSI cases	No. of BSI cases (%)
CLABSI	1,468 (47.7)
Non-CLABSI	1,166 (37.9)
Secondary BSI	446 (14.5)
Total	3,080

Table 9.5: BSI rates

Indicator	Rates
Total BSI rate(per 1,000 patient days)	**6.51**
Primary BSI rate (per 1,000 patient days)	**5.57**
CLABSI rate(per 1,000 central line days)	**3.10**
Secondary BSI rate (per1,000 patient days)	**0.94**

Table 9.6: Distribution of BSI cases by ICUs

Type of ICUs	No. of BSI cases (Percentage)	Total BSI rate (per 1,000 patient days)
Medical ICU	1,049(34)	8.50
Neonatal ICU	540(17.5)	5.09
Medical/Surgical ICU	412(13.4)	7.33
Surgical ICU	247(8.0)	8.53
Trauma ICU	236(7.7)	14.23
COVID ICU	208(6.8)	4.80
Pediatric Medical ICU (PICU)	175(5.7)	3.89
Gastrointestinal ICU	44(1.4)	7.32
Neurologic ICU	44(1.4)	13.71
Respiratory ICU	25(0.8)	7.55
High Dependency Unit (HDU)	24(0.8)	1.86
Oncologic Surgical ICU	20(0.7)	6.39
Oncologic Medical ICU	18(0.6)	6.92
Burn ICU	16(0.5)	15.37
Cardiothoracic ICU	11(0.4)	2.21
Pediatric Medical/Surgical ICU	9(0.3)	2.64
Cardiac ICU	2(0.1)	0.61
Total	**3,080**	**5.70**

Table 9.7: Distribution of BSI cases by gender and age

Gender	No. of BSI cases (%)
Males	2026 (66%)
Females	1054 (34%)
Total	3,080 (100%)

	Median (Years)	Range (Years)
Age of males	41	0–93
Age of females	41	0–93

Table 9. 8: Median and range of ICU stay for BSI cases

	Median (Days)	Range (Days)
Duration of stay in unit	55.5	3–587
Duration between date of admission and date of event	51.5	3-1,101

Table 9.9: Outcomes of BSIs

14-day outcome	No. of BSI cases (%)
Died	1,173(38.1)
Still in surveillance unit	917(29.8)
Transferred to another ward	532(17.3)
Discharged	309(10.0)
LAMA	119(3.9)
Transferred to other hospital	28(0.9)
Unknown	2(0.1)
Total	**3,080**

Table 9.10: Distribution of organisms causing BSIs

S.No.	Type of organisms	Number (%)
1	Gram negative organisms	2,548**(73.3)**
2	Gram positive organisms	629(18.1)
3	Fungi	297(8.6)
Total		**3,474**

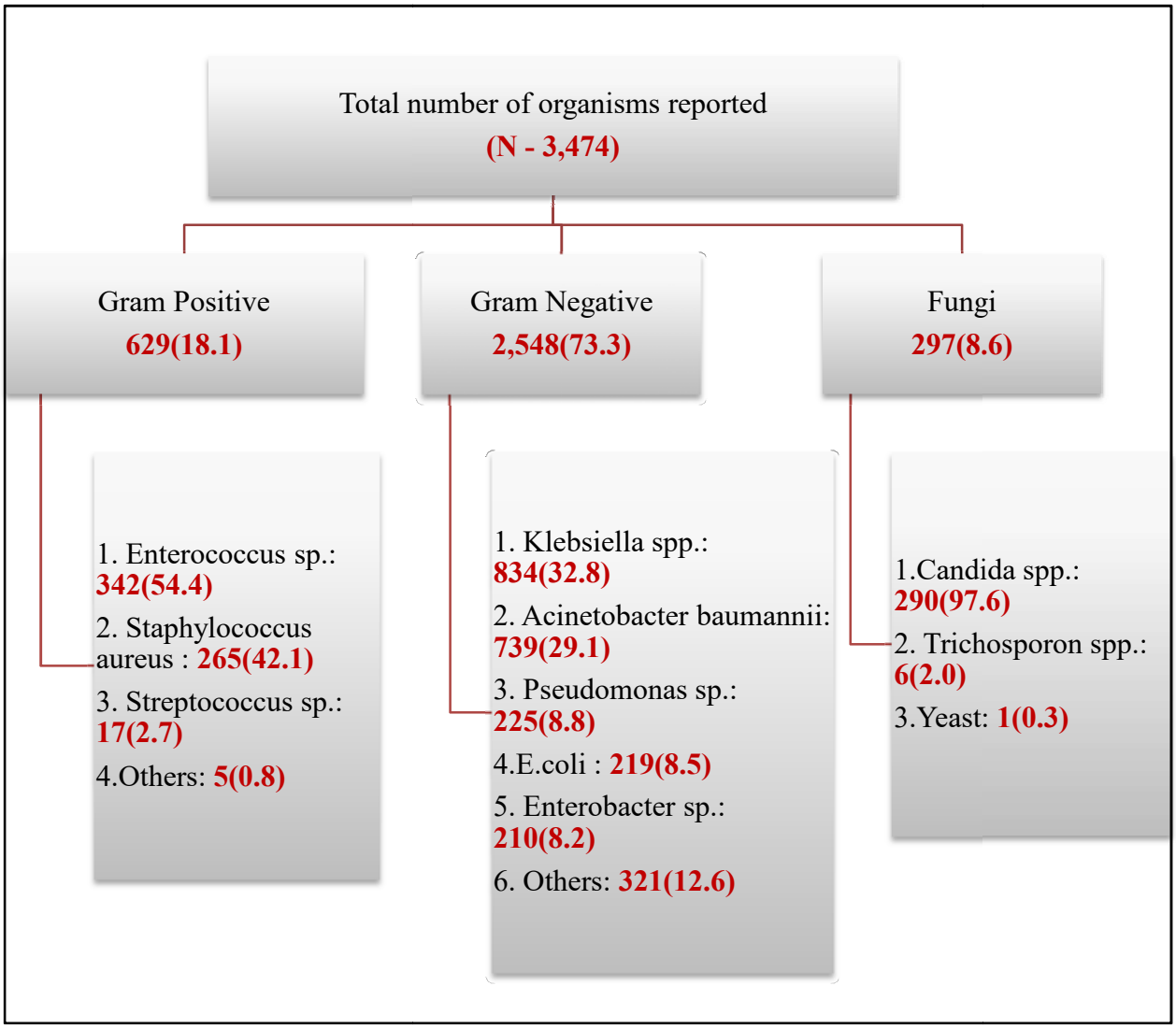


Figure 9.1: Distribution of organisms causing BSIs

Table 9.11: Distribution of Gram-positive organisms causing BSIs (Total BSIs)

S.No.	Name of organism	Number (%)
1	*Enterococcus sp.*	342(54.4)
2	*Staphylococcus aureus*	265(42.1)
3	*Streptococcus sp.*	17(2.7)
4	*Others*	5(0.8)
Total Gram Positive organisms		**629**

Table 9.12: Distribution of Gram-negative organisms causing BSI (Genus level)

S.No.	Name of organism	Number (%)
1	*Klebsiella sp.*	834(32.7)
2	*Acinetobacter sp.*	739(29.0)
3	*Pseudomonas sp.*	225(8.8)
4	*Escherichia sp.*	219(8.6)
5	*Enterobacter sp.*	210(8.2)
6	*Burkholderia sp.*	135(5.3)
7	*Stenotrophomonas sp.*	53(2.1)
8	*Serratia sp.*	33(1.3)
9	*Elizabethkingia sp.*	22(0.9)
10	*Others*	78(3.1)
Total Gram-Negative organisms		**2,548**

Table 9.13: Distribution of *Candida* sp. causing BSIs

S.No.	Name of organism	Number (%)
1	*Candidatropicalis*	74(24.9)
2	*Candida sp.*	48(16.2)
3	*Candidaparapsilosis*	40(13.5)
4	*Candida auris*	39(13.1)
5	*Candida albicans*	35(11.7)
6	Othercandida	53(17.9)
7	*Trichosporonsp.*	6(2.0)
8	Yeast£	2(0.7)
Total		**297**

£: As reported by the Centers

Central line associated bloodstream infections (CLABSIs) data

The denominator in cases of CLABSI is taken as the central line days. The risk of developing CLABSIs varies with the position of the Central lines. Table 9.14 shows the locations of Central lines in our surveillance data and table 9.15 shows the distribution of organisms causing CLABSIs. Even in CLABSIs, Gram negative pathogens predominated over Gram positives. A significant rate of CLABSI Candidemia was observed in our network. The Distribution of Gram positive, Gram negative and Candida species causing CLABSIs is shown in table 9.16-table 9.18.

Table 9.14: Location of Central lines

Location of central line	No. of CLABSI cases (%)	Total BSI rate (per 1,000 central line days)
Jugular	556(56.2)	3.69
Subclavian	281(28.4)	1.86
Umbilical	105(10.6)	0.70
Brachial	18(1.8)	0.12
Femoral	20(2.0)	0.13
HickmanLine	1(0.1)	0.01
Peripheral	5(0.5)	0.03
Mid-arm(Basilic vein)	3(0.3)	0.02
Total	**989***	**6.56**

*Multiple central lines possible in a single patient

Table 9.15: Distribution of organisms causing CLABSIs

S.No.	Name of organism	Number (%)
1	Gram positive organisms	290(17.0)
2	Gram negative organisms	1243(72.9)
3	Fungi	172(10.1)
Total organisms		**1,705**

Table 9.16: Distribution of Gram-positive organisms causing CLABSIs

S. No.	Name of organism	Number (%)
1	*Enterococcus sp.*	196(67.6)
2	*Staphylococcus Sp.*	62(27.2)
3	*Streptococcus sp.*	12(4.1)
4	*Others*	3(1.0)
Total Gram Positive organisms		**290**

Table 9.17: Distribution of Gram-negative organisms causing CLABSIs (Genus level)

S.No.	Name of organism	Number (Percentage)
1	*Klebsiella spp.*	394(31.7)
2	*Acinetobacter sp.*	328(26.4)
3	*Burkholderia spp*	117(9.4)
4	*Pseudomonas spp*	104(8.4)
5	*Escherichia sp.*	93(7.5)
6	*Enterobacter spp.*	90(7.2)
7	*Stenotrophomonas spp*	33(2.7)
8	*Serratia spp*	20(1.6)
9	*Elizabethkingia spp.*	15(1.2)
10	*Providencia spp*	10(0.8)
11	*Others*	39(3.1)
12	*Klebsiella spp.*	394(31.7)
Total Gram Negative organisms		**1243**

Table 9.18: Distribution of *Candida* sp causing BSIs

S. No.	Name of organism	Number (%)
1	*Candida tropicalis*	44(25.6)
2	*Candida sp.*	26(15.1)
3	*Candida auris*	23(13.4)
2	*Candida albicans*	21(12.2)
3	*Candida parapsilosis*	21(12.2)
4	*Candida glabrata*	14(8.1)
5	*Candida pelliculosa*	10(5.8)
6	Other candida	11(6.4)
7	*Trichosporon sp.*	2(1.2)
Total		**172**

Data of Primary Non-CLABSIs

Non CLABSI Primary BSIs are the BSI cases for which no secondary sources are traced and that do not have a central line in place for >/= two calendar days. The organism distribution of Non- CLABSI Primary BSIs is shown in table 9.19 to table 9.22.

Table 9.19: Distribution of organisms causing Non-CLABSI primary BSIs

S.No.	Name of organism	Number (%)
1	Gram positive organisms	310(24.6)
2	Gram negative organisms	857(68.0)
3	Fungi	93(7.4)
Total		1,260

Table 9.20: Distribution of gram-positive organisms causing Non-CLABSI Primary BSIs

S.No.	Name of organism	Number (%)
1	*Staphylococcus sp.*	169(54.5)
2	*Enterococcus sp.*	136 (43.9)
3	*Streptococcus sp.*	5(1.6)
Total Gram Positive organisms		310

Table 9. 21: Distribution of Gram-negative organisms causing Non-CLABSI Primary BSIs (Genus level)

S.No.	Name of organism	Number (%)
1	*Klebsiella pneumoniae*	294(34.3)
2	*Acinetobacter baumannii*	243(28.4)
3	*Escherichia coli*	96(11.2)
4	*Pseudomonas aeruginosa*	81(9.5)
5	*Enterobacter sp.*	75(8.8)
6	*Stenotrophomonas sp.*	16(1.9)
7	*Burkholderia sp.*	15(1.8)
8	*Serratia sp.*	11(1.3)
9	*Citrobacter sp.*	7(0.8)
10	*Elizabethkingia sp.*	5(0.6)
11	*Proteus sp.*	5(0.6)
12	*Others*	9(1.1)
Total Gram-Negative organisms		**857**

Table 9.22: Distribution of *Candida* sp. causing non-CLABSI Primary BSIs

S.No.	Name of organism	Number (%)
1	*Candida tropicalis*	23(24.7)
2	*Candida parapsilosis*	17(18.3)
3	*Candida spp.*	16(17.2)
4	*Candida auris*	13(14)
5	*Candida albicans*	7(7.5)
6	Other candida	15(16.2)
7	*Trichosporon sp.*	1(1.1)
8	Yeast	1(1.1)
Total		**93**

Data of Secondary BSIs

Secondary BSIs are those cases of BSIs in which a source of infection is found at some other body site and bacteremia is secondary to a primary source. The organism distribution in cases of secondary BSIs is shown in table 9.23 to table 9.26.

Table 9.23: Distribution of organisms causing Secondary BSI

S. No.	Name of organism	Number (%)
1	Gram positive organisms	29(5.7)
2	Gram negative organisms	448(88.0)
3	*Candida* sp	32(6.3)
Total		**509**

Table 9.24: Distribution of gram-positive organisms causing Secondary BSI

S.No.	Name of organism	Number (%)
1	*Staphylococcus sp.*	18 (62.1)
2	*Enterococcus sp.*	11(37.9)
Total Gram Positive organisms		**29**

Table 9.25: Distribution of Gram-negative organisms causing Secondary BSIs (Genus level)

S.No.	Name of organism	Number (%)
1	*Acinetobacter sp.*	168(37.5)
2	*Klebsiella sp.*	146(32.6)
3	*Enterobacter sp..*	48(10.7)
4	*Pseudomonas sp.*	40(8.9)
5	*Escherichia sp.*	30(6.7)
6	*Stenotrophomonas sp.*	4(0.9)
7	*Burkholderia sp.*	3(0.7)
8	*Proteus sp..*	3(0.7)
9	*Elizabethkingia sp..*	2(0.5)
10	*Serratia sp.*	2(0.5)
11	*Ralstonia sp.*	1(0.2)
12	*Salmonella sp.*	1(0.2)
Total Gram-Negative organisms		**448**

Table 9.26: Distribution of *Candida* sp. causing Secondary BSIs

S.No.	Name of organism	Number (%)
1	*Candida albicans*	7(21.9)
2	*Candida tropicalis*	7(21.9)
3	*Candida sp.*	6(18.7)
4	*Candida auris*	3(9.4)
5	Othercandida	6(18.7)
6	*Trichosporon sp.*	1(5.3)
Total		**32**

AMR in isolates causing BSIs

A high rate of resistance was seen against third generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides in *Klebsiella pneumoniae, E coli* and *Acinetobacter baumannii* causing BSIs. The rate of resistance in *Pseudomonas aeruginosa* was less as compared to these. Minocycline and Tigecycline appear to be a promising alternative in *Klebsiella* and *Acinetobacter* spp. (Table 9.27). Almost 50% strains of *E. faecium* causing BSIs were vancomycin resistant. No isolate of *S. aureus* had Vancomycin or Linezolid resistance.

Table 9.27: AMS Pattern for Gram Negative Organisms causing BSIs in HAI Surveillance Network, 2021

Antibiotics	*Klebsiella pneumoniae*	*Escherichia coli*	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*
	(N = 760)	*(N = 220)*	*(N = 460)*	*(N = 198)*
Amoxicillin-Clavulanate	19.9	31.8	1/15(6.7)	33.3
Amikacin	35.0	56.0	22.3	65.6
Ampicillin	3.9	4.8	-	-
Cefazolin	6.9	4.5	-	-
Cefepime	16.6	18.7	13.4	54.6
Cefotaxime	10.2	7.3	15.7	50.0
Ceftazidime	8.7	3.0	12.7	52.2
Ceftriaxone	10.5	8.6	12.3	35.7
Ciprofloxacin	16.6	13.4	16.0	61.4
Colistin	42.5	43.1	36.9	41.9
Ertapenem	23.9	47.1	12.5	-
Gentamicin	31.9	41.8	21.2	64.9
Imipenem	26.6	49.0	13.2	55.6
Levofloxacin	16.7	22.8	18.2	58.2
Meropenem	27.4	51.9	14.2	57.6
Minocycline	23.1	46.8	55.5	20.0
Netilmicin	22.4	28.6	19.8	55.4
Piperacillin	16.4	20.0	29.0	75.9
Tetracycline	58.5	36.7	34.0	33.3
Tigecycline	48.3	89.6	62.7	-
Tobramycin	27.4	38.1	28.6	58.9

Table 9.28: AMS Pattern for *Enterococcus species* causing BSI, 2021

Antibiotics	*Enterococcus faecalis* (N = 60)	*Enterococcus faecium* (N = 190)	*Enterococcus spp.* (N = 100)
Ampicillin	74.4	6.6	31.8
Ciprofloxacin	37.5	7.1	23.2
Gentamicin - High Level Resistance	49.1	18.5	34.6
Linezolid	100	79.2	96.4
Teicoplanin	100	52.9	81.6
Vancomycin	98.2	51.4	79.6
Nitrofurantoin	80	6.1	3/5(60.0)
Tetracycline	36.0	24.1	2/20(10.0)
Amikacin	1/1(100.0)	-	-

Table 9.29: AMS pattern for *Staphylococcus aureus* causing BSIs, 2021

Antibiotics	*Staphylococcus aureus* (N = 219)
Ampicillin	17.4
Erythromycin	22.6
Ciprofloxacin	23.8
Oxacillin	41.5
Clindamycin	49.3
Trimethoprim/Sulfamethoxazole	55.6
Tetracycline	71.6
Teicoplanin	100
Linezolid	100
Vancomycin	100

Urinary Tract Infections (UTI) data

A total of 792 cases of UTIs were reported in 2021. The distribution and profile of UTIs is shown in Table 9.30. The catheter associated UTI (CAUTI) rate was 2.97/ 1,000 urinary catheter days, as shown in table 9.31. The rates of total UTIs were compared against different types of ICUs, since the morbidity of patients varies with the different types of ICUs. Table 9.32 compares the rates of UTIs across the different ICU types in our network.

Table 9.30: Type of UTI cases

Type of UTI cases	No. of UTI cases (%)
CAUTI (catheter associated UTIs)	748 (94.4)
Non-CAUTI	44(5.6)
Total	**792**

Table 9.31: UTI rates

Indicator	Rates
UTI incidence rate (per1,000patientdays)	**1.51**
CAUTI rate(per1,000urinarycatheterdays)	**2.97**

Table 9.32: Distribution of UTI cases by ICUs

Type of ICUs	No. of UTI cases	UTI Rate (per 1000 patient days)
Medical/Surgical ICU	96 (12.1)	1.75
Neonatal ICU	3 (0.4)	0.03
Medical ICU	311 (39.3)	3.16
Surgical ICU	36 (4.5)	1.22
Pediatric Medical ICU	48 (6.1)	0.99
Anesthesia / Medical	98 (12.4)	7.59
COVID ICU	45 (5.7)	1.09
Gastrointestinal ICU	5 (0.6)	0.87
High Dependency Unit	15 (1.9)	1.21
Neurologic ICU	13 (1.6)	3.19
Oncologic Medical ICU	30 (3.8)	8.45
Respiratory ICU	8 (1.0)	2.75
Trauma ICU	84 (10.6)	5.44
Total	**792**	**1.67**

Table 9.33: Distribution of UTI cases by Gender and Age

Gender	No. of UTI cases (%)
Males	442(55.8%)
Females	350(44.2%)
Total	**792**

	Median	Range
Age of males	45.5	0 – 92
Age of females	47	0 – 86

Table 9.34 shows the duration of stay in the ICUs and the duration between ICU admission and the development of UTI. The duration of ICU stay is a risk factor for development of HAIs. Some patients had a very prolonged ICU stay and the UTI cases were found more in patients who had a longer ICU stay, across all ICU types. The 14-day mortality in cases of UTI was 27.9%. This may not be the actual attributable mortality, since severe primary illness or other underlying co-morbidities may be contributing to the fatal outcome. Only 11.4% of UTI cases were discharged at 14-day. Table 35 shows the short- term outcomes of UTI cases. A total of 883 pathogens were isolated from the UTI cases. Gram negative organisms predominated as the cause of UTIs in our network, as shown in Table 9.36-table 9.38.

Table 9.34: Duration between ICU admission and development of UTI

	Median	Range
Duration of stay in unit	16	3–454
Duration between date of admission And date of event	10	3–515

Table 9. 35: Outcome of UTI cases

14-day outcome	No. of UTI cases (%)
Died	221 (27.9)
Discharged	90 (11.4)
LAMA	33 (4.2)
Still in surveillance unit	243 (30.7)
Transferred to other hospital	7 (0.9)
Transferred to other ward/unit within the hospital	198 (25.0)
Unknown	0(0)
Total	**792**

Table 9.36: Distribution of organisms causing UTI

S.No.	Name of organism	Number (Percentage)
1	Gram Negative organisms	469(53.1)
2	Gram Positive organisms	165 (18.7)
3	Yeasts ∞	249(28.2)
Total		**883**

∞ In this surveillance network, *Candida* sp. was also included, in order to understand the epidemiology and significance of Candiduria.

Table 9.37: Distribution of organisms causing UTI (Genus level)

S. No.	Name of organism	Number (%)
1	*Candida spp.*	212(29.1)
2	*Escherichia spp.*	134(18.4)
3	*Enterococcus spp.*	123(16.9)
4	*Klebsiella spp.*	95(13.0)
5	*Pseudomonas spp.*	55(7.6)
6	*Acinetobacter spp.*	38(5.2)
7	*Proteus spp.*	14(1.9)
8	*Enterobacter spp.*	14(1.9)
9	*Myroides spp.*	12(1.6)
10	*Providencia spp.*	11(1.5)
11	*Others*	20(2.7)
Total		**883**

Table 38: Distribution of organisms (species level) causing UTI

S.No.	Name of organism	Number (%)
1	*Escherichia coli*	155(17.6)
2	*Klebsiella pneumoniae*	123(13.9)
3	*Candida spp.*	105(11.9)
4	*Enterococcus spp.*	80(9.1)
5	*Pseudomonas aeruginosa*	60(6.8)
6	*Candida albicans*	50(5.7)
7	*Enterococcus faecium*	49(5.6)
8	*Candida tropicalis*	45(5.1)
9	*Acinetobacter baumannii*	25(2.8)
10	*Enterococcus faecalis*	25(2.8)
11	*Pseudomonas spp.*	20(2.3)
12	*Candida auris*	17(1.9)
13	*Proteus mirabilis*	17(1.9)
14	*Candida glabrata*	12(1.4)
15	*Others*	100(11.3)
Total		**883**

*May not be accurate as all centres are not speciating

AMR of organisms causing UTI

A high rate of resistance was seen against third generation cephalosporins, carbapenems, fluoroquinolones, colistin, and aminoglycosides in *Klebsiella pneumoniae, E coli* and *Acinetobacter baumannii* and *Pseudomonas aeruginosa* causing UTIs; 40% isolates of *Enterococcus faecium* were vancomycin resistant.

Table 9.39: AMR Pattern for Gram Negative Organisms causing UTIs in HAI Surveillance Network, 2021

Antimicrobials	Organisms			
	Klebsiella pneumoniae (N=123)	*Escherichia coli (N=155)*	*Acinetobacter baumannii (N=25)*	*Pseudomonas aeruginosa (N=60)*
	% Susceptible			
Amikacin	27.94	61.59	20.00	39.53
Ampicillin	2.38 (1/42)	5.00	-	-
Cefazolin	9.80	4.92	-	-
Cefepime	17.28	15.05	18.18	32.31
Cefotaxime	11.11	9.17	15.00	40.00
Ceftazidime	11.11	6.38	17.65	19.74
Ceftriaxone	14.29	18.97	-	-
Ciprofloxacin	15.97	17.56	13.33	25.00
Colistin	64.71	79.37	84.62	80.85
Ertapenem	29.03	43.14	-	-
Gentamicin	29.06	54.20	6.90	30.43
Imipenem	36.80	51.80	14.29	30.12
Levofloxacin	14.29	17.65	12.50	9.52
Meropenem	33.05	55.74	21.43	33.33
Minocycline	27.27	27.78	46.15	100.00
Netilmicin	27.27	35.71	-	26.67
Piperacillin	12.00	16.00	28.57	35.71
Piperacillin/Tazobactam	27.64	38.06	27.59	41.77
Tetracycline	29.17	33.33	33.33	-
Tigecycline	70.59	79.17	33.33	-
Tobramycin	33.33	33.33	28.57	23.53
Amoxicillin/Clavulanate	14.04	18.57	-	-

Table 9.40: AMR Pattern for *Enterococcus species* causing UTI, 2021

Antimicrobials	Organisms		
	Enterococcus faecalis (N=25)	*Enterococcus faecium (N=49)*	*Enterococcus spp. (N=80)*
	% Susceptible		
Ampicillin	19.23	2.44 (1/42)	13.51
Ciprofloxacin	21.74	-	7.69
Gentamicin high level	23.81	19.51	15.28
Linezolid	95.83	84.21	93.15
Nitrofurantoin	62.50	16.13	61.64
Teicoplanin	68.42	54.29	35.29
Tetracycline	43.75	20.83	21.43
Vancomycin	76.92	60.78	70.93
Fosfomycin	82.35	87.50	100.00

Table 9. 41: Organisms causing BSIs Isolated in COVID Patients in the HAI Surveillance Network, 2021

Organism	Isolates in COVID Patients (N = 449)	% of Isolates in COVID Patients (N = 449)	Total Isolates (N = 3282)	% of Total Isolates (N = 3282)
Klebsiella pneumoniae	96	21.4	760	21.2
Acinetobacter baumannii	80	17.8	459	12.8
Acinetobacter baumannii Complex	43	9.6	190	5.3
Enterococcus faecium	31	6.9	190	5.3
Pseudomonas aeruginosa	29	6.5	198	5.5
Staphylococcus aureus	20	4.5	219	6.1
Escherichia coli	19	4.2	214	6.0
Candida tropicalis	18	4.0	81	2.3
Enterococcus sp.	15	3.3	100	2.8
Stenotrophomonas maltophilia	12	2.7	51	1.4
Enterococcus faecalis	9	2.0	60	1.7
Burkholderia cepaciae	7	1.6	128	3.6
Enterobacter cloacae	6	1.3	84	2.3
Klebsiella sp.	6	1.3	90	2.5
Candida albicans	5	1.1	36	1.0
Candida auris	5	1.1	37	1.0
Candida glabrata	5	1.1	18	0.5
Acinetobacter app.	4	0.9	105	2.9
Candida parapsilosis	4	0.9	41	1.1
Serratia marcescens	4	0.9	30	0.8
Streptococcus pneumoniae	3	0.7	5	0.1
Burkholderia cepacia	2	0.4	3	0.1
Candida sp.	2	0.4	48	1.3
Elizabethkingia meningoseptica	2	0.4	20	0.6
Enterobacter aerogenes	2	0.4	61	1.7
Staphylococcus haemolyticus	2	0.4	15	0.4
Staphylococcus hominis	2	0.4	5	0.1
Chryseobacterium indologenes	2	0.4	2	0.4
Non-Fermenting Gram Negative Bacilli	2	0.4	2	0.4
Aeromonas sp.	1	0.2	1	0.0
Candida krusei	1	0.2	4	0.1
Candida lusitaniae	1	0.2	1	0.0
Enterobacter kobei	1	0.2	1	0.0
Kluyvera sp.	1	0.2	1	0.0
Kodamaeaohmeri	1	0.2	1	0.0
Providencia rettgeri	1	0.2	5	0.1
Providencia stuartii	1	0.2	5	0.1
Salmonella sp.	1	0.2	2	0.1
Sphingomonas paucimobilis	1	0.2	1	0.0
Staphylococcus cohnii	1	0.2	1	0.0
Streptococcus infantarius	1	0.2	1	0.0

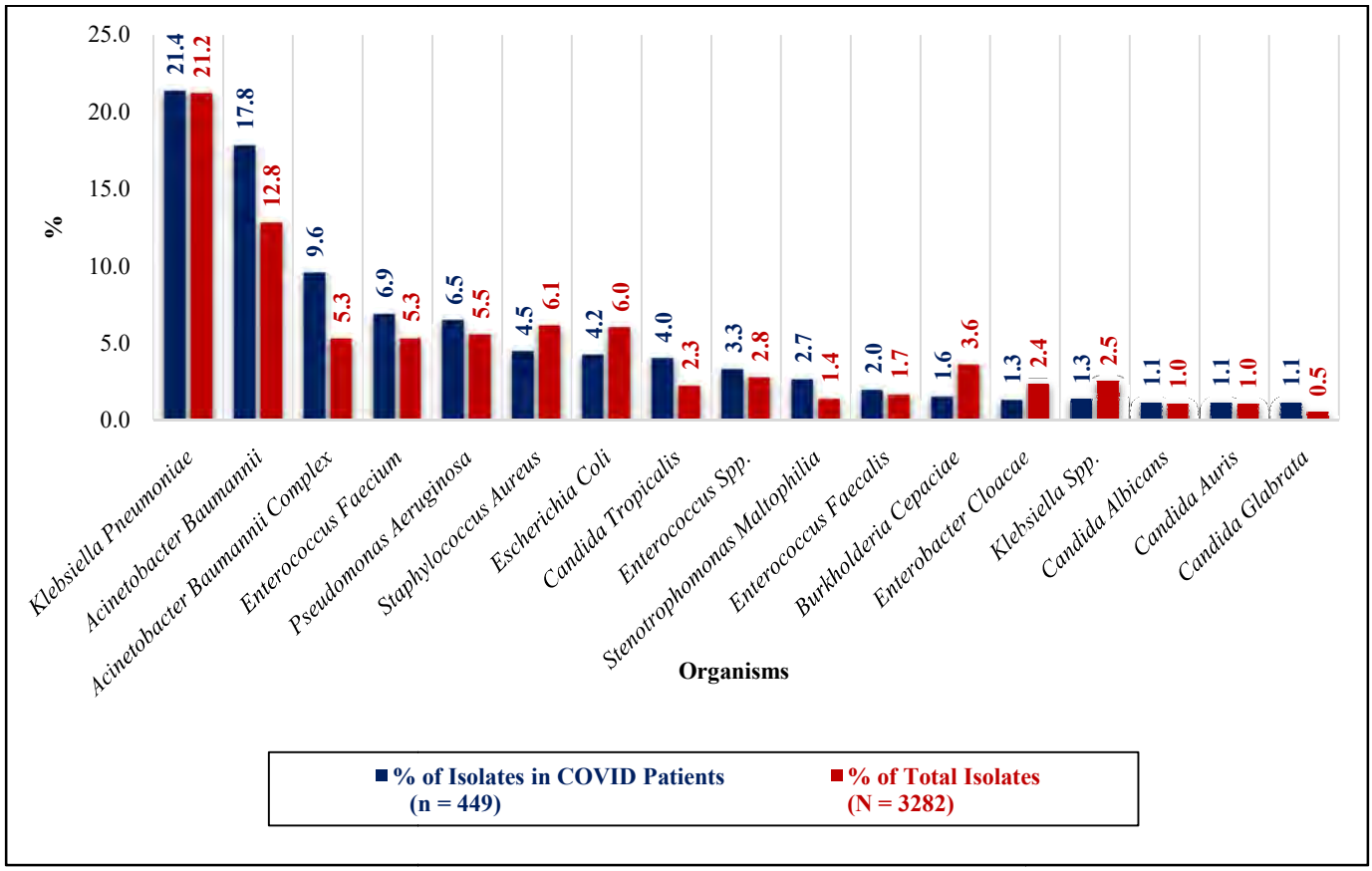


Figure 9.2: Organisms causing BSIs in COVID Patients in the HAI Surveillance Network, 2021

Table 9.42: Organisms causing UTIs Isolated in COVID Patients in the HAI Surveillance Network, 2021

	Isolates in COVID Patients (N = 83)	% of Isolates in COVID Patients (n = 83)	Total Isolates (N = 939)	% of Total Isolates (N = 939)
Candida sp.	16	19.3	106	11.3
Candida albicans	11	13.3	55	5.9
Escherichia coli	11	13.3	161	17.2
Klebsiella pneumoniae	10	12.1	133	14.2
Enterococcus faecium	8	9.6	53	5.6
Candida tropicalis	7	8.4	50	5.3
Enterococcus sp.	5	6.0	86	9.2
Candida glabrata	4	4.8	13	1.4
Candida auris	3	3.6	17	1.8
Pseudomonas aeruginosa	2	2.4	69	7.4
Acinetobacter baumannii	1	1.2	26	2.8
Citrobacter freundii	1	1.2	2	0.2
Enterococcus faecalis	1	1.2	28	3.0
Klebsiella sp.	1	1.2	11	1.2
Myroides species	1	1.2	1	0.1
Proteus mirabilis	1	1.2	17	1.8

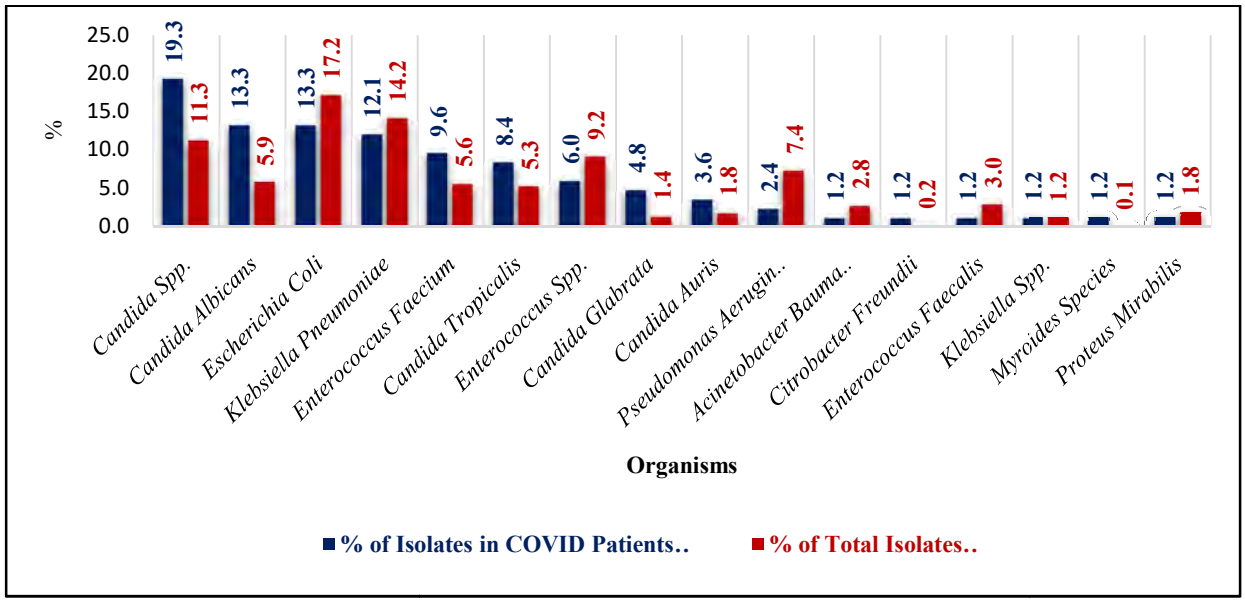


Figure 9.3: Organisms causing UTIs Isolated in COVID Patients in the HAI Surveillance Network, 2021

Appendix A

Compilation team (ICMR)

Dr Kamini Walia, Ph.D, MPH
Dr (Maj Gen) Vinod Ohri, MD
Dr. Nitin Bansal, MD
Dr Sonam Vijay, PhD
Ms Jasmine Kaur, MSc

List of contributors

Dr Arti Kapil
AIIMS, New Delhi
Dr Pallab Ray
PGIMER, Chandigarh
Dr V Balaji
CMC Vellore
Dr Sujatha Sistla
JIPMER, Pondicherry
Dr Purva Mathur
JPN Trauma Center, AIIMS, Delhi
Dr Shivaprakash
PGIMER, Chandigarh

Participating centers

Nodal centers

Dr Arti Kapil
Typhoidal Salmonella
AIIMS, New Delhi

Dr Pallab Ray
Enterobacterales
PGIMER, Chandigarh

Dr Sujatha Sistla
Staphylococci and Enterococci
JIPMER, Pondicherry

Dr V Balaji
Diarrheal pathogens
CMC ,Vellore

Dr V Balaji
Nonfermeting Gram negative bacteria
CMC ,Vellore

Dr V Balaji
Streptococcus pneumoniae
CMC ,Vellore

Regional Centers

Gp Capt SP Singh
AFMC, Pune
Dr. Vibhor Tak
AIIMS, Jodhpur
Dr. T. Karuna
AIIMS, Bhopal
Dr. Nandini Sethuraman
Apollo Hospitals, Chennai
Dr Arunjyoti Sarmah
Assam Medical College, Dibrugarh
Dr. Camilla Rodrigues
Hinduja hospital, Mumbai
Dr. Raja Ray
IPGMER, Kolkata
Dr. Purva Mathur
JPN Trauma Center, AIIMS, Delhi
Dr. Prashant Gupta
KGMU, Lucknow
Dr. Chiranjay Mukhopadhyay
KMC, Manipal
Dr.Dilip Turbadkar
LTMGH Sion Hospital, Mumbai
Dr. Vijayshri Deotale
MGIMS, Wardha

Dr Shivaprakash
Fungal pathogens
PGIMER, Chandigarh

Dr Purva Mathur
HAI surveillance
JPN Trauma Center, AIIMS, Delhi

Dr. Harpreet Singh
Data management unit
Bioinformatics Division, ICMR Hqrs

Dr. S. Sukanya
NIMS, Hyderabad
Dr Mamta Kshetrimayum
RIMS, Imphal
Dr. Bashir Ahmad Fomda
SKIMS, Srinagar
Dr. Chand Wattal
Sir Ganga Ram Hospital, New Delhi
Dr. Sanjay Bhattacharya
Tata Medical Centre, Kolkata
Dr Namita Jaggi
ARTEMIS Hospital, Gurgaon
Dr Anita Arora
Fortis Memorial Research Institute, Gurgaon
Fortis Escorts Heart Instiute, New Delhi
Fortis Hospital, Noida
Dr Anita Sharma
Fortis Hospital, Mohali
Dr B R Das
SRL Lab, Mumbai Dr Lal Path Lab, New Delhi

Image credits

Dr Pallab Ray
PGIMER, Chandigarh
Dr. Sujatha Sistla
JIPMER, Puducherry
Dr Nandini
Apollo Chennai