Prevalence and emergence of high drug resistant bacterial isolates from clinical samples in a tertiary care hospital of Chidambaram: a cross sectional study

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ABSTRACT

Background: This research aimed to study the prevalence and emergence of high drug-resistant bacterial isolates from clinical samples in a tertiary care hospital and their antibiogram profile. The existence of multidrug-resistant organisms is on the rise across the globe and is a severe problem. Knowledge of the prevalence and antibiogram profile of such isolates is essential to develop an appropriate treatment methodology.

Methods: A cross-sectional study was conducted in the Department of Microbiology, RMMCH, from July 2022 to December 2022. Different clinical specimens were collected from patients who were suspected of infections. A total of 470 clinical samples were collected and identified using conventional microbiological, biochemical tests following the Clinical and Laboratory Standard Institute (CLSI) guidelines. Antimicrobial susceptibility testing (AST) was performed using the *in vitro* Kirby-Bauer disk diffusion method. The screening test for ESBL producers was performed phenotypically using the double disk diffusion method.

Results: Overall, 316 bacterial isolates were isolated from 470 patients who had visited the hospital. Of these, 263 (83.2%) were gram-negative and the remaining 53 (16.8%) were gram-positive. The most common isolates were *Escherichia coli* 84 (26.6%), *Klebsiella pneumoniae* 73 (23.1%), *Staphylococcus aureus* 53 (16.8%) and *Pseudomonas aeruginosa* 42 (13.3%). The overall rate of MDR, XDR and PDR bacterial isolates from RMMCH were found to be 89 (28.16%), 157 (49.68%), and 33 (10.44%) respectively. Highest MDR 23 (25.84%), XDR 55 (35.03%), and PDR 7 (21.21%) showed *E.coli* Prevalence of ESBL, AmpC, and Carbapenemase-producing isolates. *Escherichia coli* and *Klebsiella pneumoniae* were the predominant isolates and were also the major ESBL producers. Besides Clindamycin, Erythromycin, Polymyxin B, Piperacillin/Tazobactam, Meropenem and Imipenem showed high efficacy.

Conclusions: In this study, a high antimicrobial resistance rate was observed like MDR, XDR, PDR, ESBL, AmpC and carbapenem which resistance rate is worrisome. We concluded that multidrug resistance was very high; carbapenems are the choice of drugs against ESBL producers but should be used as reverse drugs.

Keywords:

Antibiotics, Multidrug resistance, extensively resistance, Pan-drug resistance, Extended-spectrum beta-lactamase, Carbapenem resistance.

INTRODUCTION

Antimicrobial drugs have helped many individuals worldwide by saving lives and easing their suffering. However, the rise of drug-resistant bacteria has put into question the tremendous advantages of antibiotics in lowering morbidity and death [1]. Over the past 20 years, antibiotic-resistant bacteria have become a major worldwide threat [2]. Due to a shortage of antibiotic alternatives, illogical medication usage, poor drug quality, poor sanitation, starvation, poor and insufficient healthcare systems, and a lack of antibiotic stewardship programs, the issue is especially severe in developing nations [3]. In recent years, varieties of bacteria are becoming resistant to two or more classes of antibiotics as a result of selective pressure or horizontal gene transfer [4].

These resistant bacteria's recent discovery and spread have raised severe public health concerns. Particularly in nations with few resources, the proliferation of such germs [5]. Antibiotic resistance has been deemed a "global public health concern" by some significant organizations, including the World Health Organization (WHO), the Infectious Diseases Society of America, the World Economic Forum, and the Centers for Disease Control and Prevention (CDC). WHO has been commissioned by the World Health Assembly to provide a worldwide action plan to address the issue of antibiotic resistance [6]. Penicillin, broad-spectrum cephalosporin, aztreonam, and monobactam can all be hydrolyzed by ESBLs, a class of plasmid-mediated, varied, complicated, and quickly developing enzymes. Antibiotics called carbapenem are also ineffective against ESBL-producing bacteria. Bacteria that produce ESBLs and are carbapenem- and methicillin-resistant are widely spread [7]. In underdeveloped nations, the issue of ESBL-producing microbes is particularly severe.

The majority of Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* produce ESBLs [8]. The treatment of infections brought on by bacteria that are resistant to antibiotics is more challenging and, in some cases, impossible. These infections increase morbidity and death, which has a significant financial impact on healthcare [9]. Over the years, several new classes of β-lactam antibiotics have been created, but their excessive usage has led to the development of novel β-lactamases [1]. As a result, ESBL producers have emerged as an important multidrug-resistant pathogen, and during the past 20 years, considerable shifts in ESBL-producing isolates have been seen globally [10]. Multidrug-resistant bacterial infections are thought to be responsible for 23,000 fatalities annually in the USA and 25,000 deaths annually in the European Union (EU) [11]. It has been observed that the prevalence of isolates that produce MDR and ESBL is growing globally, and the majority of investigations in recent years have demonstrated this tendency [10].

Large levels of antibiotic resistance are caused by several types of antibiotic abuse, including the widespread illogical and careless use of large dosages of antibiotics, easy access to medicines, the practice of self-medication without a prescription, and prescriptions made even before the findings of an AST test. Clinicians typically struggle with choosing an appropriate empirical therapy for infections brought on by isolates that produce MDR and ESBL [12]. Clinicians must be knowledgeable about the clinical importance of ESBLs and possible management techniques. The optimal antibiotic therapy may be chosen with the aid of several additional parameters and knowledge of local epidemiology [13]. To direct effective antimicrobial therapy and reduce the danger of quickly developing drug resistance, regular monitoring of MDR and ESBL development is required.

Therefore, our goal was to identify the prevalence and antimicrobial susceptibility patterns (MDR, XDR, PDR, ESBL induced, and Carbapenem resistance patterns) of bacteria isolated from various clinical samples at Chidambaram's tertiary care facilities.

METHODS

Study design

A cross-sectional study was conducted for 6 months (July to December 2022) at the Department of Microbiology, Government Cuddalore Medical College Formerly Rajah Muthiah Medical College and Hospital which is located in Chidambaram, Cuddalore district, Tamil Nadu, India. It operates as Government Cuddalore Medical College and Hospital a 1200-bed tertiary level centre with 24-hour emergency department and coronary care unit. It is affiliated with Tamil Nadu Dr. M.G.R. Medical University from 2021.

Different clinical samples (blood, urine, Endotracheal aspirate, pus, and sputum) were collected aseptically from patients admitted to the hospital and from visiting outpatient departments of the hospital were included. The sample's data accessed from the Microbiology lab. In the case of urine and sputum, proper information about sample collection was given. Improperly collected samples or those lacking proper labelling were excluded from the study.

Isolation and identification of the isolates

A total of 470 samples [urine (177); blood (104); pus (90); sputum (53); and Endotracheal aspirate (46)] were processed and cultured by the following Clinical and Laboratory Standard Institute (CLSI) guidelines. The specimens were cultured on brain heart infusion (BHI) broth (only for blood samples), MacConkey agar, and blood agar. The isolates were identified based on colony morphology, Gram's stain result, and conventional biochemical methods.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was done by using the in vitro Kirby-Bauer disk diffusion technique using Muller Hinton agar (MHA) following Clinical and Laboratory Standards Institute guidelines. The antibiotics used were Amikacin (30 µg), Amoxicillin-Clavulanic acid (20/10 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Erythromycin (15 µg), Gentamycin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Nalidixic acid (30 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg), Oxacillin (1 µg), Piperacillin/Tazobactam (100/10 µg), Polymyxin B (100 IU), Tetracycline (30 µg), Trimethoprim- sulfamethoxazole (23.75/1.25 µg). All the antibiotic disks were procured from Himedia, Mumbai. The inhibition zone diameter was measured using an antibiotic zone scale and recorded on an Excel sheet.

Screening and confirmation of ESBL producers

ESBL Detection

All isolates from the primary screening of clinical specimens were used for the screening of ESBL detection by using the Double Disc Diffusion test. ESBL-positive *K. pneumonia* ATCC 700603 was used as a control [14]. For this, the test isolates were seeded on Mueller-Hinton agar, 30 µg ceftazidime and cefotaxime apart from a 24mm distance each disc was placed on the surface of the seeded MHA plate. 20 µg Amoxycillin with 10 µg clavulanate placed in the center of the plate. The plate was incubated at 37ºC overnight. The zone diameter was measured and β-lactamase was inferred if the zone of the disc Amoxycillin combined with the clavulanic acid was more than 8mm which was registered as resistant.

Test for Plasmid AmpC β-Lactamase

The cefoxitin-cloxacillin inhibition test was performed. The test isolates were seeded on Mueller-Hinton agar and two 30 µg cefoxitin discs were placed on the surface of the seeded MHA plate. One of the cefoxitin discs was supplemented with 200 µg cloxacillin. The plate was incubated at 37ºC for 24hrs. The zone diameter was measured and AmpC β-lactamase was inferred if the zone of the cefoxitin disc supplemented with cloxacillin was ≥4 mm greater than that of cefoxitin alone [14].

Tests for Inducibility of Chromosomal β-Lactamases (AmpC β-Lactamases)

MHA plates were inoculated with test organisms for ESBL detection. A 30µg ceftazidime and ceftriaxone disc were placed 20-24 mm apart from the cefoxitin 30 µg discs with cefoxitin disc at their center. The plates were incubated at 37ºC for 24hrs. Inducible AmpC β-lactamase was inferred if blunting was observed in the ceftazidime and ceftriaxone

zone of inhibition adjacent to the cefoxitin disc. A positive *Pseudomonas aeruginosa* isolate and a negative control strain *Escherichia coli* ATCC 25922 were used [14].

Metallo-β-Lactamase Detection (MBL)

Each test isolate was seeded on the surface of the MHA plate for ESBL detection. Imipenem and Meropenem discs (10 µg each) were placed on either side of a 1900 µg Ethylene Diamine Tetra Acetic acid (EDTA) disc, 10 mm apart from the EDTA disc (edge–to–edge) on the seeded plate. The plate was incubated at 37ºC for 24hrs. A synergistic zone of inhibition between the EDTA disc and one or both discs was taken as positive for metallo-β lactamase when compared with the negative control strain E. coli ATCC 25922 which did not show any synergism [14].

Data quality control

All batches of the culture media and chemical reagents were processed with aseptic techniques following CLSI guidelines and applying a standard aseptic procedure. Standard operating procedures (SOPs) were strictly followed while we did all bacteriological procedures starting from sample collection, isolation, identification and antibiotic susceptibility testing. To standardize the inoculum density of bacterial suspension for a susceptibility test, 0.5 McFarland standards, which is comparable with the approximate number of bacterial suspensions (1.0×10^8 to $2.0x10^8$ bacteria/mL), was used [15].

Data analysis and statistical tests

Data were entered into Microsoft Excel software version 2021 and transferred to SPSS version 20 for analysis. The results were presented as tables, pie charts and graphs. p-values < 0.05 were considered statistically significant.

Ethical consideration

The ethical approval of the study was got from the Human Institutional Ethics Committee (IHEC), at Annamalai University.

RESULTS

In the present study, 470 samples were collected from males 226 (48%) and females 244 (52%) $p<0.001$ (Table 1). A total of 470 samples were processed, out of which 316 (67.23%) were culture positive with significant growth and 154 (32.77%) had no growth (culture negative) $p<0.001$ (Table 2).

Table 1. Gender wise population

Gender	No.	Percentage
Male	226	48%
Female	244	52%
Total	470	100%

Table 2. Percentage of culture positives

Total No. of samples	Number	Percentage
No. of culture positive	316	67.23%
No. of culture negative	154	32.77%

Out of 316 cultures obtained or isolated from females 179 (57%) and males 137 (43%). There was no significant difference in growth positivity between males (43%) and females (57%) showed in Figure 1 ($p<0.001$). In the present study, maximum positivity of 263 (83.2%) Gram-negative bacteria were isolated from various clinical specimens. Escherichia coli 73 (23.1%), and Klebsiella pneumonia 53 (16.8%) were the predominant isolates. A total of 53 (16.8%) Gram-positive bacteria were isolated from various clinical specimens. Staphylococcus aureus, 42 (13.3%) were the predominant isolates. The highest number of organisms was isolated from the age- group 11–20 followed by 21-30 and the least from the age group 1 day – 1 year (Table. 3, & Fig. 3). However, growth positivity in urine was significantly higher in females 67 (37%) than in males 45(33%) ($p<0.001$) (Table. 4). The highest number of the organism was isolated from urine samples (112) followed by blood (73) shown in Table.5 & Fig.4. A total of 105 and 211 organisms were isolated from the inpatient department and outpatient department, respectively.

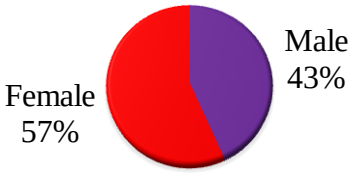


Figure 1. Over All Gener wise distribution of positive growth

Out of the total 316 culture-positive samples, 84 (26.6%) were *Escherichia coli*, 73 (23.1%) *Klebsiella pneumonia*, 53 (16.8%) *Staphylococcus aureus*, 42 (13.3%) *Pseudomonas aeruginosa*, 21 (6.6%) *Acinetobacter* species, 19 (6.0%) *Proteus vulgaris*, 13 (4.1%)

Enterobacter species, 7 (2.2%) *Citrobacter* species, and 4 (1.3%) *Serratia* species (Fig. 2).

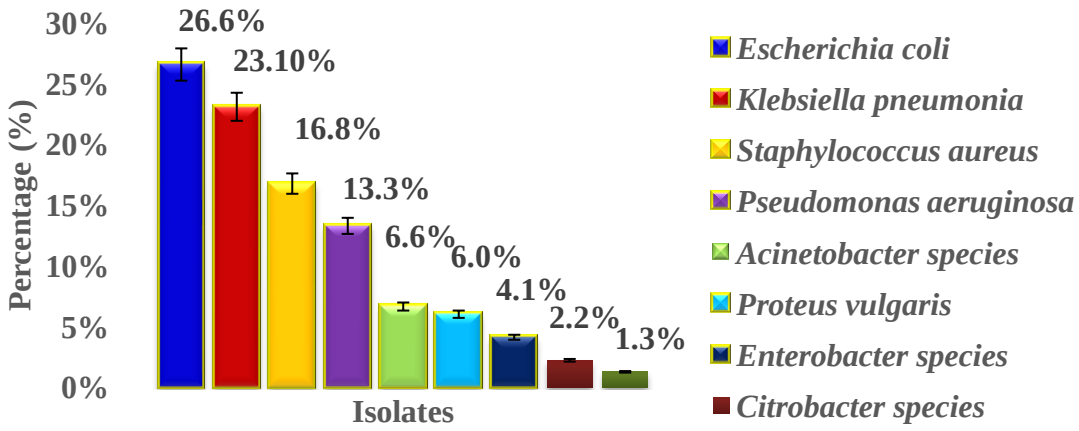


Fig.2 Distribution of various isolates

Table 3. Overall Age wise distribution of growth

Age	Growth	No Growth	p-value	Total
1 Day – 1 Year	13	7	0.101	20
01– 10 Years	29	15	<0.001	44
11– 20 Years	75	28	<0.001	103
21– 30 Years	64	25	<0.001	89
31 – 40 Years	43	17	<0.001	60
41 – 50 Years	41	25	<0.001	66
51 - 60 Years	33	21	0.003	54
> 60 Years	18	16	0.571	34
Total	316	154	<0.001	470

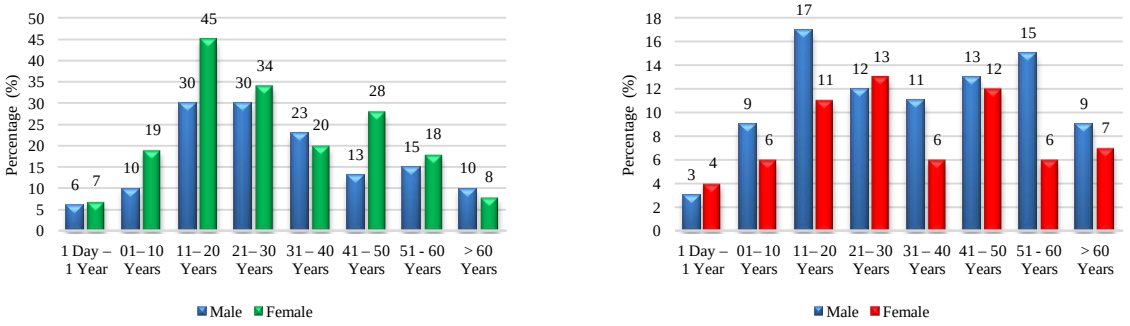
Table 4. Distribution of positive growth among various clinical specimens

Specimen	Male	%	Female	%	p-value	Total	%
Endotracheal aspirate	17	12%	14	8%	0.398	31	10%
Sputum	10	7%	24	13%	<0.001	34	11%
Pus	31	23%	35	20%	0.264	66	21%
Blood	34	25%	39	22%	0.167	73	23%
Urine	45	33%	67	37%	<0.001	112	35%
Total	137	100%	179	100%	<0.001	316	100%

Table 5. Distribution of cultures among various clinical specimens

Specimen	Positive	Negative	Total
Endotracheal aspirate	31	15	46
Sputum	34	19	53
Pus	66	24	90

Blood	73	31	104
Urine	112	65	177
Total	316	154	470



a (n=316) b (n=154)

Figure 3. Age and sex-wise distribution of positive growth (a) and no growth (b)

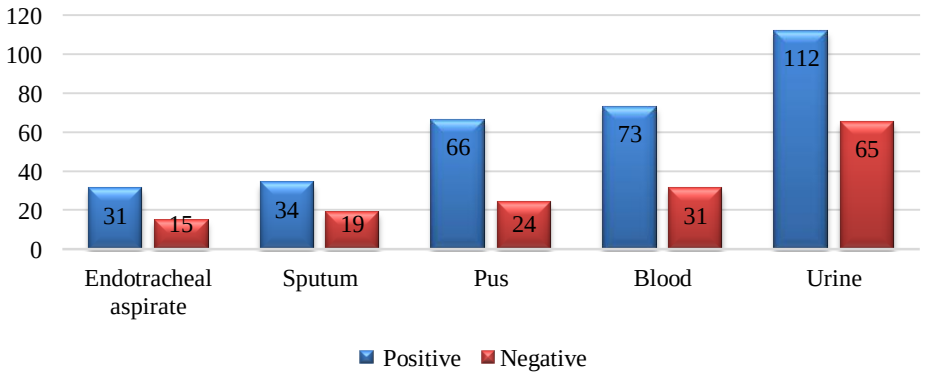


Figure 4. Overall growth from different specimens

Table 6 Sample-wise distribution of isolates

		Specimens					
S.No.	Microorganisms	Urine	Blood	Pus	Sputum	Endotracheal aspirate	Total
1	*E. coli*	29	20	15	10	10	84
2	*K. pneumoniae*	27	21	13	4	8	73
3	*Staphylococcus aureus*	14	12	20	2	5	53
4	*P. aeruginosa*	19	6	7	9	1	42
5	*Acinetobacter* spp.	6	5	4	4	2	21
6	*Proteus vulgaris*	10	3	2	1	3	19
7	*Enterobacter* species	4	3	3	2	1	13
8	*Citrobacter* species	2	2	1	1	1	7
9	*Serratia* species	1	1	1	1	0	4
	Total	112	73	66	34	31	316

Antibiotic susceptibility pattern

Antibiotic susceptibility pattern of clinical isolates of the 25 different antibiotics used. All isolates were tested for different categories of antimicrobials such as penicillin, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems (Table.7). E. coli isolates were the highest rate of resistance to AK 56 (66.67%) followed by IPM 38 (45.24%), NIT 31 (36.90%), NX/COT 20 (23.81%) and sensitive to CD/E. *Klebsiella pneumonia* isolates were the highest rate of resistance to TE 27 (36.99%), followed by COT 24 (32.88%), AK 22 (30.14%), GEN 21 (28.77%), AMP/C 20 (27.40%) and sensitive to OX/CD/E. *Staphylococcus aureus* isolates were the highest rate of resistance to PB 26 (49.06%), followed by NA 25 (47.17%), AK 21 (39.62%), COT/E 19 (35.85%) and sensitive to MRP/CAZ/AMC/IMP/AMP. P. aeruginosa isolates were the highest rate of resistance to NA 24 (57.14%), followed by AK 21 (50%), PIT 15 (35.71%), NX/IPM 12 (28.57%) and sensitive to IT/OX/CD/E. *Acinetobacter* spp. isolates were the highest rate of resistance to IPM/COT 16 (76.19%), followed by NA/GEN 14 (66.67%), MRP 13 (61.90%) and sensitivity to OX/CD/PIT. *Proteus* vulgaris isolates were the highest rate of resistance to AMC/TE 6 (31.58%), followed by AMP 5 (26.32%), IPM/AK/CAZ/CB/CTR 4 (21.05%) and sensitive to OX/CD/PIT/E. *Enterobacter* species isolates were the highest rate of resistance to AMC 6 (46.15%), followed by AMP/CAZ 5 (38.46%), TE/CTR/NA 4 (30.77%) and sensitive to OX/CD/E. *Citrobacter* species isolates were the highest rate of resistance to AMC/AMP/GEN 7 (100%), followed by CAZ/CTR/CIP 6 (85.71%) and were sensitive to OX/CD/E. *Serratia* species isolates were the highest rate of resistance to AMC/AMP/GEN/CAZ/CTR/CIP/TE/C/COT/MRP/NX 1 (25%) and sensitive to OX/CD/E/PB/IPM/NIT/NA/PIT/AK.

Table 7. Frequency of antimicrobial-resistant bacterial isolates for selected antibiotics

S.No.	Antibiotics	Symbol	*E. coli* (84)	*Klebsiella* sp (73)	*S. aureus* (53)	*P. aeruginosa* (42)	*Acinetobacter* sp (21)	*Proteus* sp (19)	*Enterobacter* sp (13)	*Citrobacter* sp (7)	*Serratia* sp (4)
1	Amikacin (30 µg)	AK	56	22	21	21	12	4	3	4	0
2	Amoxicillin-clavulanic acid (20/10 µg)	AMC	18	19	0	9	7	6	6	7	1
3	Ampicillin (10 µg)	AMP	19	20	0	9	1	5	5	7	1
4	Ceftazidime (30 µg)	CAZ	6	17	0	8	9	4	5	6	1
5	Ceftriaxone (30 µg)	CTR	5	16	15	9	3	4	4	6	1

6	Chloramphenicol (30 µg)	C	9	20	15	9	1	3	1	5	1
7	Ciprofloxacin (5 µg)	CIP	4	12	16	6	8	3	3	6	1
8	Clindamycin (2 µg)	CD	0	0	17	0	0	0	0	0	0
9	Erythromycin (15 µg)	E	0	0	19	0	1	0	0	0	0
10	Gentamycin (10 µg)	GEN	12	21	13	8	14	3	3	7	1
11	Imipenem (10 µg)	IPM	38	19	0	12	16	4	1	1	0
12	Meropenem (10 µg)	MRP	5	9	0	4	13	3	1	2	1
13	Nalidixic acid (30 µg)	NA	13	14	25	24	14	1	4	1	0
14	Nitrofurantoin (300 µg)	NIT	31	5	8	0	1	1	2	1	0
15	Norfloxacin (10 µg)	NX	20	15	16	12	2	2	1	1	1
16	Oxacillin (1 µg)	OX	17	0	14	0	0	0	0	0	0
17	Piperacillin/Tazobactam (100/10 µg)	PIT	12	7	10	15	0	0	1	2	0
18	Polymyxin B (100 IU)	PB	19	4	26	7	6	4	1	1	0
19	Tetracycline (30 µg)	TE	18	27	17	9	5	6	4	5	1
20	Trimethoprim- sulfamethoxazole (23.75/1.25 g)	COT	20	24	19	4	16	2	2	4	1

Based on the AST test the isolates were categorized as MultiS - susceptible to all antibiotic classes; MoDR - resistant to a single antibiotic class; MDR - resistant to at least one agent in three or more antimicrobial categories; XDR - resistant to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories); PDR - resistant to almost all antibiotic classes. The overall rate of MDR, XDR and PDR bacterial isolates from RMMCH were found to be 89 (28.16%), 157 (49.68%), and 33 (10.44%) respectively. The highest MDR 23 (25.84%), XDR 55 (35.03%), and PDR 7 (21.21%) showed *E.coli* followed by *K. pneumoniae*, *Staphylococcus aureus*, *P. aeruginosa*, *Acinetobacter* spp. *Serratia* species did not show any activity and *Citrobacter* species MDR 2 (6%), XDR 1 (3.03), and PDR 1 (3.03%) have shown the least rate (Fig.5).

Prevalence of ESBL, AmpC, and Carbapenemase-producing isolates of the 316 isolated, 69 (21.83%), 15 (4.74%) and 36 (11.39%) were positive for extended-spectrum beta-lactamase, AmpC and Carbapenemase respectively. Highest ESBL 29 (42.03%), AmpC 6 (40%), and Carbapenemase 11 (30.56%) showed by *E.coli*. In *Serratia* species ESBL, AmpC, and Carbapenemase 1 (2.78%) have shown the least rate. The prevalence of MDR was higher in males, whereas ESBL production was dominant in females. MDR prevalence was very high in all age groups. ESBL production was higher in the age groups 11–20 and 21-30 years. The percentage of MDR isolates was higher in outpatients than in inpatients, whereas ESBL production was higher in isolates from inpatients than the outpatient.

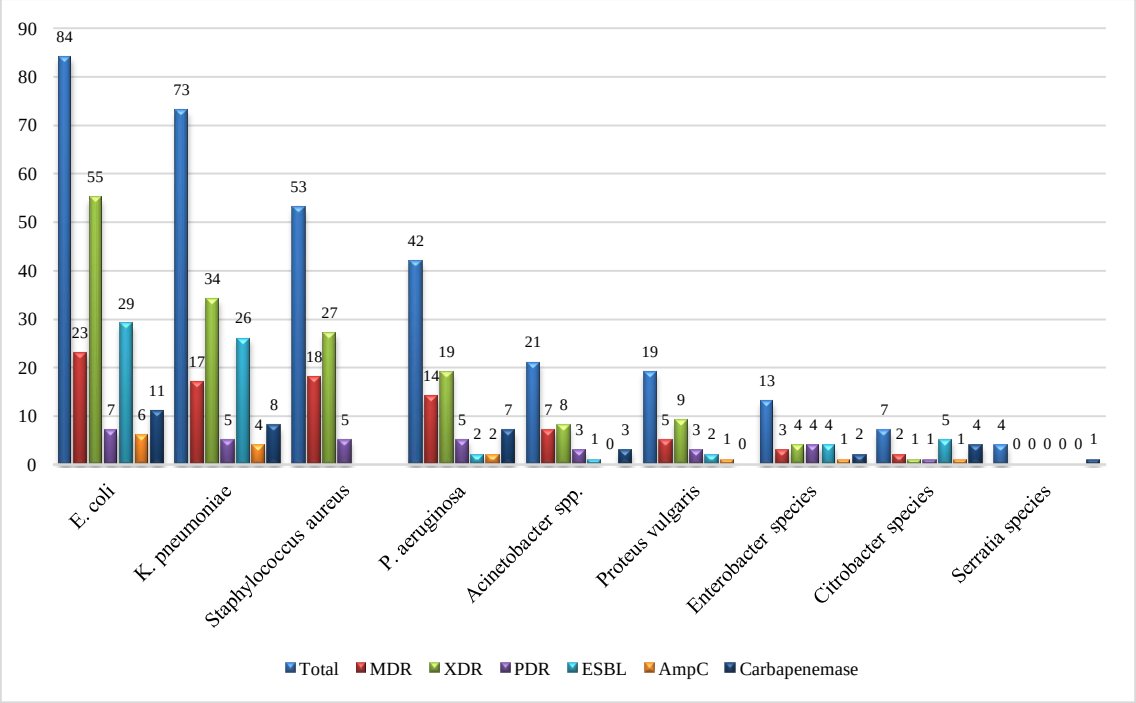


Figure 5 Frequency of MDR, XDR, PDR, ESBL, AmpC and Carbapenemase Production

DISCUSSION

In this study, 67.23% of the samples showed growth positivity. However, higher rates of growth positivity have been reported in similar studies retrospective record-based cross-sectional study conducted by Negam et al., (2021) [16] and Warda et al., (2022) [17]. The major proportion of the sample in our study included the urine sample. Hence, the highest number of growths was observed in the urine sample and is parallel to the finding of others. To compare with other similar studies Grundmann et al., 2017 [18] and De Laveleye et al. (2017) and [19] reported a higher prevalence of *K. pneumoniae* which Carbapenemase-producing isolates showed high resistance to last-line antibiotics. followed by. *E. coli*

Gram-negative microorganisms were the most common pathogens Klebsiella pneumoniae and Escherichia coli [16]. Gram-positive S. aureus most common pathogens sensitive to tobramycin, gentamicin, chloramphenicol, vancomycin, and ceftriaxone. In contrast, 94.0% of these gram-positive isolates showed resistance to penicillin. Multidrug resistance was observed in both gram-positive and negative bacteria. Our results of the overall MDR rate among the isolates was 73.4% [20].

Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. Carbapenems are widely regarded as the drugs of choice for the

treatment of severe infections caused by ESBL-producing *Enterobacter*iaceae, although comparative clinical trials are scarce [7]. Continuous and frequent surveillance for resistance patterns is necessary for the judicious and evidence-based use of antibiotics [21]. Comprehensive efforts are needed to minimize the pace of resistance by studying emergent microorganisms, resistance mechanisms, and antimicrobial agents. Multidisciplinary approaches are required across healthcare settings as well as the environment and agriculture sectors [3]. Measures to control the emergence and the spread of antibacterial resistance ABR are presented [5]. ESBL-producing isolates were characterized phenotypically for ESBL production using the double disc synergy test. Similarity of research done by [8]. ESBLs producers the highest sensitivity was observed with Imipenem, while the highest resistance was revealed with ceftriaxone [8].

The most frequent isolates were S. aureus, K. pneumoniae, *Proteus* spp., S. pneumoniae, *Citrobacter* spp., *Enterobacter* and P. aeruginosa. Overall, 45.2% of the isolates were multi-drug resistant [22]. rapid diagnostic plays a pivotal role in the treatment of bacterial infection. [12]. β-Lactams are the most widely used antibiotics, and β-lactamases are the greatest source of resistance to them. Resistance to ceftazidime or cefpodoxime implies extended-spectrum β-lactamase production in Escherichia coli and Klebsiella spp., especially if susceptibility to cefoxitin is retained. [23].

Antibiotic sensitivity testing showed that polymyxin B was the most effective antibiotic against Gram-negative bacteria, whereas vancomycin and linezolid were the most active antibiotics against Gram-positive pathogens. [17].

On the other hand, the observed MDR, XDR and PDR rate at the hospital indicates that the problem of AMR is increasing at an alarming rate and pathogenic bacteria that circulate in RMMCH are becoming more resistant to all available antibiotics.

CONCLUSIONS

Routine surveillance of MDR and ESBL producers and implementation of hospital infection control policies to prevent the transmission of such isolates is much required. Besides clindamycin, erythromycin, polymyxin B, Piperacillin/Tazobactam, meropenem and imipenem showed high efficacy and seem to be the choice of drugs against ESBL producers and Gram-negatives but should be considered alternatives until we have other sensitive drugs that could be administered safely. In this study, a high antimicrobial resistance rate was demonstrated. The observed high MDR, XDR, PDR, ESBL, AmpC and carbapenem resistance rate is worrisome. A coordinated effort is needed from all stakeholders working in the health system in Chidambaram to tackle this important public health problem. Immediate

action should be taken at the hospital to start an antibiotic stewardship program to reduce the observed antibiotic resistance and prevent further complications.

Limitations

There were a few limitations to our study. Firstly, the sample size was around 470, which seemed significantly less than in other studies. The study was conducted within a short duration, i.e., 6 months. The data taken was purely obtained from only one hospital, which might not represent the whole population. Only a phenotypic study was performed. Studies on the molecular level would have strengthened the findings.

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Authors' contributions

All authors made substantial contributions to conception and design, research work, and data analysis; took part in drafting the article or revising it; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

References

1. Miethke M, Pieroni M, Weber T, et al (2021) Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem 5:726–749
2. Ventola CL (2015) The Antibiotic Resistance Crisis: Part 1: Causes and Threats. Pharmacy and Therapeutics 40:277. https://doi.org/Article
3. Aslam B, Wang W, Arshad MI, et al (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645. https://doi.org/10.2147/IDR.S173867
4. Gashaw M, Berhane M, Bekele S, et al (2018) Emergence of high drug resistant bacterial isolates from patients with health care associated infections at Jimma University medical center: a cross sectional study. Antimicrob Resist Infect Control 7:. https://doi.org/10.1186/S13756-018-0431-0

5. Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109:309. https://doi.org/10.1179/2047773215Y.0000000030
6. Aslam B, Wang W, Arshad MI, et al (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645. https://doi.org/10.2147/IDR.S173867
7. Shaikh S, Fatima J, Shakil S, et al (2015) Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci 22:90. https://doi.org/10.1016/J.SJBS.2014.08.002
8. Yousefipour M, Rasoulinejad M, Hadadi A, et al (2019) Bacteria Producing Extended Spectrum β-lactamases (ESBLs) in Hospitalized Patients: Prevalence, Antimicrobial Resistance Pattern and its Main Determinants. Iran J Pathol 14:61. https://doi.org/10.30699/IJP.14.1.61
9. La Rosa R, Johansen HK, Molin S (2022) Persistent Bacterial Infections, Antibiotic Treatment Failure, and Microbial Adaptive Evolution. Antibiotics 11:. https://doi.org/10.3390/ANTIBIOTICS11030419
10. Shilpakar A, Ansari M, Rai KR, et al (2021) Prevalence of multidrug-resistant and extended-spectrum beta-lactamase producing Gram-negative isolates from clinical samples in a tertiary care hospital of Nepal. Trop Med Health 49:. https://doi.org/10.1186/S41182-021-00313-3
11. Yang X, Ye W, Qi Y, et al (2021) Overcoming Multidrug Resistance in Bacteria Through Antibiotics Delivery in Surface-Engineered Nano-Cargos: Recent Developments for Future Nano-Antibiotics. Front Bioeng Biotechnol 9:696514. https://doi.org/10.3389/FBIOE.2021.696514/BIBTEX
12. Khan ZA, Siddiqui MF, Park S (2019) Current and Emerging Methods of Antibiotic Susceptibility Testing. Diagnostics 2019, Vol 9, Page 49 9:49. https://doi.org/10.3390/DIAGNOSTICS9020049
13. Rawat D, Nair D (2010) Extended-spectrum β-lactamases in Gram Negative Bacteria. J Glob Infect Dis 2:263. https://doi.org/10.4103/0974-777X.68531
14. Ibadin EE, Omoregie R, Igbarumah IO, et al (2017) Prevalence of Extended Spectrum β-Lactamase, AmpC β-Lactamase and Metallo-β-Lactamase Among Gram Negative Bacilli Recovered From Clinical Specimens in Benin City, Nigeria. Int J Enteric Pathog 5:85–91. https://doi.org/10.15171/ijep.2017.20
15. Clinical and Laboratory Standards Institute - CLSI (2016) M100S Performance Standards for Antimicrobial
16. Negm EM, Mowafy SMS, Mohammed AA, et al (2021) Antibiograms of intensive care units at an Egyptian tertiary care hospital. The Egyptian Journal of Bronchology 15:. https://doi.org/10.1186/S43168-021-00059-W
17. Warda EA, Torti C, Subbian S, et al (2022) Emergence of High Antimicrobial Resistance among Critically Ill Patients with Hospital-Acquired Infections in a Tertiary Care Hospital. mdpi.com. https://doi.org/10.3390/medicina58111597
18. Grundmann H, Glasner C, Albiger B, et al (2017) Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective,

multinational study. Lancet Infect Dis 17:153–163. https://doi.org/10.1016/S1473-3099(16)30257-2

19. De Laveleye M, Huang TD, Bogaerts P, et al (2017) Increasing incidence of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in Belgian hospitals. Eur J Clin Microbiol Infect Dis 36:139–146. https://doi.org/10.1007/S10096-016-2782-X
20. Woreta AN, Kebede HB, Tilahun Y, et al (2022) Antibiotic Susceptibility Pattern and Bacterial Spectrum Among Patients with External Eye Infections at Menelik II Referral Hospital in Addis Ababa, Ethiopia. Infect Drug Resist 15:765–779. https://doi.org/10.2147/IDR.S352098
21. Chakraborty A, Pal NK, Sarkar S, Gupta M Sen (2015) Antibiotic resistance pattern of Enterococci isolates from nosocomial infections in a tertiary care hospital in Eastern India. J Nat Sci Biol Med 6:394. https://doi.org/10.4103/0976-9668.160018
22. Ayehubizu Z, Mulu W, Biadglegne F (2021) Common bacterial causes of external ocular infections, associated risk factors and antibiotic resistance among patients at ophthalmology unit of Felege Hiwot Referral Hospital, Northwest Ethiopia: a cross-sectional study. J Ophthalmic Inflamm Infect 11:. https://doi.org/10.1186/S12348-021-00238-2
23. Livermore DM, Brown DFJ (2001) Detection of beta-lactamase-mediated resistance. J Antimicrob Chemother 48 Suppl 1:59–64. https://doi.org/10.1093/JAC/48.SUPPL_1.59