Surveillance of Antimicrobial Susceptibility and Resistance Among Enterobacteriaceae Isolates from The Clinical Specimens in Secondary Referral Hospital

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Surveillance of Antimicrobial Susceptibility and Resistance Among Enterobacteriaceae Isolates from The Clinical Specimens in Secondary Referral Hospital

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Abstract: The aim of our study is to assess the pattern of antimicrobial susceptibility and resistance to the bacterial pathogens isolated from patient specimens. The main objectives are to evaluate the pattern of antimicrobial susceptibility and resistance to the bacterial pathogens isolated from patient specimens, to determine the proportion of antimicrobial sensitivity and resistance against specific antibiotics by bacterial pathogens isolated from various specimens collected and to assess the difference in Resistance of bacterial isolates to various antibiotics within different years. Emergence of antimicrobial resistance is a major public health problem worldwide. Antimicrobial resistance is one of the ten threats identified by the World Health Organization in 2019. Approximately 0.7 million people die every year from AMR. The WHO estimates 350,000,000 deaths could be caused by AMR by 2050. For three years the retrospective observational study was conducted among all the age groups of people. Antibiograms were used based on CLSI guidelines. A total of 2430 samples, 1226 males and 1204 females, are there. The samples were collected by different types like Urine Blood, Pus, Tissue culture, Stool culture, swab culture, CSF, and other fluids. Among all the isolated bacteria *E. coli, Klebsiella, and Enterobacter* were more isolated and were more resistant to the penicillins and cephalosporins category of drugs. The study shows that isolated gram-negative bacteria were resistant to ampicillin, amoxicillin, cephalothin, ciprofloxacin, cefuroxime, cefepime, ceftazidime, and ceftriaxone. So, these drugs can be replaced with organism-sensitive antibiotics like amikacin, chloramphenicol, colistin, and gentamicin to treat bacterial infections. When compared to year to year, the resistance will be increased.

Keywords: Antimicrobial Resistance, Enterobacteriaceae, Isolated Bacteria, And Sensitivity.

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1.   INTRODUCTION

Antimicrobial resistance (AMR) is a critical problem in the 21st century [1]. Approximately 0.7 million people die every year worldwide from drug-resistant strains of microbes. The number is estimated to increase to 10 million by 2050 [2]. Antimicrobial resistance (AMR) poses health and economic burdens for patients and healthcare systems globally. India has a large burden of infectious diseases and is one of the largest consumers of antibiotics in the world [3]. The efficacy interlinked factors, including the high burden of illness, poor public health infrastructure, lack of appropriate diagnostic support, poor infection control practices, and the tendency of clinicians to continue empirical treatment practices, have amplified the crisis of AMR in India [4] Unregulated over-the-counter availability of antibiotics and non-compliance to the recommended treatment duration have been recognized as critical drivers for the emergence of resistance in India[5] The resistant bacterial strains emerging out of selection pressure to spread either through hospital-acquired infections or from the community. Non-availability of nationwide data on estimates of the extent of drug resistance significantly limits the concerted response against AMR in India. The problem of antimicrobial resistance is not only the development of the resistance but also the transmission of the resistant strains from one person to another. Most of the AMR data available in the past have been from individual hospitals and from small networks, which did not represent the national picture [6] Among the available approaches, surveillance has been reported to be the best approach for reducing infection spread [7.] It is believed that identifying resistance patterns and factors contributing to AMR, together with the reduced consumption of antimicrobials, may help control the emergence and spread of AMR in pathogens [8]. As per the 'scoping reports of antimicrobial resistance (2017), the government of India, among the Gram-negative bacteria, more than 70% of isolates *E. coli, Klebsiella pneumonia,* were resistant to the fluoroquinolones and third-generation cephalosporins, geographical variations in sensitivity are also noted by studies were conducted in the North side of India. The Geographical and time-based variations in antibiotic resistance and sensitivity have been reported by the many studies[9.] In India the treatment of most bacterial infections is usually made empirically in which the etiologic agents are rarely identified. So, identifying the most common bacterial pathogens and their respective AMR profile would be valuable to optimize treatment and ultimately to reduce morbidity and mortality associated with infectious diseases. Therefore, this study aimed to assess the type of pathogenic bacterial isolates and their antimicrobial resistance profile from different kinds of clinical samples at secondary care referral hospitals in India.

2.   MATERIALS AND METHODS

The retrospective observational study was conducted at the secondary care referral hospital three years later. Bacteriological data recorded from April 2018 to October 2020 were retrieved for analysis using a predefined data extraction sheet. In addition, patient-related data (age and sex) with a full record of bacteriological culture and antimicrobial resistance profile were retrieved from the laboratory records. A total of 2440 specimens were collected. Out of all the collected reports, those specimens positive to at least a single bacterial pathogen were classified as either susceptible or resistant to specific antibiotics tested. In addition, the difference in resistance of bacterial isolates to various antibiotics within different years was analysed.

3.   STATISTICAL ANALYSIS

MS - Excel spreadsheets and SPSS version20 (Statistical software) were utilized for data analysis. Percentages were calculated for all categorical variables.

3.1   Study Criteria

3.1.1   Inclusion Criteria

a. All the non-repeated culture and sensitivity test reports will be included for analysis.
b. The clinical specimen collected from urine, stool, pus, sputum, or blood will be included for analysis.
c. All the test reports belonging to either gender and at any age will be included.

3.1.2   Exclusion Criteria

a. The test reports with improper/missing data like age, gender, sample type, and the test result will be excluded.

3.2   Ethical Considerations

Ethical clearance has been obtained from the institutional review board (RIPER/IRB/PP/2020/007).

3.3   Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests were carried out using the Kirby–Bauer disc diffusion method as per the Clinical Laboratory Standards Institute (CLSI) guidelines on Muller–Hinton agar. Suspension of 3–5 pure colonies of freshly grown test organisms was prepared equivalent to 0.5 McFarland standards. The Muller–Hinton agar surface was then completely covered by rotating the swab with the suspension. Muller–Hinton agar supplemented with 5% lysed/defibrinated whole blood was used for fastidious microorganisms. Plates were allowed to dry for 3–5 minutes; then, discs were evenly distributed 24mm apart on the inoculated plate using sterile forceps and incubated at 37°C for 18–24 hours. The diameter of the zone of inhibition around the disc was measured using a ruler. Results were interpreted as sensitive, intermediate, and resistant based on the CLSI 2016 guideline. Following routinely used antimicrobials were tested: ampicillin (10 μ g), amoxicillin-clavulanic acid (30μ g), cephalothin (30) mcg, ceftriaxone (30μ g), ciprofloxacin (5μ g), chloramphenicol (30) μ g), gentamicin (10 μ g), piperacillin (100μ g), amikacin(30mcg), penicillin10 IU), vancomycin (30μ g), oxacillin (1μ g), clindamycin (2μ g), and cefoxitin (30μ g). Cefoxitin disc (30μ g), nalidixic acid (30mcg), doxycycline (30mcg). linezolid (30mcg), ceftazidime(30mcg).

4. RESULTS

Table 1 : Age and sex distribution of the study participants 2018–2020			
Age in Years	Males	Females	Total
0-12	123(40.19)	183(59.8)	306(12.59)
13-18	147(51.04)	141(48.95)	288(11.5)
19-59	820(59.59)	655(44.4)	1475(60.95)
≥ 60	136(36.67)	225(62.32)	361(14.85)
Total	1226(50.45)	1204(49.54)	2430(100)

p< 0.05

A total of 2430 samples were collected among 1226 males and 1204 females; samples were divided into the age group into the four categories like 0-12 years,13-18 years,19-59 years, and >60 years. Among these age groups, both males and females, 0-12 years of age group have 306 samples,13-18years have 288 samples,19-59 have 1475 samples and >60 age group samples are 361. Among all the age groups, the 19- 59 age group has a greater number of samples found, and compared to the gender, and males are more samples than females. Therefore, our study shows that the 15- 59 people are more affected by the infections, as shown in Table 1.

Table 2 The distribution of identified bacterial pathogens from different clinical samples 2018–2020										
Isolated bacteria	Blood	Urine	Stool	Sputum	Pus	Swab culture	Any other fluids	Tissue culture	CSF	Other	Total
E. Coli	86 (5.23)	848(51.64)	1(0.06)	26(1.58)	216(13.15)	183(11.14)	24(1.46)	227(13.82)	7(0.42)	24(1.46)	1642(16.57)
K.Pneumonia	47(10.51)	134(29.97)	0(0)	38(8.50)	46(10.29)	89(19.91)	19(4.25)	69(15.43)	1(0.22)	4(0.89)	447(18.39)
Citrobacter species	1(6.6)	0	0	0	6(40)	5(33.3)	1(6.6)	1(6.6)	1(6.6)	0	15
Shigella species	1(6.25)	2(12.5)	9(56.2)	0	0	3(18.7)	0	0	1(6.25)	0	16
Salmonella typhi	87(95.600)	1(1.09)	1(1.09)	0(0)	1(1.09)	0(0)	0(0)	1(1.09)	0(0)	0(0)	91(3.74)
Enterobacter Species	17(10.2)	16(9.6)	0	6(3.6)	43(25.9)	42(25.3)	31(18.6)	26(15.6)	2(1.2)	3(1.8)	166
Serratia Marcescens	6(30)	1(5)	0(0)	1(5)	3(15)	1(10)	0(0)	3(15)	0(0)	4(20)	20(0.82)
Proteus species	0	1(7.6)	0	0	7(53.8)	4(30.7)	0	1(7.6)	0	0	13
Providensia species	3(23)	0	0	2(15.3)	5(38.4)	2(15.3)	0	8(61.5)	0	0	20

The values in the brackets are percentages for sample type and isolated bacterial pathogens.

Samples were collected from different methods like blood, urine, stool pus swab culture, tissue culture, CSF, peripheral fluids, other fluids, etc,. Among all the different clinical samples, all were isolated bacteria only. The majority of the bacteria were isolated from urine culture 846(51.64), tissue culture 227(13.82), pus culture 216 (13.15), and swab culture 183 (11.13). Among all these culture samples, the isolated bacteria were *E. coli, klebsiella pneumonia, Citrobacter freundii, Shigella dysenteriae, Salmonella typhi, Enterobacter cloacae, Enterobacter* .

species, Serratia Marcescens, Shigella species, Proteus penneri, Proteus Vulgaris, Providencia rettegiri, Providencia stuartii, Providencia Analifaiens, Shigella flexineria, Enterobacter asburiae, and *Citrobacter Koseri*. Of all these isolated bacteria E. *coli and Klebsiella pneumonia* bacteria were more found. For *E. coli* only two years (2019-2020) of data will be collected due to the unavailability of clear data. For all these samples, percentage calculations were done with clinical samples with isolated pathogens

Table 3 Antimicrobial resistance profile of the isolated organism and its tested antimicrobial agents									
Isolated bacteria	E.coli	Klebsiella Pneumonia	Citrobacter freundii	Shigella species	Salmonella typhi	Enterobacter species	Proteus species	Providence species	Seratia marcescens
Antibiotics	1641	448	9	15	91	166	13	20	20
AMX	1438 (87.6)	381 (85)	7 (77.5)	3 (20)	0	144 (86.7)	11 (84.6)	12 (60)	17 (85)
AMK	90 (5.4)	55 (12.2)	1 (11.1)	0	0	23 (13.8)	0	4 (20)	1 (5)
AMP	1555 (94.7)	427 (95.3)	7 (77.5)	8 (53.3)	5 (5.4)	146 (87.9)	12 (92.3)	16 (80)	17 (85)
CPL	1498 (91.2)	331 (73.8)	6 (66.6)	1 (6.6)	0	132 (79.5)	12 (92.3)	12 (60)	13 (65)
CIP	1114 (67.8)	222 (49.5)	3 (33.3)	9 (60)	42 (46.1)	54 (32.5)	3 (23)	6 (30)	0
CFX	1395 (85)	220 (49.1)	5 (59.56)	1 (6.6)	0	126 (75.9)	10 (76.9)	15 (75)	15 (75)
CHF	89 (5.4)	57 (12.72)	1 (11.1)	4 (26.6)	0	25 (15)	2 (15.3)	6 (30)	3 (15)
clt	12 (0.7)	4 (0.8)	2 (22.2)	0	0	3 (1.8)	9 (69.2)	13 (65)	10 (50)
CFP	1110 (67.6)	192 (42.8)	4 (44.4)	1 (6.6)	0	68 (40.9)	4 (30.7)	6 (30)	13 (65.5)
CTX	905 (55.1)	59 (35.4)	2 (22.2)	8 (53.3)	1 (1.1)	45 (27.1)	6 (46.1)	8 (40)	0
GEN	370 (22.5)	82 (82.5)	1 (11.1)	0	0	27 (16.2)	1 (7.6)	4 (20)	0
MER	92 (5.6)	41 (9.5)	1 (11.1)	0	0	8 (4.8)	0	4 (20)	0
PIP	250 (15.2)	86 (19.2)	2 (22.2)	0	0	44 (26.5)	1 (7.6)	6 (30)	1 (5)
PG	34 (2)	26 (5.8)	0	0	0	2 (1.2)	1 (7.6)	0	0

The percentages for the isolated organisms and testing drugs R% resistance rate, AMX-amoxicillin, AMP-ampicillin, AMK-amikacin, CPL-cephalothin , CIP- ciprofloxacin, CFX- cefuroxime, CHF- chloramphenicol, CLT- colistin, CFP- cefepime, CTX- cotrimoxazole, GEN- gentamicin, MER- meropenem, PIP- piperacillin- tazobactam, PG- penicillin G, DOX- doxycycline, SPT- streptomycin, LNZ- linezolid, NFT- nitrofurantoin, CTZ-ceftazidime, CRO- ceftriaxone , VAN- vancomycin, CL- clindamycin, RIF- rifampicin

We have collected the *Enterobacteriaceae family* samples among the 17 different types of organisms found, among which *E. coli, Klebsiella Pneumonia, Enterobacter SPS, and salmonella typhi* were more isolated. For these organisms' we have done the antibiotic sensitivity test for commonly used drugs in our area drugs like amoxicillin, ampicillin, amikacin, cephalothin/cefadroxil, chloramphenicol colistin co-trimoxazole, gentamicin, meropenem, piperacillin, penicillin - G, doxycycline, streptomycin, linezolid, nitrofurantoin, vancomycin clindamycin, cefepime, ciprofloxacin, ceftazidime, and ceftriaxone and rifampin. Among all these drugs amoxicillin ampicillin, amikacin, cefuroxime, and chloramphenicol are more resistant. other drugs like cephalothin, cefepime, co-trimoxazole, meropenem, ceftazidime, and ceftriaxone are moderate resistance and others are mild resistance .so that this data is helped in the drugs which are effective to treat in the empirical therapy because of most of the drugs are used in the empirical therapy. For all these drugs we have taken drug classification like penicillin, cephalosporins, and aminoglycosides, and one or two drugs taken as others for these drugs, we have done the percentage calculations for the organisms which are more isolated and more resistant.

Table 4 : Antimicrobial resistance profile of Isolated bacteria from 2018 to 2020

E.coli	Penicillin	Cephalosporin	Aminoglycoside	Others
2018	0	0	0	0
2019(n=1033)	49.85%	58.21%	9.366%	12.35%
2020(n=630)	26.34%	65.33%	11.58%	23.99%
Klebsiella pneumonia				
	Penicillin	Cephalosporin	Aminoglycoside	Others
2018(n=191)	53.12%	39.65%	15.22%	91.6%
2019 (n=37)	39.06%	56.14%	14.2%	19.89%
2020 (n=33)	82.4%	59.6%	14.8%	43.2%
Enterobacter species				
	Penicillin	Cephalosporin	Amino glycosides	Others
2018(n=57)	70.17%	44.55%	4.38%	11.83%
2019(n=27)	49.99%	52.58%	12.34%	15.42%
2020(n=5)	73.33%	76%	20%	45%
Enterobacter cloacae				
	Penicillin	Cephalosporin	Amino glycoside	Others
2018 (n=5)	46.66%	48%	20%	30%
2019 (n=37)	78.37%	55.15%	45.94%	25.33%
2020 (n=33)	49.8%	52.42%	15.5%	11.41%

Among all the isolated bacteria in 2430 samples, the *E. coli* has a more significant number of isolated bacteria. In 2019 the samples were 1033 and in 2020 the samples were 630 found. Among these two years of study, cephalosporin was more resistant and increased resistance had been seen in these two years. so that these classes of drugs are less effective to treat the infections caused by *E. coli*. For *Klebsiella pneumonia*, in 2018 the samples were found to be 191, in 2019 there were 37 samples, and in 2020 there were 33 samples found. Among these samples, penicillins have more resistance. For *Enterobacter cloacae,* in 2018 there were 5 samples, in 2019 there were 37 samples, and in 2020 there were 33 samples. Among these samples, Penicillin and cephalosporin have more resistance. For *Enterobacter SPS,* in 2018 the samples were found to be 57, in 2019 there were 27 samples, and in 2020 there were five samples. Among these samples, penicillin and cephalosporins have more resistance. When compared to the years of resistance. In 2018, *Klebsiella pneumonia* had more resistance to the penicillin category of drugs [53.12%], *Enterobacter cloacae* had more resistance to cephalosporins [48%], and *Enterobacter species* had more resistance to the penicillin category of drugs [70.71%]. In 2019, *E. coli* had more resistance to cephalosporins [58.21%], *klebsiella pneumonia* had more resistance to cephalosporins [56.145], *Enterobacter cloacae* had more resistance to penicillin [78.37%], *Enterobacter species* had more resistance to cephalosporins [52.58%]. In 2020, E. coli had more resistance to cephalosporin [65.33%], *klebsiella pneumonia* had more resistance to cephalosporin [59.6%], and *Enterobacter cloacae* had more resistance to cephalosporins [52.42%], and *Enterobacter species* had more resistance to cephalosporins [76%].

5. DISCUSSION

The emergence and spread of drug-resistant pathogens are one of the significant challenges for providing good quality health services in hospitals [10]. Successful management of patients with different kinds of infectious diseases depends on the identification of bacterial pathogens and the proper selection of antimicrobials effective against the organisms[11]. The present study's overall proportion of only culture-positive results was taken. According to previous reports, the presence of drug-resistant strains of these isolates has been associated with prolonged hospital stays, higher healthcare costs, and increased morbidity and mortality in resource-limited settings, including India[12]. The present study has a total of 2430 samples, among which 1226 were males [50.45%], and 1204 were females [49.54]. In the present study, the majority of the clinical isolates were recovered from urine samples [41.27%]; others were tissue culture [13.82%], swab culture [13.58%], pus [13.45%], stool. *E. coli* and *K. pneumonia* were the major identified etiologic agents from our clinical specimens. Our study results indicate that the antibiotic resistance pattern varied across the studies. This variation was found depending on the type of isolate, the source of the sample, type of infection, type of antibiotics, and the geographical difference used in each study[13]. The same type of study was done in India up to know the broad-spectrum antibiotics, including third-generation cephalosporins and

fluoroquinolones was 75-80 percent in *E. coli*, 65-77 percent in *K. pneumoniae*, 73-87 percent. Compared to our study, results were the same: cephalosporins are more resistant to *E. coli* and Klebsiella pneumonia, and another study was done in Dhaka city, a single-centered study. In this study, it was found that ceftriaxone and gentamicin were effective against gram-negative bacteria *E. coli, Klebsiella, Pseudomonas,* and *Salmonella* Typhi. In contrast, ampicillin shows maximum resistance (100%) against all gram-negative bacteria except *Salmonella* Typhi. In this study, ceftriaxone (95.45%) and gentamicin (72.72%) When compared to our study ampicillin and ceftriaxone showed high resistance and gentamicin also but compared to their study gentamicin has a low resistance in our study so effective to treat. This is due to the prescribing pattern and self-medication in that area [14,15]. Even though it is difficult to discuss the average resistance pattern of gram-negative bacteria with a single study for various antibiotics, a study in Gondar, Northwest Ethiopia, showed 20%– 100% for gram-negative bacteria, respectively. If we look at the overall resistance pattern of the above studies, it ranges from 10% to100% when compared to our study, we are only done for gram-negative bacteria where the resistance was high [16,17]. The same type of study was done in Tanzania. While opposition to ampicillin, tetracycline, and sulphonamides in Gram-negative bacteria was frequent already in the seventies, it is worrying that resistance to trimethoprim-sulfamethoxazole, chloramphenicol, nitrofurantoin, nalidixic acid, and amoxicillin-clavulanate appear to have increased compared to previous studies and our study also shows resistance to the amoxicillin and chloramphenicol [18,19]. Although still low, it is of concern that the rate of gentamicin resistance in *E. coli* has increased from zero in 1978–79 to 2% in 1995 and 8% in the current study. In neighboring Kenya, the rate of gentamicin resistance in *E. coli* has increased from 2% in the late seventies to 20% and above in recent studies in our study show that gentamicin resistance had increased slowly [20]. Several countries reported over the past years have shown widespread resistance to commonly available first-line antimicrobial agents (cephalosporins and fluoroquinolones) in sub-Saharan Africa[21]. Likewise, our findings indicate a gradual increase in resistance of bacterial species to these classes of drugs, and penicillins have also increased their resistance gradually. This variation was found depending on the type of isolate, the source of the sample, type of infection, type of antibiotics, geographical difference used in each study [22].

In the year 2020 NARS-net collates national AMR surveillance data and shares the resistance profile of commonly used antibiotics with stakeholders at the national and state level, which shows *E. coli* and klebsiella pneumonia has more resistance similarly our study also shows that these two organisms have more resistance to commonly used drugs [23,24].

5.1. Limitations

There are some limitations in this study. The susceptibility of some antibiotics are not tested in our research they are macrolids and other categories of drugs.

6. CONCLUSION

The three years' longitudinal study shows that isolated gram-negative bacteria were resistant to ampicillin, amoxicillin, cephalothin, ciprofloxacin, cefuroxime, cefepime, ceftazidime, and ceftriaxone. So, these drugs can be replaced with organism-sensitive antibiotics like amikacin, chloramphenicol, colistin, and gentamicin during the treatment of gram-negative bacterial infections. Our study differs resistance of the drugs for various geographical reasons, which may be due to the prescribing pattern of drugs and their usage. So, there is no well-standardized bacteriological and AMR surveillance system in the study area. Regular monitoring of the etiologic agents and their antibiotic resistance profile should be evaluated for better patient management. Moreover, actions to contain the impact of AMR should be assessed and strengthened in the study area.

7. ACKNOWLEDGMENTS

8. AUTHORS CONTRIBUTION STATEMENT

Raghu Prakash reddy conceptualized and designed the study and D C S Naveen kumar curated the data and prepared the original draft. Sai Tharun vijay mandala and Harshitha gonuguntla discussed the methodology and analysed the data. Narayana Goruntla and Bogireddy Sahithi provide valuable inputs towards designing of the manuscript. All authors read and approve the final version of the manuscript.

9. CONFLICT OF INTEREST

The authors declare no conflict of interest

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