Section: Microbiology

Original Research Article

DRUG RESISTANCE PROFILE OF ENTEROBACTERALES WITH EMPHASIS ON MULTIDRUG RESISTANT E.COLI AND KLEBSIELLA PNEUMONIAE IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

Background: Multidrug resistant (MDR) Enterobacterales which are resistant to third-generation cephalosporins and Carbapenems are critical microorganisms that require urgent attention. The study aimed to identify Enterobacterales from clinical samples and to determine their drug resistance profile.

Materials and Methods: A total of 228 Enterobacterales isolates were included in this study. Antimicrobial susceptibility profile was determined by Kirby bauer disk diffusion method as per CLSI guidelines.

Results: The predominant organism isolated were E.coli followed by Klebsiella pneumoniae, Proteus species and Citrobacter species. The overall drug resistance pattern showed that more than 30% E.coli isolates were resistant to Carbapenems and Betalactam/betalactamase inhibitor combination, > 70% to Co-trimoxazole and 60% to fluoroquinolone, 30% to Amikacin and resistance to Gentamicin was observed among 50 % E.coli isolates. About 50% Klebsiella pneumoniae isolates showed resistance to Carbapenems, Betalactam/betalactamase inhibitor combination, and Amikacin and >50% isolates showed resistance to fluoroquinolone and Co-trimoxazole.

Conclusion: The rise of MDR E.coli and Klebsiella pneumoniae strains poses significant challenges for treatment. Effective antimicrobial stewardship programs are crucial in reducing antibiotic resistance rates and promoting effective treatment options for infections caused by MDR strains.

Keywords: MDR Enterobacterales, drug resistance, antimicrobial stewardship.

INTRODUCTION

Enterobacterales are an extensively distributed heterogeneous group of bacteria that are causative agents of urinary tract infections (UTIs), hospital and healthcare-associated pneumonia, diarrhea, meningitis, bloodstream infections, sepsis, endotoxic shock and intra-abdominal infections. The predominant species that cause human infections are Escherichia, Klebsiella, Salmonella and Yersinia, Proteus, Enterobacter, Shigella and Citrobacter.[1] Globally, multi-drug resistant Enterobacterales infection are recognized as a severe threat to patients' health. Enterobacterales resistant to third-generation cephalosporins and Carbapenems are critical microorganisms that require urgent attention.[2]

The epidemiology of multi-drug resistant Enterobacterales infection varies significantly across geographical regions, as it is influenced by local antibiotic usage patterns, infection control measures, and healthcare infrastructure quality. The dissemination of Carbapenem-resistant Enterobacterales (CRE) frequently challenge the efficacy of carbapenem as the last line drug against multi-drug resistant (MDR) organisms.[3] The recently approved agents for the treatment of Carbapenem-resistant Enterobacterales, include

ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, cefiderocol, and novel aminoglycosides and tetracyclines.[4]

WHO Bacterial priority pathogen list 2024 has stratified the bacterial pathogens into three priority groups as critical group, High group and medium group. Under critical group, Bacterial pathogens that pose the highest threat to public health due to limited treatment options, high disease burden (mortality and morbidity) and increasing trends in antimicrobial resistance, with few or no promising candidates in the pipeline are included. Infections with pathogens in the critical category may also be uniquely difficult to prevent and are highly transmissible. Carbapenem resistant Enterobacterales, ESBL producing Enterobacterales, Carbapenem resistant Acinetobacter baumannii, Rifampicin resistant Mycobacterium tuberculosis are included under critical group.

Among multi-drug resistant organisms, Extended-spectrum beta lactamase (ESBL)-producing Klebsiella pneumoniae and Carbapenem-resistant Klebsiella pneumoniae (CRKP) are of specific concern because of their potential for community transmission and limited treatment options available.[5] It has been reported that Klebsiella pneumoniae strains have wide variation by geographic region in the specimen source most frequently associated with ESBL non-Carbapenem-resistant (non-CRE) phenotypes.[6]

MATERIALS AND METHODS

A cross sectional study was done on Enterobacterales obtained from various clinical samples such as urine, pus, tissue, blood, sputum, body fluids etc. collected from October 2023 to September 2024.Samples were collected under strict aseptic conditions and were transported to the Microbiology laboratory for further processing.

Enterobacterales were identified by gram staining, colony morphology and standard biochemical reactions. Antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method was performed using the following discs (Hi Media Laboratories Pvt. Ltd., Mumbai, India); Amikacin (30µg), Cefotaxime (30µg), Cefepime (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Co-trimoxazole (25 µg), Gentamicin (10µg), Imipenem (10µg), Meropenem(10µg), Piperacillin/ tazobactam (100/10µg), Nitrofurantoin ((300µg), Norfloxacin (10µg), Doxycycline (30µg), Ampicillin(10µg) as per CLSI guidelines.[7,8]

IEC approval: This study was conducted after obtaining clearance from the Institutional Ethics Committee (Ref No AMCH/IEC/Proc .no 51/2023).

RESULTS

A total of 228 Enterobacterales which includes 127 E.coli isolates ,74 Klebsiella pneumoniae isolates, 21 Proteus species,5 Citrobacter species and 1 Morganella morganii were isolated.Out of 228 samples which yielded Enterobacterales, 91 were urine samples,79 were pus samples,26 from tissue,14 were from sputum,12 blood samples, 2 each were from body fluids,2 from vaginal swab and 2 from Endotracheal aspirate. 184 isolates were obtained from inpatients and 44 were from outpatients.

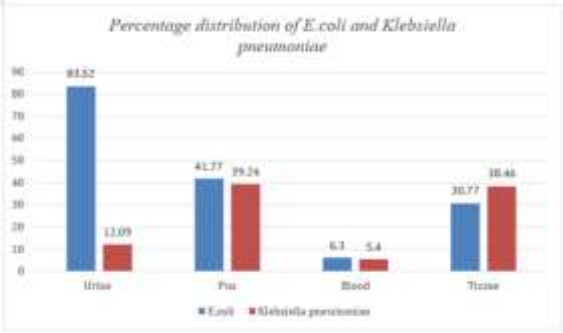

Figure 1: Distribution of E. coli and Klebsiella pneumoniae isolates from clinical samples.

Out of 127 E.coli isolates studied, 76 were from urine samples, 33 were from pus samples,8 from tissue bits, 8 were from blood samples and 2 were from vaginal swab. Out of 74 Klebsiella pneumoniae isolates,31 from pus samples,14 were from sputum,11 from urine, 10 from tissue bit,4 from blood, 2 from endotracheal aspirates, one from synovial fluid and one from body fluid. [Fig:1]

Thirteen Proteus isolates were from pus samples, 2 from urine and 6 were from tissue samples. Out of 5 Citrobacter isolates,1 was from pus,2 from urine samples and 2 were from tissue bit. A single isolate of Morganella morganii was from pus sample. The predominant organism isolated in urine was E.coli and in tissue bits, Klebsiella pneumoniae were predominant.

A total of 94 isolates of E.coli and 64 isolates of Klebsiella pneumoniae were obtained from inpatients and 32 isolates of E.coli and10 isolates of Klebsiella pneumoniae were from outpatients.

The overall drug resistance pattern showed that more than 30% E.coli isolates were resistant to Carbapenems and betalactam/betalactamase inhibitor combination.[Table 1]. More than 70% E.coli isolates showed resistance to Co-trimoxazole. About 60% isolates showed resistance to fluoroquinolone. More than 50% E.coli urinary isolates showed resistance to Nitrofurantoin and about 76% isolates showed resistance to Norfloxacin. More than 30% E.coli isolates showed resistance to Amikacin and resistance to Gentamicin was observed among 50 % E.coli isolates. More than 80% E.coli isolates were resistant to third generation cephalosporins and more than 50 % resistance for cefepime was seen.

Among Klebsiella pneumoniae isolates, about 50% isolates showed resistance to Carbapenems and betalactam/betalactamase inhibitor combination and Amikacin [Table 2]. More than 50% isolates showed

resistance to fluoroquinolone and Co-trimoxazole. [Figure 2].

78 E.coli and 21 Klebsiella pneumoniae isolates showed resistance to Doxycycline. A single isolate of Klebsiella pneumoniae from synovial fluid showed resistance to all tested antibiotic including Carbapenems except Co-trimoxazole and Doxycycline. More than 90% Klebsiella pneumoniae isolates were resistant to third generation cephalosporins and more than 70 % resistance for cefepime was seen.

Among the urinary isolates of E.coli from inpatients,22(42.31%) out of 52 were resistant to Nitrofurantoin and 41(78.85%) showed resistance to Norfloxacin, whereas 17(70.83%)out of 24 E.coli isolates from outpatients showed resistance to Nitrofurantoin and Norfloxacin. Two E.coli isolates from outpatient samples showed intermediate resistance to Nitrofurantoin and a single isolate showed resistance to Norfloxacin. Seven out of eleven urinary isolates of Klebsiella pneumoniae showed resistance and two isolates were intermediately resistant to Nitrofurantoin. Six Klebsiella isolates showed resistance to Norfloxacin. Thirty seven (48.68%) E.coli isolates from urine samples showed resistance to Ciprofloxacin and 49(64.47%) were resistant to Co-trimoxazole. Six (54.55%) Klebsiella pneumoniae isolates from urine showed resistance to Ciprofloxacin and 7(63.64%) showed resistance to Co-trimoxazole.

Two isolates of Citrobacter from urine samples showed susceptibility to Aminoglycosides, Doxycycline, Co-trimoxazole, Carbapenems, Piperacillin /tazobactam and Nitrofurantoin. A single isolate of Proteus from urine sample showed susceptibility to Aminoglycosides, Cefepime, Carbapenem and Piperacillin/tazobactam. [Table:3] More than 70% proteus isolates were resistant to third generation cephalosporins.

A single isolate of Morganella morganii from pus sample showed susceptibility to aminoglycosides, Co-trimoxazole, Carbapenems and Piperacillin/Tazobactam.

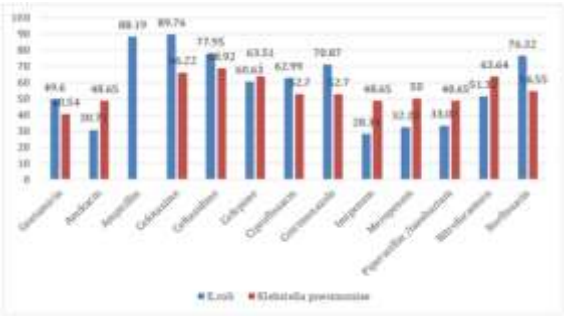

Figure 2: Drug resistance profile of E.coli and Klebsiella pneumoniae isolates.

Table 1: Comparison of drug resistance profile of E.coli isolates from inpatients and outpatient units

Antibiotics	Inpatients(n=95)		Outpatients(n=32)	
	Resistant	Intermediate resistant	Resistant	Intermediate resistant
Gentamicin	36(37.89%)	9(9.47%)	17(53.13%)	0(0%)
Amikacin	7(7.37%)	0(0%)	16(50%)	1(3.25%)
Cefotaxime	85(89.47%)	0(0%)	29(9.06%)	0(0%)
Ceftazidime	77(81.05%)	1(1.05%)	21(65.63%)	2(6.25%)
Cefepime	59(62.11%)	0(0%)	17(53.13%)	0(0%)
Ciprofloxacin	73(76.84%)	0(0%)	26(81.25%)	2(6.25%)
Co-trimoxazole	67(70.53%)	1(1.05%)	22(68.75%)	1(3.25%)
Imipenem	26(27.37%)	3(3.16%)	10(31.25%)	1(3.25%)
Meropenem	28(29.47%)	1(1.05%)	13(40.63%)	0(0%)
Piperacillin tazobactam	29(30.53%)	0(0%)	13(40.63%)	2(6.25%)

The most effective antibiotics against E.coli isolates were found to be Amikacin(81.10%), followed by Meropenem(66.92%),Imipenem(68.50%) and Piperacillin Tazobactam(65.35%).

Table 2: Comparison of drug resistance profile of Klebsiella pneumoniae isolates from inpatients and outpatient units

Antibiotics	Inpatients(n=64)		Outpatients(n=10)	
	Resistant	Intermediate resistant	Resistant	Intermediate resistant
Gentamicin	25(39.06%)	1(1.56%)	5(50%)	0(0%)
Amikacin	32(50%)	2(3.13%)	4(40%)	0(0%)
Cefotaxime	42(65.63%)	0(0%)	7(70%)	0(0%)
Ceftazidime	42(65.63%)	0(0%)	9(90%)	0(0%)
Cefepime	33(51.56%)	0(0%)	7(70%)	0(0%)
Ciprofloxacin	35(54.69%)	1(1.56%)	2(20%)	1(10%)
Co-trimoxazole	40(62.50%)	0(0%)	5(50%)	0(0%)
Imipenem	30(46.88%)	5(7.81%)	6(60%)	0(0%)
Meropenem	33(51.56%)	0(0%)	4(40%)	0(0%)
Piperacillin -tazobactam	31(48.44%)	0(0%)	5(50%)	0(0%)

The most effective antibiotic against Klebsiella pneumoniae was found to be Gentamicin (58.11%), Piperacillin tazobactam (51.35%) and Meropenem (50%).

Table 3: Comparison of drug resistance profile of Proteus isolates from inpatients and outpatient units

Antibiotics	Inpatients(n=20)		Outpatients(n=1)	
	Resistant	Intermediate resistant	Resistant	Intermediate resistant
Gentamicin	8(40%)	4(20%)	1(100%)	0(%)
Amikacin	10(50%)	3(15%)	0(%)	0(%)
Cefotaxime	14(70%)	0(%)	0(%)	0(%)

Ceftazidime	15(75%)	0(%)	0(%)	0(%)
Cefepime	9(45%)	1(5%)	1(100%)	0(%)
Ciprofloxacin	17(85%)	0(%)	0(%)	0(%)
Co-trimoxazole	16(80%)	0(%)	0(%)	0(%)
Imipenem	7(35%)	1(5%)	1(100%)	0(%)
Meropenem	5(25%)	0(%)	1(100%)	0(%)
Piperacillin -tazobactam	3(15%)	0(%)	1(100%)	0(%)

The most effective antibiotics against Proteus was Piperacillin/tazobactam (80.95%) and Meropenem. (71.43%).70% Carbapenem resistant E.coli isolates showed resistance to Piperacillin/tazobactam, Cephalosporins, Co-trimoxazole, Ciprofloxacin and aminoglycosides. 82.93% Carbapenem resistant Klebsiella pneumoniae isolates showed resistance to Cephalosporins, Piperacillin/ tazobactam, Co-trimoxazole, Ciprofloxacin and aminoglycosides.

DISCUSSION

Multidrug-resistance (MDR) has emerged as one of the most serious public health threats in the 21st century, where the bacteria exhibit resistance to multiple antimicrobial agents,resulting in prolonged hospitalization, unresolved infections, increased utilization of healthcare resources and medical costs, as well as elevated morbidity and mortality.[5]

In the present study we have evaluated the antimicrobial profile of Enterobacterales with emphasis on multi-drug resistance among E.coli and Klebsiella pneumoniae isolates. A total of 228 Enterobacterales were analysed in our study. The predominant isolate was E.coli followed by Klebsiella pneumoniae, Proteus species and Citrobacter species.

The overall drug resistance profile showed 33.33% of isolates showed resistance to Amikacin and Meropenem,39.91% to Imipenem,42.54% to Gentamicin,67.11% to Co-trimoxazole and 70.61% to Ciprofloxacin. Murray et al. have reported fluoroquinolones resistance among 70-90% Enterobacterales and third generation cephalosporin resistance among 70-100% isolates.[9] Datta Sangeetha et al. reported Enterobacterales with high resistance to fluoroquinolones, third- and fourth-generation cephalosporins and monobactams, as well as notable resistance to colistin.[10]

In our study, majority of the isolates were from inpatients (80.70%) and a major proportion of isolates from inpatients showed resistance to Cephalosporins, Ciprofloxacin and Co-trimoxazole. Akhavizadegan H et al. reported a greater proportion of inpatient samples with resistance to ceftriaxone, cefixime, sulfamethoxazole-trimethoprim, Ciprofloxacin and nalidixic acid.[11]

A significant proportion of isolates from outpatient unit showed resistance to Cephalosporins, Ciprofloxacin and Co-trimoxazole. Thirty seven (48.68%) E.coli isolates from urine samples showed resistance to Ciprofloxacin and 49(64.47%) were resistant to Co-trimoxazole. Six (54.55%) Klebsiella pneumoniae isolates from urine showed resistance to Ciprofloxacin and 7(63.64%) showed resistance to Co-trimoxazole. Kaye, K.S et al. reported, high prevalence of non-susceptibility to trimethoprim/ sulfamethoxazole and fluoroquinolones among E. Coli urinary isolates from outpatient unit.[12] Among the urinary isolates of E.coli from inpatients,22(42.31%) out of 52 were resistant to Nitrofurantoin and 41(78.85%) showed resistance to Norfloxacin, whereas 17(70.83%) each out of 24 isolates from outpatients showed resistance to Nitrofurantoin and Norfloxacin. Seven out of eleven urinary isolates of Klebsiella pneumoniae showed resistance to Nitrofurantoin and six isolates showed resistance to Norfloxacin. Neha Tiwari et al. reported Escherichia coli from urine samples with100% resistance to Norfloxacin and 48.28% to Nitrofurantoin, while Klebsiella pneumoniae isolates from urine exhibited 100% resistance to both antibiotics.[13]

The most effective antibiotics against E.coli isolates were found to be Amikacin(81.10%), followed by Meropenem(66.92%), Imipenem (68.50%) and Piperacillin/Tazobactam (65.35%) whereas for Klebsiella pneumoniae, Gentamicin (58.11%), Piperacillin tazobactam (51.35%) and Meropenem (50%) were found to be effective. The most effective antibiotics against Proteus were Piperacillin/tazobactam (80.95%) and Meropenem (71.43%). Bandy A et al. reported aminoglycosides as effective drugs against E. coli(96.5%) and Klebsiella pneumoniae(74.9%). Highest resistance exhibited by Proteus mirabilis (76.8%) towards cefepime (fourth-generation cephalosporin) was reported in their study.[14]

In the present study majority of Proteus isolates (61.90%) were obtained from Pus samples. More than 60% Proteus isolates showed resistance to third and fourth generation cephalosporins. Fahim Alam et al. reported multidrug resistance among 91.6% Proteus isolates from wound infection.[15] Among Proteus isolates highest susceptibility levels were observed for piperacillin-tazobactam, Carbapenems, and cephalosporins antibiotics.[16]

In our study,70% Carbapenem resistant E.coli isolates showed resistance to Piperacillin/tazobactam, Cephalosporins, Co-trimoxazole, Ciprofloxacin and aminoglycosides.

82.93% Carbapenem resistant Klebsiella pneumoniae isolates showed resistance to Cephalosporins, Piperacillin/tazobactam, Co-trimoxazole, Ciprofloxacin and aminoglycosides. Among Carbapenem resistant Klebsiella pneumoniae, more resistance to Amikacin and Co-trimoxazole was observed when compared to E.coli

isolates. This is in concordance to the study finding by Aiesh, B.M et al.[17]

In our study, resistance to multiple antibiotics was observed among Klebsiella pneumoniae isolates, when compared to other members of Enterobacterales.

CONCLUSION

The present study evaluated the drug resistance profile of Enterobacterales for one year period. High degree of resistance to multiple antibiotics was observed among Klebsiella pneumoniae isolates, when compared to other members of Enterobacterales. High resistance rates to commonly used antibiotics, including third-generation cephalosporins, fluoroquinolones, and even Carbapenems, underscore a critical threat to effective antimicrobial therapy

The emergence of MDR E.coli and Klebsiella pneumoniae strains poses significant challenges for treatment as the continued spread of MDR organisms will severely compromise patient outcomes and burden healthcare systems. These findings underscores the importance of judicious use of antimicrobial agents, stringent infection control practices, and continuous local surveillance to monitor resistance trends. Effective antimicrobial stewardship programs are crucial in reducing antibiotic resistance rates and promoting effective treatment options for infections caused by MDR strains.

Limitations of the Study

This is a Single-Centered study. The findings may not be generalizable to other hospitals or regions, as the resistance patterns can vary by geographic location and healthcare practices. The other limitation is lack of molecular characterization for identifying specific resistance genes (e.g., blaNDM, blaKPC, blaCTX-M) which limits our understanding of transmission dynamics and clonal spread. Tertiary hospitals often see more severe or referred cases, potentially overestimating resistance rates compared to community settings. To overcome the limitations in future studies, it is recommended to conduct multicenter or region-wide surveillance and to incorporate molecular diagnostics to detect resistance genes.

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