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Original Research Article

Detection of colistin susceptibility in extended spectrum β lactamases positive *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates by broth disc elution method

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ABSTRACT

Background: Antibiotic resistance is one of the greatest threats in human health. Extended spectrum β lactamases mediated resistance is prevalent worldwide, *Klebsiella pneumoniae* and *Escherichia coli* leap out as this significant ESBL producers conferring resistance to the expanded spectrum cephalosporins. Colistin is being administered as last line therapy for patients that have failed to respond to other available antibiotics that are active against Gram-negative bacteria.
Methods: The present study was conducted at school of medical education Kottayam, Kerala from January 2023 to November 2023.During the period of study 150 isolates of *K. pneumoniae* and 136 isolates of *E. coli* were collected from various diagnostic microbiology laboratories in Kerala. The colistin susceptibility pattern of ESBL producing isolates was detected by broth disc elution method recommended by CLSI.
Results: In this study prevalence of multi-drug resistant is 6% and 9.6% and Extensively-drug resistant is 62% and 63.9% for *K. pneumoniae* and *E. coli* respectively. ESBL production was detected as 72% in *K. pneumoniae* and 79% in *E. coli.* The colistin susceptibility pattern of ESBL producing *K. pneumoniae* and *E. coli* was detected as 76.9% and 87.9% respectively
Conclusions: Our result demonstrated that the recent use of colistin as last resort treatment for extensively drug resistant gram-negative bacilli, it is essential to know the prevalence of susceptibility pattern to this antibiotic.

Keywords: *Klebsiella pneumoniae*, *Escherichia coli*, Multi-drug resistance, Extensively-drug resistance, Extended spectrum β lactamases, Colistin

INTRODUCTION

Escherichia coli and *Klebsiella* species are the most common causative pathogens for most of the infections, especially in countries with poor health care system.[1] *E. coli* is a normal flora of human and animal gut but can also be found in water, soil, and vegetation.[2] *Klebsiella* species are considered as major opportunistic pathogens that can cause infections mostly in children. *Klebsiella pneumoniae* is an important cause of human infections among all *Klebsiella* species, followed by *Klebsiella oxytoca*, *Klebsiella ozaenae*, and *Klebsiella rhinoscleromatis.* Several common bacterial infections such as gastroenteritis, urinary tract infection (UTI), septicemia, and neonatal meningitis are mainly caused by *E. coli* and *Klebsiella* spp in children.[3,4] Antimicrobial resistance (AMR) is one of the top ten global health threats to humans.[5] AMR, due to extended-spectrum beta-lactamase (ESBL) producing bacteria, has escalated over the past years, both in hospitals and in communities.

Currently, many Gram-negative bacteria can produce ESBL enzymes, conferring resistance to penicillins, first-, second, and third-generation cephalosporins, and aztreonam (but not carbapenems or cephamycins).[6] ESBL genes, which were first reported among *Klebsiella* spp. and *E. coli*, are rapidly spreading among other bacteria through plasmid-mediated horizontal gene transfer.[7] In addition, ESBL encoding plasmids can also code for other non-beta-lactam resistance genes, leading to multi-drug resistance.[8] In SSA, infections caused by ESBL-producing bacteria, including *E. coli* (ESBL-EC) and *K. pneumoniae* (ESBL-KP), are of great concern.[9]

The inexorable rise of antibiotic resistance and the paucity of new antimicrobials have led to a renewed interest in the use of the polymyxin group of antibiotics for the treatment of infections due to MDR bacteria.[10,11] It have been used for over 50 years in human medicine.[12] Polymyxins are multicomponent polypeptide antibiotics that act primarily on the Gram-negative bacterial cell wall, leading to rapid permeability changes in the cytoplasmic membrane and ultimately to cell death.[13] There are five types of polymyxins, from A to E, but only colistin (also known as polymyxin E) and polymyxin B were clinically used in the 1950s, as they were found to be the least nephrotoxic.[14] Ultimately, these antibiotics fell out of favour, and their systemic use was reduced due to their considerable adverse effects, particularly their potential for nephrotoxicity and neurotoxicity.[15] Colistin is a decapeptide administered either as colistin sulphate, an oral prodrug, or as colistin methane sulfonate (CMS) when used intravenously.[16] Colistin have occasionally been used to treat infections caused by Gram-negative bacteria (GNB) that are resistant to aminoglycosides, cephalosporins, anti-*Pseudomonas* penicillins, quinolones, monobactams and carbapenems.[17,18] Thus, they are being used as a last resort drug for the treatment of life-threatening infections.[19] The aim of this study is to assess the prevalence of polymyxin resistance in ESBL-positive *K. pneumoniae* and *E. coli* clinical isolates since its reintroduction in the era of multidrug resistance.

METHODS

The present cross-sectional study was conducted at school of medical education (SME) Kottayam, Kerala from January 2023 to November 2023. During the period of study isolates of *K. pneumoniae* and *E. coli* were collected from various diagnostic microbiology laboratories in Kerala. All *K. pneumoniae* and *E. coli* isolates which deemed clinically significant were included in the study; irrespective of age, gender, and underlying disease. Isolates that did not show clinically significant were excluded from the study

Identification of isolates and antimicrobial susceptibility testing

All the isolates were identified by routine biochemical testing. Antimicrobial susceptibility testing by disc diffusion as prescribed by Clinical Laboratory Standards Institute (CLSI) guidelines M02-A13.20,21 The following antibiotics were tested; Gentamicin (10 µg), Amikacin (10 µg), Imipenem (10 µg), Cefuroxime (30 µg), Cefoxitin (30 µg), Ciprofloxacin (5 µg), Aztreonam (30 µg), Ampicillin (10 µg), Amoxyclav (20/10 µg), Tetracycline (30 µg), Cefixime (5 µg). Based on the recommendations of the Centre for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC), isolates were termed as MDR which are non-susceptible to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (Bacterial isolates remain susceptible to only one or two categories). Non-MDR is defined as susceptibility to all the agents in all antimicrobial categories.[22]

Detection of ESBL production in K. pneumoniae and E. coli

ESBL production was detected phenotypically by disc combination method as recommended by guidelines of CLSI by using Ceftazidime (30 µg), Ceftazidime/Clavulanic acid (30/10 µg), Cefotaxime (30 µg), Cefotaxime/Clavulanic acid (30/10 µg). A difference of ≥5 mm between cephalosporin discs and their respective cephalosporin/clavulanic acid disc was taken as ESBL producing strain

Detection of colistin susceptibility in K. pneumoniae and E. coli by broth disc elution method

Based on CLSI guidelines M02-A13, using a loop picked 3-5 colonies from a fresh (18 to 24 hours) non selective agar plate and was transferred to peptone water. Adjusted turbidity to equivalent of 0.5 McFarland turbidity standard. 10 ml of the cation adjusted Mueller Hinton broth (CA-MHB) was transferred to the test tubes. Labelled the four tubes of CAMHB for each isolate tested with 1, 2, 4 µg/ml and control. Colistin discs (10 µg) were warmed to room temperature and were added using aseptic techniques as follows: 1 colistin disc to the tube labelled "1 µg/ml", 2 colistin discs to the tube labelled "2 µg/ml" and 4 colistin discs to the tube labelled "4 µg/ml". One tube was kept as control which contained only CA-MHB and inoculum. Gently vortexed the tubes with the added disc and the colistin was let elute from the disc for 30 minutes. Added 50 µg standardized inoculum to the control and all the tubes with discs attained a final inoculum concentration of approximately 7.5×10^5 CFU/ml. Incubated the tubes at 35°C for 16 to 20 hours. On the very next day the incubated tubes were examined for growth and turbidity in the control tube. Read the MIC as the lowest concentration that completely inhibited the growth of the test isolates such as ≤2 µg/ml = intermediate, ≥4 µg/ml = resistant. All reagents, cultural media and antibiotic discs were purchased from HiMedia Laboratories Pvt. Ltd India. The data was tabulated and analysed by using Microsoft Excel 2019.

RESULTS

In the present study, a total of 286 isolates were obtained, including 150 isolates of *K. pneumoniae* and 136 isolates of *E. coli*, sourced from various clinical samples. *K. pneumoniae* isolates were obtained from urine (N=83), sputum (N=29), blood (N=21), pus (N=12), and exudate (N=5), while *E. coli* isolates were derived from urine (N=109), sputum (N=9), blood (N=12), pus (N=2), and exudate (N=4).

Of the 150 *K. pneumoniae* isolates, 81 were from females and 69 were from males (Figure 1). In the case of *E. coli*, 112 were from females and 24 were from males.

Table 1: Antibiogram of *K. pneumoniae* and *E. coli*

Antibiotics	*Klebsiella pneumoniae*			*Escherichia coli*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Gentamicin	66	1.3	32.7	78.7	3.7	17.6
Amikacin	55.3	8.7	36	69.1	12.5	18.4
Imipenem	48.7	15.3	36	76.4	11.8	11.8
Cefuroxime	20.7	20	59.3	29.4	14.7	55.9
Cefoxitin	35.3	5.3	59.4	64.7	1.5	33.8
Ciprofloxacin	28	10	62	36.8	8.8	54.4
Aztreonam	40.7	4.6	54.7	39.7	13.2	47.1
Ampicillin	-	-	-	23.5	1.5	75
Amoxiclav	-	-	-	40.4	18.4	41.2
Tetracycline	46	9.3	44.7	55.1	9.6	35.3
Cefixime	36.7	2	61.3	28.7	5.1	66.2
Ceftazidime	22.6	12.6	64.8	16.2	30.9	52.9
cefotaxime	23.3	9.3	67.4	11	16.2	72.8

Antimicrobial susceptibility pattern of K. pneumoniae and E. coli

The susceptibility of *K. pneumoniae* and *E. coli* isolates to commonly used antibiotics is depicted in (Table 1). In the current study, *K. pneumoniae* exhibited 66% (n=99) sensitivity, 1.3% (n=2) intermediate and 32.7% (n=49) resistance to gentamicin, 55.3% (n=83) sensitive, 8.7% (n=13) intermediate and 36% (n=54) resistance to amikacin, 48.7% (n=73) sensitive, 15.3% (n=23) intermediate and 36% (n=54) resistance against imipenem, 20.7% (n=31) sensitive, 20% (n=30) intermediate and 59.3% (n=89) resistance to cefuroxime, 35.3% (n=53) sensitive, 5.3% (n=8) intermediate and 59.4% (n=89) resistance against cefoxitin, 28% (n=42) sensitive, 10% (n=15) intermediate and 62% (n=93) resistance to ciprofloxacin, 40.7% (N=61) sensitive, 4.6% (n=7) intermediate and 54.7% (n=82) resistance to aztreonam, 46% (n=69) sensitive, 9.3% (n=14) intermediate and 44.7% (n=67) resistance to tetracycline, 36.7% (n=55) sensitive, 2% (n=3) intermediate and 61.3% (n=92) resistance to cefixime, 22.6% (n=34) sensitive, 12.6% (n=19) intermediate and 64.8% (n=97) resistance to ceftazidime, 23.3% (n=35) sensitive, 9.3% (n=14) intermediate and 67.4% (n=101) resistance to cefotaxime. The highest rate of susceptibility was observed to Gentamicin 66% (n=99), followed by Amikacin 55.35% (n=83) and Imipenem 48.7% (n=73) and highest rate of resistance was observed in following order Cefotaxime 67.4% (n=101), Ceftazidime 64.8% (n=97) and Ciprofloxacin 62% (n=93).

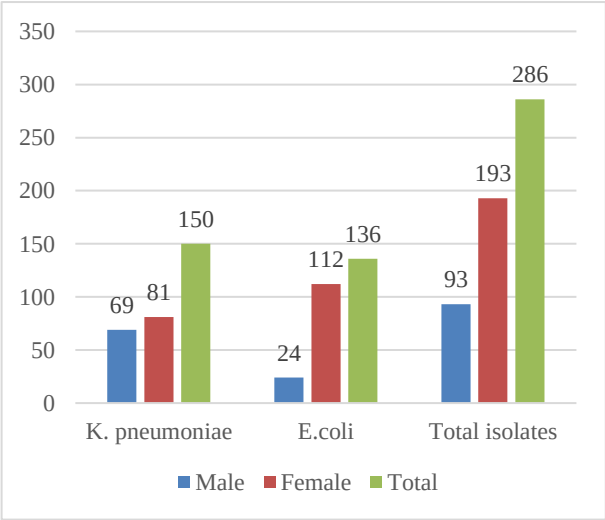

Figure 1: Gender wise distribution *K. pneumoniae* and *E. coli.*

E.coli exhibited 78.7% (n=107) sensitivity, 3.7% (n=5) intermediate and 17.6% (n=24) resistance to Gentamicin, 69.1% (n=94) sensitive, 12.5% (N=17)intermediate and 18.4% (n=25) resistance to Amikacin, 76.4% (n=104) sensitive, 11.8% (n=16) intermediate and 11.8% (n=16) resistance against Imipenem, 29.4% (n=40) sensitive, 14.7% (n=20) intermediate and 55.9% (n=76) resistance

to Cefuroxime, 64.7% (n=88) sensitive, 1.5% (n=2) intermediate and 33.8% (n=46) resistance against Cefoxitin, 36.8% (n=50) sensitive, 8.8% (n=12) intermediate and 54.4% (n=74) resistance to Ciprofloxacin, 39.7% (n=54) sensitive,13.2% (n=18) intermediate and 47.1% (n=64) resistance to Aztreonam, 23.5% (n=32) sensitive, 1.5% (n=2) intermediate and 75% (n=102) resistant to Ampicillin,40.4% (n=55) sensitive , 18.4% (n=25) intermediate and 41.2% (n=56) resistance to Amoxyclav, 55.1% (n=75) sensitive, 9.6% (n=13) intermediate and 35.3% (n=48) resistance to Tetracycline, 28.7% (n=39) sensitive, 5.1% (n=7) intermediate and 66.2% (n=90) resistance to cefixime, 16.2% (n=22) sensitive, 30.9% (n=42) intermediate and 52.9% (n=72) resistance to Ceftazidime, 11% (n=15) sensitive, 16.2% (n=22) intermediate and 72.8% (n=99) resistance to Cefotaxime.

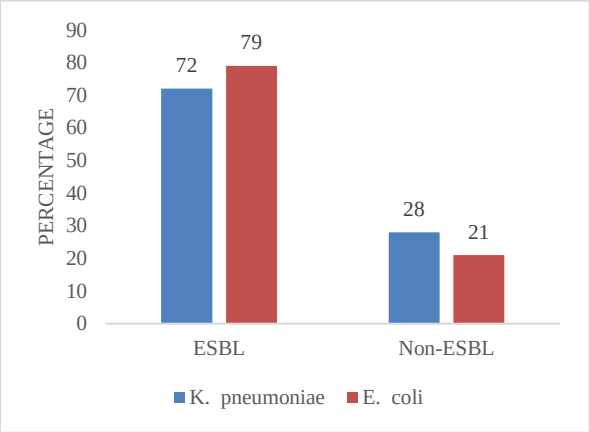

Figure 2: Prevalence of ESBL production among *K. pneumoniae* and *E. coli.*

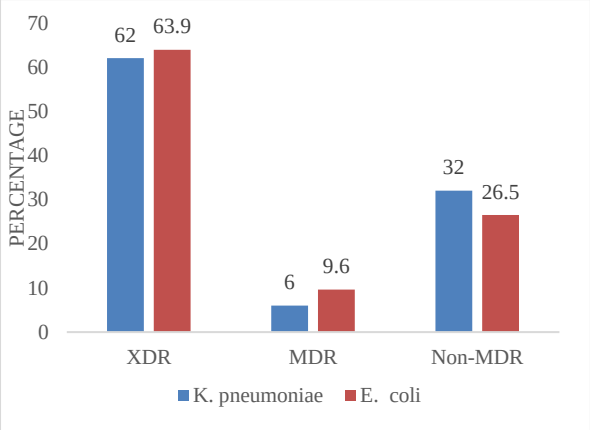

Figure 3: Prevalence of XDR, MDR and Non-MDR strains of *K. pneumoniae* and *E. coli.*

The highest rate of susceptibility was observed to Gentamicin 78.7% (n=107), followed by Imipenem 76.4% (n=104) and Amikacin 69.1% (n=94). The highest rate of resistance was observed in following order Ampicillin 75% (n=102), Cefotaxime 72.8% (n=99) and 66.2% (n=90).

Prevalence of ESBL production in K. pneumoniae and E. coli

In this study, the ESBL production of *K. pneumoniae* was detected as 72% (n=108) and *E. coli* showed 79% (n=107) as shown in (Figure 2).

Prevalences of XDR, MDR and Non-MDR strains of K. pneumoniae and E. coli

For *K. pneumoniae* 62% (n=93) XDR, 6% (n=9) MDR and 32% (n=48) non-MDR were revealed and *E. coli* exhibited 63.9% (n=87) XDR, 9.6% (n=13) MDR and 26.5% (n=36) Non-MDR as shown in (Figure 3). The highest rate of XDR strains shown by *K. pneumoniae* (62%) and highest rate of MDR strains shown by *E. coli* (9.6%).

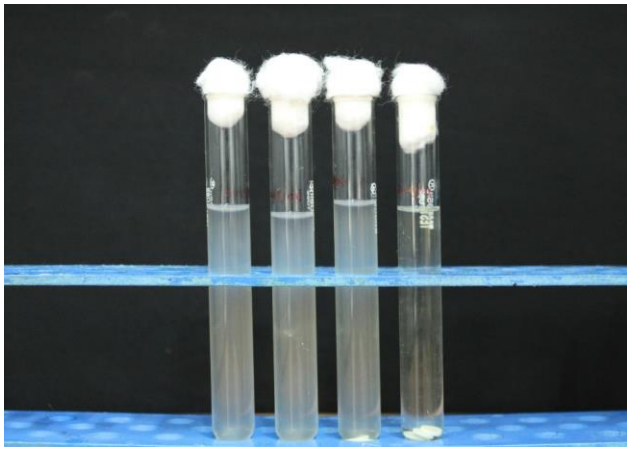

Figure 4: Broth disc elution method *K. pneumoniae* exhibiting intermediate susceptibility to colistin (From left to right, tube 1 showing negative control and tube 2, 3, 4 showing intermediate susceptibility to 1µg, 2 µg, 4 µg).

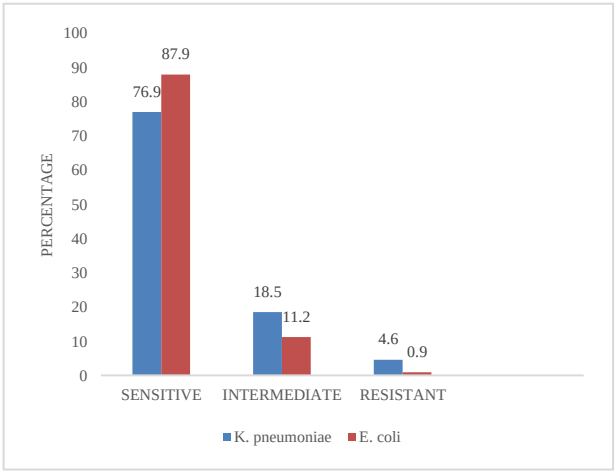

Figure 5: Colistin susceptibility pattern of *K. pneumoniae* and *E. coli.*

Colistin susceptibility pattern of *K. pneumoniae* and *E. coli*

In this study *K. pneumoniae* exhibited 76.9% (n=83) sensitive, 18.5% (n=20) intermediate and 4.6% (n=5) resistance to colistin. *E. coli* exhibited 87.9% (n=94) sensitive, 11.2% (n=12) intermediate and 0.9% (n=1) resistance to colistin as shown in (Figure 5).

DISCUSSION

Colistin is an antibiotic that has recently been 'rediscovered' in human medicine due to the problems encountered in the treatment of extremely drug-resistant and multidrug-resistant Gram-negative bacteria, for which it is a last-resort drug.[23] Failure of 3rd generation cephalosporins and carbapenems against Gram-negative bacteria has led to the unprecedented increase in the use of colistin and subsequent emergence and dissemination of colistin resistance.[24] As an increased use of colistin is being documented, the present study evaluated the presence of colistin susceptibility in ESBL positive Enterobacterales namely *K. pneumoniae* and *E. coli*.

In the present study prevalence of colistin resistance was 4.6% in *K. pneumoniae* and 0.9% in *E. coli* was less and in accordance with NARS-Net India AMR annual report 2021.[25] These results are also consistent with the studies of Nirmal et al and Chauhan et al.[26,27] But in the present study a higher number of intermediately susceptible *K. pneumoniae* (18.5%) and *E. coli* (11.2%) was encountered, which is lesser than Nirmal et al were reported as 100% intermediate susceptibility to *K. pneumoniae* and *E. coli*.[26] These contesting results could be due to a lesser sample size in their study, *K. pneumoniae* (N=64) and *E. coli* (N=6). While the present study colistin resistance was evaluated in ESBL positive *K. pneumoniae* (N=108) and *E. coli* (N=107). In the present study several limitations were present as only *K. pneumoniae* and *E. coli* isolates were included from Enterobacterales and other Gram-negative bacilli like *Pseudomonas*, *Acinetobacter* were excluded. The susceptible isolates were disproportionately more, so a larger sample size with more bacterial species may provide more insights into colistin resistance. The present study employed only phenotypic method for detection of colistin susceptibility that is broth disc elution method.

CONCLUSION

In conclusion, given the recent use of colistin as a last resort treatment for extensively drug resistant gram-negative bacilli, it is essential to know the prevalence of susceptibility pattern to this antibiotic. Though resistance to colistin was less in *K. pneumoniae* (4.6%) and *E. coli* (0.9%) but intermediate susceptibility of 18.5% and 11.2% for *K. pneumoniae* and *E. coli* respectively could be a concern. These results support the idea to regulate the use of life saving drug Colistin.

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