Change in Carbapenem Susceptibility Patterns among Urinary *Escherichia coli* isolates at a Tertiary Care Centre

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ABSTRACT

Introduction: *Escherichia coli* (*E.coli*) is the major pathogen causing urinary tract infections (UTIs), and carbapenems are prescribed frequently to treat UTIs at tertiary care settings. It is, therefore, of clinical importance to determine the change in carbapenem susceptibility of *E.coli* isolated from urinary samples after adopting a new cleaning policy. Study aimed to determine the change in antimicrobial susceptibility of *E.coli* strains isolated from urinary samples towards carbapenems.

Material and methods: A retrospective comparison of carbapenems susceptibility profile of *E.coli* isolated from urine cultures at a tertiary care centre was carried out for two years between 2017 and 2018. *E.coli* isolates were identified from positive cultures as per the conventional microbiological methods. Carbapenem susceptibility was exhibited using Meropenem (10 µg) disk on Mueller-Hinton Agar (MHA) using Kirby Bauer disk diffusion method.

Results: In a total of 1219 urinary *E.coli* isolates, sensitivity towards carbapenems has increased from 81.3% to 84.2% (overall 83.2%). The value of chi-square for the difference in the distribution of Meropenem susceptibility for 2017 and 2018 was found to be <0.001, which is highly significant. Hence, the susceptibility profile of Meropenem changed significantly in two consecutive years in this tertiary care hospital after adopting a new cleaning policy.

Conclusion: *E.coli* is the leading pathogen causing UTIs, and carbapenems are prescribed frequently, so regular monitoring of antimicrobial susceptibility is recommended. Better cleaning policies can improve the sensitivity patterns of isolates towards antimicrobials.

Keywords: *E.coli*, Carbapenem, ABST, Urine

INTRODUCTION

Escherichia coli (*E.coli*) belongs to the family *Enterobacteriaceae* and comprises part of the healthy gastrointestinal tract flora of humans and animals.[1] *E.coli* is the prime pathogen causing urinary tract infections (UTIs) and leads to causing bloodstream infections, wounds, otitis media and other complications in humans.[2] Increased risk of UTI in extreme ages, pregnant women, patients with spinal cord injuries, diabetes, immune deficiency disorders is reported more often. The basis of proper treatment in complicated urinary tract infections is the selection of an appropriate antibiotic with high efficacy.[3]

Antimicrobial drugs have provided physicians with the ability to treat and prevent many infectious diseases, but, there has been an inappropriate increase in carbapenems consumption in recent times;[4] unfortunately, the injudicious use of these drugs has been accompanied by the rapid

appearance of resistant strains.[5] The emergence and the spread of multi-drug resistant Gram-negative pathogens is a significant public health concern and leads to increased morbidity and mortality, especially in hospitals and health care settings.[6] Carbapenem resistance patterns have shown vast inter-regional diversity. The suitable choice of antibiotic needs to be custom-made based on the local susceptibility pattern.[7] It is, therefore, of clinical importance to determine the change in antimicrobial susceptibility of strains isolated from urinary samples towards carbapenems and frequent cleaning of the healthcare institutes can have a positive impact on decreasing environmental contamination and improving antimicrobial susceptibility.[8] Study aimed to determine the change in antimicrobial susceptibility of *E.coli* strains isolated from urinary samples towards carbapenems.

MATERIAL AND METHODS

In this study, a retrospective comparison of urine cultures was carried out for two years at a tertiary care centre in the northern part of India between 2017 and 2018. The urinary catheter or suprapubic samples using appropriate aseptic methods and clean-catch midstream morning urine specimens in sterile wide-mouth containers were collected. Urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar using calibrated wire loops. These were incubated under the aerobic atmosphere at 37°C for 24 hours. *E.coli* isolates were identified from positive cultures as per the conventional microbiological methods.

Antimicrobial susceptibility tests were performed on Mueller-Hinton Agar (MHA) using Kirby Bauer disk diffusion method. Meropenem (10 µg) along with other antimicrobials was inoculated, and each inhibition zone

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diameter size was measured and interpreted according to the standard given in the CLSI guidelines 2013.[9] An isolate showing a zone of ≥23 mm around Meropenem disk was considered sensitive, 20-22 mm as intermediate, and ≤19 mm as resistant to carbapenems. Pearson chi-square test was used to compare the susceptibility patterns of bacterial isolates with patient gender and age; and a comparison of carbapenem resistance after implementing a new cleaning policy in the hospital.

In the second year of study, the centre adopted a new frequent cleaning policy inside the hospital with daily cleaning of inanimate equipment and mopping of floors with detergent and water after every two hours in various wards and after every hour in the ICU. Compliance with cleaning was supervised by senior nurses every day.

RESULTS

The centre received a total of 29022 samples in 2017 and 2018. Out of a total of 2868 positive urine cultures, *E.coli* was isolated in 1648 samples with 57.4% positivity for this bacteria among all urinary isolates. Among 1648 *E.coli* isolates, Meropenem disk was tested in a total of 1219 samples. Gender-wise, 536 (44%) isolates were from females, and 683 (56%) were from males. Location-wise 46 (3.8%) positive isolates were from ICU, 818 (67.1%) from OPDs and 355 (29.1%) from various wards.

Out of a total of 1219 urinary *E.coli* isolates, 83.2% were

Susceptibility pattern	2017 (n=416)	2018 (n=803)	Total (n=1219)
Intermediate	4 (1%)	32 (4%)	36 (3%)
Resistant	74 (17.8%)	95 (11.8%)	169 (13.9%)
Sensitive	338 (81.3%)	676 (84.2%)	1014 (83.2%)

Table-1: Year-wise Meropenem susceptibility patterns

Test	Value (n=1219)	Asymptotic Significance (2-sided)
Pearson Chi-Square	15.782[a]	0.000
Likelihood Ratio	17.245[b]	0.000

a,b - degree of freedom (df) = 2

Table-2: Chi-square test for year-wise statistical significance of Meropenem susceptibility

Year	Mean Age	Std. Deviation	Std. Error Mean
2017 (n=416)	50.37	23.085	1.132
2018 (n=803)	50.44	22.473	0.793

Table-3: Age- and year-wise statistical significance

susceptible to Meropenem, 3% intermediate, and 13.9% were resistant to Meropenem. In the year 2017, a total of 416 *E.coli* isolates were identified among various uropathogens with 81.3% sensitivity towards Meropenem, and in the year 2018, this number was 803 with 84.2% sensitivity towards Meropenem, as illustrated in Table 1. The value of chi-square for the difference in the distribution of Meropenem susceptibility for 2017 and 2018 was found to be <0.001, which is highly significant as summarised in Table 2. Hence, the susceptibility profile of Meropenem changed significantly in two consecutive years in this tertiary care hospital after adopting a new cleaning policy.

The distribution of isolates, age-wise and year-wise is depicted in Table 3. Since the t-value for the difference in age of patients in two consecutive years, 2017 and 2018 was found to be -0.049, which is less than 1.96. Hence the difference between the age of patients was not significant as exhibited in Table 4.

Location-wise and year-wise distribution of samples are shown in Table 5. The value of chi-square for the distribution of the location of patients in the two consecutive years 2017 and 2018 was found to be 26.382, which was highly significant for <.0001 level of significance as shown in Table 6.

Location-wise susceptibility of Meropenem among *E.coli* isolates is depicted in Table 7. Since the value of chi-square for change in Meropenem susceptibility for different locations was found to be 80.711, which is highly significant at <.0001 level of significance. Hence, Meropenem susceptibility profile was significantly different for different areas viz., ICU, Outdoor (OPDs) and Wards as depicted in Table 8.

DISCUSSION

Species in genus *Escherichia* are Gram-negative rods 2-4 μm x 0.6 μm, oxidase-negative, that grow well on MacConkey agar or Cysteine Lactose Electrolyte Deficient (CLED) agar media on which they exhibit large 2-3 mm, low convex, glossy, round, smooth-surfaced with regular margins, lactose fermenting pink and yellow coloured colonies, respectively. Most strains of *E.coli* ferment lactose. All ferment D-glucose and are indole positive, methyl-red (MR) positive, motile with peritrichous flagella.[10,11] Antimicrobial resistance in *E.coli* has risen globally, and its susceptibility patterns exhibit substantial geographic variation as well as differences in population and environment. Resistance to carbapenems is often due to decreased outer membrane permeability, increased efflux systems, alterations of penicillin-binding proteins, and carbapenem hydrolysing enzymes carbapenemases.[12]

Isolates resistant to carbapenems produce β-lactamases

Age-wise tests	t-test for Equality of Means						
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Equal variances assumed	-0.049	1217	0.961	-0.067	1.370	-2.755	2.621

Table-4: Age-wise statistical significance with t-test for Equality of Means

Location	2017 (n=416)	2018 (n=803)	Total (n=1219)
ICU	29 (7%)	17 (2.1%)	46 (3.8%)
OPD	248 (59.6%)	570 (71%)	818 (67.1%)
Wards	139 (33.4%)	216 (26.9%)	355 (29.1%)
Table-5: Location- and year-wise distribution of *E. coli* isolates			

Test	Value (n=1219)	Asymptotic Significance (2-sided)
Pearson Chi-Square	26.382[c]	0.000
Likelihood Ratio	25.236[d]	0.000
c,d - degree of freedom (df) = 2		
Table-6: Chi-square test for location- and year-wise statistical significance		

Location	Intermediate (n=36)	Resistant (n=169)	Sensitive (n=1014)	Total (n=1219)
ICU	0	17	29	46
OPDs	30	65	723	818
Wards	6	87	262	355
Table-7: Location-wise meropenem susceptibility patterns among *E. coli* isolates				

Test	Value (n=1219)	Asymptotic Significance (2-sided)
Pearson Chi-Square	80.711[e]	0.000
Likelihood Ratio	75.365[f]	0.000
e,f - degree of freedom (df) = 4		
Table-8: Chi-square test for location-wise statistical significance of Meropenem susceptibility		

which are classified according to their molecular structure and functional properties by Ambler and Bush, respectively.[13-15] Two different families of β-lactamases have evolved in bacteria: the serine-beta-lactamases (SBL) and the metallo-beta-lactamases (MBL). The difference between SBL and MBL indicates their different molecular structures and phylogeny. The SBLs are structurally and mechanically related to the penicillin-binding proteins. The metallo-β-lactamases, which were discovered in the mid-1960s (about 25 years after the SBL), were first detected in only species with low pathogenic potential. In the 1990s, with the spread of genes encoding MBL carried on mobile deoxyribonucleic acid (DNA) elements among major Gram-negative pathogens, these enzymes increased in clinical importance culminating in the recent crisis resulting from international dissemination of Carbapenem-Resistant *Enterobacteriaceae* (CRE) producing the VIM and NDM types MBLs.[16] The metallo-beta-lactamases efficiently hydrolyse all β-lactams, except for aztreonam, in vitro. Therefore, detection of Gram-negative bacilli (GNB) resistant to carbapenems is crucial for the optimal treatment of patients and to control the spread of resistance.[17]

Although UTIs are treated empirically, increasing carbapenem resistance in uropathogens requires clinicians to incorporate epidemiologic characteristics and susceptibility patterns into their prescribing practices. Inappropriate prescribing of carbapenems is especially concerning, given that region with high rates of carbapenems prescribing show higher *E.coli* resistance rates.[18]

In this study, a total of 416 *E.coli* isolates were identified in the year 2017 among various uropathogens with 81.3% sensitivity towards Meropenem, and in the year 2018, this number was 803 with 84.2% sensitivity towards Meropenem. The value of chi-square for the difference in the distribution of Meropenem susceptibility for 2017 and 2018 was found to be <0.001, which is highly significant. Hence, the susceptibility profile of Meropenem changed significantly in two consecutive years in this tertiary care hospital.

In the second year of study, the centre adopted a new frequent cleaning policy inside the hospital with daily cleaning of inanimate equipment and mopping of floors with detergent and water after every two hours in various wards and after every hour in ICU. Compliance with cleaning was supervised by senior nurses every day. Multiple studies have proved that frequent cleaning can decrease environmental contamination and hand carriage of microbes, but it may not have high impact cost-wise, and clinically.[8] This study has a few limitations. The data is from a single centre and analysed urinary strains of *E.coli* only, with a maximum of one isolate per patient. Further studies are required to study the prevalence of various pathogens and their susceptibility patterns.

CONCLUSION

E.coli is the leading pathogen causing UTIs, and carbapenems are prescribed frequently, so regular monitoring of antimicrobial susceptibility is recommended, and frequent cleaning of the healthcare institutes can have a positive impact on decreasing environmental contamination and improving antimicrobial susceptibilities.

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