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Colistin susceptibility profile among MDR Gram-negative bacteria from urine samples in a tertiary care hospital

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Abstract :

Background: The study aimed to determine the colistin susceptibility profile among multidrug-resistant Gram-negative bacteria from patients with UTI. Materials and Methods: A prospective study was conducted for 9 months. In total, 60 urine samples were screened for Gram-negative bacteria. Isolates were subjected to antimicrobial susceptibility testing through the Kirby-Bauer method. Colistin susceptibility was determined through the E-test method.Results: In this study, 60 multidrug-resistant isolates were obtained from May 2022-Jan 2023 from urine samples received in the microbiology department, SRMMCH and Research Center for routine diagnosis. Among them, 75% were Escherichia coli, 15% were Klebsiella pneumonia, 6.6% were Pseudomonas spp and 3.3% were Proteus spp. All study isolates were resistant to ampicillin, cefoxitin, ceftriaxone, ceftazidime, and cefepime. For colistin, 90% of the isolates showed intermediate resistance and 10% showed complete resistance. Conclusion: This study documented 100% non-susceptibility to colistin among Gram-negative bacteria from urine samples. Notably, 60% of the strains were ESBL producers followed by 40% of AmpC producers. This study highlights the fact that the colistin non-susceptibility was higher among Gram-negative bacteria in UTI.

IndexTerms **- Gram negative bacilli, colistin resistance, UTI, MDR, ESBL**

I. INTRODUCTION

Gram-negative bacteria pose a major health problem in the community and hospital settings as they rapidly acquire resistance to multiple antimicrobial agents [1]. The incidence of multidrug-resistant (MDR) organisms producing ESBL, AmpC, and carbapenemase enzymes is increasingly reported in clinical isolates. The treatment of MDR Gram-negative bacterial infections among critically ill patients has presented major challenges in recent years [2].

Remarkably, the incidence of urinary tract infection (UTI) caused by MDR Gram-negative bacteria is a major issue around the world, as these bacteria developed resistance against fluoroquinolone, cephalosporins and carbapenems [3]. Various clinical manifestations of UTI include cystitis, pyelonephritis, asymptomatic bacteriuria, chronic and recurrent UTI [4]. Mainly Escherichia coli, Klebsiella spp., and Proteus mirabilis are the common pathogens causing UTI [5]. However, uncomplicated UTIs are generally community-acquired and largely caused by uropathogenic E. coli (UPEC) and Klebsiella spp., accounting for about 75–95% of the total reported cases [6]. Other uncommonly reported organisms include Enterobacter spp., Proteus spp., Pseudomonas spp., Enterococcus spp., and Staphylococcus spp., [7].

Notably, colistin and polymyxin are the last line of drugs for critical infections caused by Gram-negative bacteria [8]. Colistin (Polymyxin E) is the Polymyxin family of antibiotics. It is the old polypeptide antibiotic discovered from the organism Paenibacillus polymyxa subspecies colistinus by Y. Koyama in 1947 [9]. In general, colistin has activity against many Gram-negative bacteria mainly Enterobacteriaceae and some non-fermentative Gram-negative bacteria (NFGNB) such as P. aeruginosa and A. baumannii [9]. To date, colistin resistance (CLR) is reported to be mediated by chromosomal mutations in the genes associated with LPS alteration. In addition, the presence of two plasmids-mediated CLR genes such as mcr-1 and mcr-2 also contributed to colistin resistance [10].

This increasing resistance to last-resort antibiotics in Gram-negative bacteria is a significant public health concern. Therefore, the present study aimed to determine the prevalence of CLR in MDR Gram-negative bacteria obtained from patients with UTI.

II. NEED OF THE STUDY

The alarming rise in multidrug-resistant (MDR) Gram-negative bacteria, particularly in urinary tract infections (UTIs), has significantly limited treatment options, especially in hospital settings. Colistin, once considered a last-resort antibiotic, is increasingly showing reduced efficacy due to emerging resistance. Despite its critical role, surveillance data on colistin susceptibility among MDR uropathogens in many regions, including India, remains sparse. Understanding the current resistance patterns is essential to guide clinicians in empirical therapy, optimize antimicrobial stewardship, and implement effective infection control strategies. This study was therefore undertaken to evaluate the susceptibility of MDR Gram-negative urinary isolates to colistin, with the aim of informing local treatment protocols and contributing to broader antimicrobial resistance surveillance efforts.

III. RESEARCH METHODOLOGY

3.1 Study design

A prospective study was carried out for 9 months between May 2022 and Jan 2023 in the Department of Microbiology at SRMMCH and Research Center. MDR Gram-negative bacteria that are not intrinsically resistant to colistin were included while MDR Gram-negative bacteria with intrinsic resistance to colistin were excluded from the study.

3.2 Bacterial Identification and Antimicrobial susceptibility testing (AST)

Gram-negative bacteria were identified using standard clinical microbiological techniques. Urine samples were cultured on Blood agar, MacConkey agar, and UTI chrome agar and incubated for 24 hours at 37°C under aerobic conditions. Biochemical assays such as gram staining, oxidase, catalase, triple sugar iron test, indole, citrate, urease, mannitol, and motility tests were used for species identification.

AST was done using Kirby Bauer, disk diffusion method with bacterial growth adjusted to 0.5 McFarland standard solutions and streaked on Muller Hinton agar. Antibiotics tested were ampicillin, cefoxitin, ceftriaxone, ceftazidime, and cefepime. Clinical and Laboratory Standards Institute (CLSI) 2022 recommendations were used to interpret the results of the AST. Colistin MIC was determined using both the E-test strip and the Vitek 2 method [11].

For ESBL testing, K. pneumoniae ATCC 700603 was used as a positive control and E. coli ATCC 25922 as a negative control strain in this study. Similarly, for AmpC beta-lactamases, Enterobacter cloacae ATCC BAA 1143 and E. coli ATCC 25922 were used as positive and negative control strains in this study.

3.3 Statistical Analysis

All the data was entered, maintained, and analysed in Microsoft Excel 2018. Descriptive data was analysed as percentages and presented as graphs.

3.4 Ethics statement

The study was approved by the Institutional Ethics Committee (8447/IEC/2022).

IV. RESULTS AND DISCUSSION

4.1 Result with descriptive analysis

In this study, 60 MDR isolates were obtained from 60 urine samples screened from May 2022 – Jan 2023 in the Department of Microbiology, SRM Medical College and Research Centre. On microbiological analysis, Escherichia coli was found in 75% (n=45) of the isolates, Klebsiella pneumoniae in 15% (n=9), Pseudomonas spp. in 6.6% (n=4), and Proteus spp. in 3.3% (n=2) of the isolates. Figure 1 displays the distribution of MDR Gram-negative bacteria that were isolated from a urine sample. The Proteus spp. was excluded from further analysis due to its intrinsic resistance to colistin.

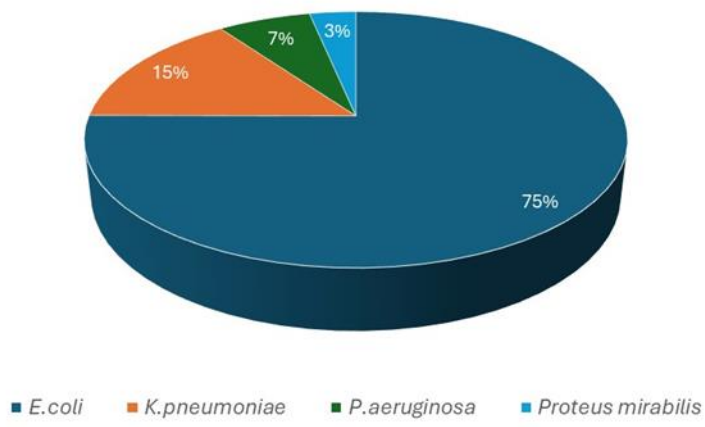

Fig 1: Distribution of MDR Gram-negative bacteria from urine sample

Among the identified MDR Gram-negative bacteria, 60% (n=36) of them were found to be ESBL producers and 40% (n=24) were AmpC producers as shown in Figure 2.

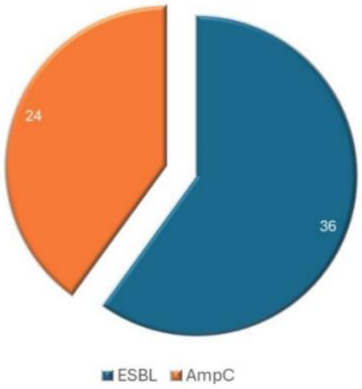

Fig 2: Distribution of enzyme producers among the study isolates

Colistin susceptibility testing results showed that 90% of the isolates were intermediate resistant and 10% of the isolates showed complete resistance by both E-strip and Vitek 2 methods as shown in Figure 3

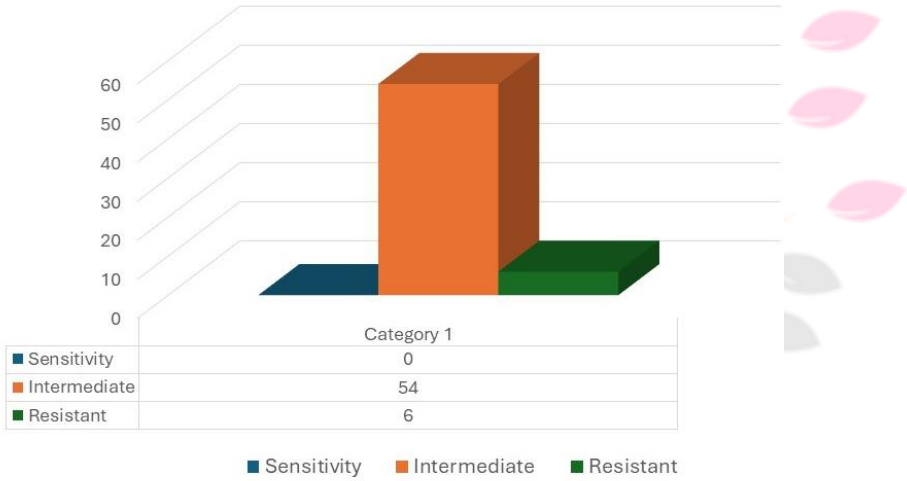

Fig 3: Colistin susceptibility testing by E-strip and Vitek 2 method

The susceptibility profile of the study isolates to other tested antibiotics is shown in Figure 4.

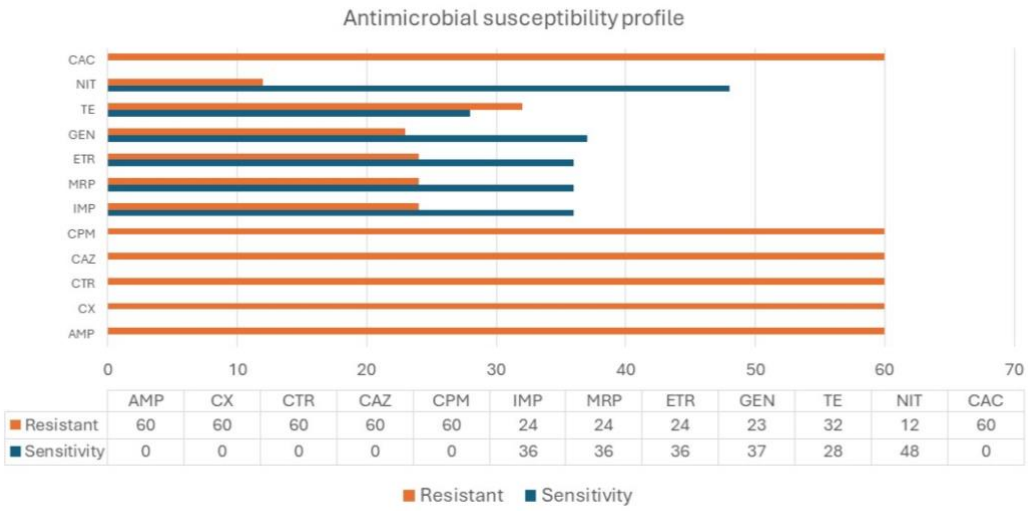

Fig 4: Antimicrobial susceptibility profile of MDR Gram-negative bacteria

All study isolates showed 100% resistance to ampicillin, cefoxitin, ceftriaxone, ceftazidime, and cefepime. Whereas carbapenem resistance was found in 24% of the isolates, while gentamicin, tetracycline, and nitrofurantoin resistance were observed in 23%, 32%, and 12% of the isolates respectively.

4.2 Discussion

UTI is the second most common infectious disease in both healthcare and community settings worldwide affecting 150 million people each year. UTI is more prevalent among women than men with 50-fold higher among the 20–50 years age group [12]. Here we present the prospective study data on the prevalence and antimicrobial susceptibility profile of MDR GNB causing UTI at SRM Medical College and Research Centre.

A total of 60 MDR GNB had been identified from urine samples attending the OPD of SRM Medical College and Research Centre representing the local epidemiology of UTI. In recent studies from India, E. coli was reported as the predominant isolate (72%) followed by Klebsiella spp. (15%). A similar observation was also reported from northern India [13]. This observation was concordant with our study results.

Further, UTIs caused by ESBL-producing E. coli and K. pneumoniae have been increasingly reported from many parts of the world including India which pose a major challenge for the clinician to start the empirical therapy. In this study, the majority of the isolates (60%) were ESBL producers followed by AmpC producers (40%). However, a recent multi-centre study from India reported only 48% of ESBL-producing GNB [14]. The variations in the ESBL prevalence rate might be due to the difference in the target population in different geographical regions. This implies that the study of UTI at a larger scale reflecting the national epidemiology is essential. Moreover, the resistance of MDR GNB against tetracycline, aminoglycosides, carbapenems, and nitrofurantoin was found to be 32%, 23%, 24% and 12% respectively which is lower compared to other studies from India.

Limited therapeutic options forced the clinicians and microbiologists to apply high drugs like colistin and polymyxin antibiotics [15]. Particularly, colistin is one of the important antibiotics used for treating MDR GNB infection in recent days. However, issues like pharmacokinetics, nephrotoxicity, lack of susceptibility data, dosing, and resistance development widely limited their usage. A similar study from India reported colistin resistance in 50%, 23% and10% of the GNB isolates from blood, respiratory and urine cultures respectively [16].

Nitrofurantoin is widely recommended as first-line empiric therapy for the treatment of UTI as per the European Association of Urology guidelines. In the current study, resistance to nitrofurantoin was observed to be 12%. This favours the use of nitrofurantoin as an effective agent for UTI caused by MDR GNB. While the rates of nitrofurantoin resistance from different European countries were reported below 1.5% [17]. Therefore, restricted usage is warranted to prevent further emergence of resistance against these antibiotics.

The study represents only single-centre data which is the limitation of this study. A wider geographical perspective on the significance of ESBLs and AmpC-producing GNB in UTI and the incidence of colistin resistance could have been gained if samples were included from multiple tertiary care hospitals.

4.3 Conclusion

Here we report an increased incidence of ESBLs and AmpC producers in UTI with increased colistin non-susceptibility. E. coli was the predominant species identified followed by K. pneumoniae. The increasing resistance to last-resort antibiotics such as colistin among GNB calls for the need for continuous monitoring and antimicrobial stewardship in hospitals. Colistin is the last line of drugs prescribed by clinicians for the treatment of MDR infections in many hospitals. The study concludes that in our hospital, most of the MDR Gram-negative fall under intermediate resistance and wise use of colistin helps in reducing the mortality among the patients infected with MDR bacteria.

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