ORIGINAL RESEARCH PAPER

Microbiology

PHENOTYPIC DETECTION OF ESBL AND MBL PRODUCERS IN COMMUNITY-ACQUIRED URINARY TRACT INFECTIONS – A PROSPECTIVE STUDY FROM WAYANAD

KEY WORDS: UTI, CAUTI, ESBL, MBL, Carbapenems

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ABSTRACT

Background: Community-acquired urinary tract infection (CA-UTI) endures the communal diseases diagnosed in community health practices. Urinary tract infections are commonly encountered by Escherichia coli and Klebsiella pneumoniae. Antibiotic therapy is an important step in UTI treatment but, the increase in the spread of resistance become a major concern. ESBL and MBL are the most common among antibiotic resistance. Aims and objectives: The major objective of the study is to detect the prevalence organisms causing CA-UTI and to find out the prevalence of ESBL and MBL isolates among drug-resistant organisms. **Materials and methods:** The study was conducted at the Microbiology Department of Dr. Moopens Medical College, Wayanad, Kerala, over a period of 3 months. All urine samples obtained from outpatients were processed for identification by culture, morphological and biochemical tests. Antibiotic sensitivity was performed using Kirby Bauer disc diffusion and the isolates were tested for ESBL and MBL producers. **Results:** Out of 876 urine samples cultured, 285 (32.5%) of the samples showed significant growth of organisms, in which 130 (44.6%) E.coli, 48 (16.8%) Klebsiella spp, 21 (7.4%) Citrobacter spp, and 18 (6.3%) Pseudomonas spp, were the most frequently isolated organisms. Among 285 samples ,79 were identified as ESBL isolates and 16 were identified as MBL isolates. Maximum ESBL and MBL activity was shown by E.coli (72.1% and 43.7%) respectively. The ESBL producers showed higher sensitivity towards amikacin, polymyxin, colistin, imipenem, meropenem, and ertapenem. MBL producers showed sensitivity also towards, gentamycin and nitrofurantoin. **Conclusion:** The prevalence of ESBL producers in the study is high in comparison with MBL producers. ESBL isolates in the present study show resistance to cephalosporins, ampicillin, ciprofloxacin, and norfloxacin. Routine testing for ESBL and MBL producers is necessary in order to optimize antibiotic management and cost-effective UTI therapy.

INTRODUCTION

Urinary tract infections are one of the most common infectious diseases.[1] *Escherichia coli* and *Klebsiella pneumoniae* are the commonly encountered bacteria in UTIs along with other *Enterobacteriaceae* members.[2] Community-acquired urinary tract infection (CA-UTI) are the communal diseases which is diagnosed in community health practices. In order to prevent the advancement of other diseases and to recognise the increase in upper urinary tract infections, an accurate and precise diagnosis of UTIs is essential. Antibiotic therapy is an important step in UTI treatment, especially using oral antibiotics such as nitrofurantoin, quinolones, trimethoprim, and oral cephalosporins.[3,4] It has been an important concern for the health care settings as there is a surge in the spread of resistance to the extended generation cephalosporin group of antibiotics in both hospital and community settings. Given their broad-spectrum activity, β -lactam antibiotics makes the choice of drugs in the treatment of UTIs. The β-lactam resistance mechanism includes alternations in the drug target site, reducing drug permeability and activity of the drug efflux pump. ESBL and MBL are the most common among antibiotic resistance.[5]Extended-spectrum cephalosporin and carbapenem resistance is brought on by beta-lactamases called ESBLs and MBL. In addition to being inhibited by -lactam inhibitors like clavulanic acid and sulbactam, ESBLs are a class of heterogeneous enzymes that can hydrolyse β-lactam antibiotics with oxyiminogroups (third-generation cephalosporin and aztreonam). As per studies, several ESBL-producing *Enterobacteriaceae* (ESBL-PE) infections are associated with carbapenem treatment and remain the gold standard in treating serious and invasive ESBL-PE infections.[6] The increase in the ESBL producers increases the use of carbapenems which is intended to prevent the emergence of bacteria that are resistant to them by producing metallo β-lactamase (MBL), a carbapenem hydrolyzing enzyme. *Enterobacteriaceae* such as *E.coli* was reported as the primary producer of MBL.[6] Their ability to resist phagocytosis and enhancing the membrane permeability results in a reduction in the amount of drugs that may penetrate the cells.[7,8] The treatment options for ESBL and MBL producing bacteria is also limited. Thus, it can be beneficial to regularly monitor the clinical isolates for drug resistance.[9] The current study was designed to detect the ESBL and MBL producers in community-acquired urinary tract infections which will also help to improve the treatment of UTIs.

AIMS AND OBJECTIVES

- To find out the most prevalent organisms causing CA-UTI.
- To detect the most common drug resistance among the isolates.
- To detect the prevalence of ESBL and MBL isolates among drug-resistant organisms.

MATERIALS AND METHODS

The study was conducted at the Microbiology Department of Dr. Moopens Medical College, Wayanad, Kerala, over a period of 3 months from March 2023 to May 2023. During the period, a total of 876 urine samples were collected from patients visiting to the outpatient department. Positive bacterial culture obtained from patients attending outpatient departments (OPDs) with complaints of UTIs is preferred.

Sample collection and bacterial identification

Urine samples were collected by the clean catch midstream method in a clean, leakproof, wide-mouthed container. The specimens are then inoculated into the blood and MacConkey agar plates. The plates are then incubated at 37°C for 24 hours. The presence of more than 10^5 CFU/ml bacteria indicates UTI. Isolates are identified based on colony morphology, wet mount, and biochemical methods such as catalase, oxidase,

coagulase, citrate, indole, mannitol, and urease.

Inclusion criteria

Urine samples collected from community-acquired UTI patients in dry, clean, leakproof containers with proper labeling were included in the study.

Exclusion criteria

Urine samples collected in leaking containers with improper labelling and patients admitted for more than 48 hours and patients already on antibiotics are excluded from the study.

Antibiotic susceptibility test

Antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method on Muller-Hinton agar (MHA) plates as per CLSI guidelines[10]. The bacterial isolates are swabbed on the MHA plates to make a lawn culture and antibiotic disks are placed at equidistance on the agar surface and then incubated for 24 hours at 37°C. The plates are then monitored for zone of inhibition.

Phenotypic detection of ESBL

Isolates that are resistant to cephalosporins like cefuroxime, ceftazidime, cefotaxime were then subjected to a confirmatory test for ESBL using a double disk synergy test(DDST). In DDST, Muller-Hinton agar plates were inoculated with each isolate to form a lawn culture. Antibiotic disks containing ceftazidime with and without clavulanic acid are placed on the lawn culture. The zone of inhibition if greater than or equal to 5 mm can be considered as ESBL producers as per CLSI guidelines.[10]

Phenotypic detection of MBL

The isolates that are resistant to imipenem or meropenem were tested phenotypically for MBL production. It was done using imipenem-EDTA combined disk synergy test. Two imipenem discs were placed on the surface of Muller-Hinton agar plates and 10 µl of EDTA solution was added to one of them. EDTA disk itself is used as a negative control. Plates are incubated for 16 to 18 hours at 37°C. The difference of 7 mm or greater between the inhibition zone diameter of the imipenem-EDTA disk and that of the imipenem alone constitutes MBL positivity.[1]

RESULTS

Out of 876 urine samples cultured, 285 (32.5%)of the samples showed significant growth of organisms. 290 (33.1%) samples came negative with no significant growth,whereas 274 (31.3%)samples showed no growth, and 27 (3.1%)samples showed polymicrobial growth (Fig 1). Out of 285 isolates with significant growth, 18 different types of bacteria were identified. In which 130 (44.6%) *E.coli*, 48 (16.8%), *Klebsiella spp*, 21 (7.4%) *Citrobacter spp,* and 18 (6.3%) *Pseudomonas spp*, were the most frequently isolated organisms (Fig 2).

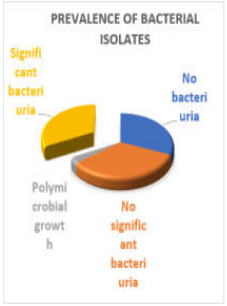

Fig 1

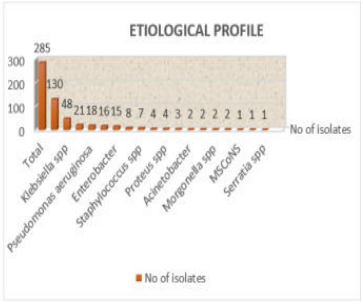

Fig 2

Among 285 samples ,79 were identified as ESBL isolates and 16 were identified as MBL isolates. In the present study maximum ESBL activity was shown by *E.coli* (72.1%), *K.pneumonia* (13.9%), citrobacter spp (8.6%), *Enterobacter* (3.7%) and *Acinetobacter spp* (1.2%). Out of 16 MBL isolates identified, 43.7% were *E.coli*, 25% were *K.pneumoniae* and 31.2% were *Pseudomonas aeruginosa.* (Fig 3).

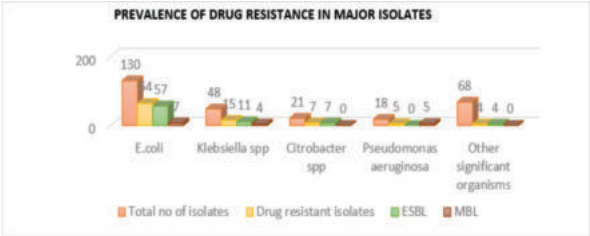

Fig 3

Most of the ESBL *Escherichia coli* and *Klebsiella* isolates in the present study showed resistance to cephalosporins-Cefuroxime, cefotaxime, ceftazidime, and ampicillin. The majority of isolates showed sensitivity to amikacin and gentamycin. Quinolones like ciprofloxacin and norfloxacin showed resistance to *E,coli* and *Klebsiella* isolates. Other classes of drugs which showed maximum sensitivity to ESBL *Escherichia coli* and *Klebsiella spp* include amikacin, polymyxin, colistin, imipenem, meropenem, and ertapenem.

The present study showed that MBL-producing *E,coli* and *Klebsiella* isolates were resistant to Imipenem, Meropenem, Piperacillin-tazobactam, Ampicillin, and Cephalosporins. The least resistance was shown by amikacin, gentamycin, polymyxin, colistin, and nitrofurantoin in the case of *Escherichia coli* whereas polymyxin and colistin shows the least resistance to MBL *Klebsiella* spp.

DISCUSSION

In the present study a total of 876 urine samples from outpatients were analysed, in this 285 of them showed significant growth with 18 different types of bacteria. In which *E. coli* and *K. pneumoniae* were the most frequently isolated gram negative bacteria. Similar rates of isolation of these organisms were reported by shreshtha *et al.*,[4] The identification of ESBL and MBL is critical since the enzymes are linked with either false susceptibility or false resistance[2]. The present study showed lower percentage of ESBL and MBL producers among the samples obtained from outpatients which is ranging from 28 % to 5 % respectively. Similar results showing the lower rate of MBL production was shown in a study conducted by Shahendeh *et al.*,[6]. The present study showed 72.1% of the ESBL isolates are *Escherichia coli* followed by 13.9% of *K. pneumoniae*, 8.6% *Citrobacter species,* 3.7% *Enterobacter* and 1.2% *Acinetobacter* species being the least prevalent. According to his study conducted by S A Khan *et al.*, ESBL positive isolates were mostly found in *K. pneumoniae* (72.1%), *E. coli* (53.5%), *P. aeruginosa* (20%), and *Acinetobacter* (20%), which contradicts the current study (44). our study the percentage of MBL producers was high in *E. coli* which is 43.7% followed by 31.2% *P. aeruginosa* and 25% *K. pneumoniae*. Shrestha *et al* .,in their study showed maximum MBL activity in *E. coli* (38%), *Pseudomonas spp* (31%), which is followed by *K. pneumoniae* (19%), and *Proteus spp* (12%). The results of this study are simliar to the present study.[7]

Infections produced by ESBL-producing bacteria are problematic because they may co-occur with factors that contribute to resistance to other antimicrobial drugs due to restricted treatment options and the failure of broad-spectrum antibiotic therapy. As a result, they have become a significant and severe challenge for public health practitioner.[8]

CONCLUSION

Drug resistance among *E.coli* and *K.pneumoniae* are widely prevalent and their isolation in the community-acquired UTI is a matter of major concern. The present study demonstrates the presence of ESBL producers as high in comparison with MBL producers. ESBL isolates in the present study show

resistance to cephalosporins, ampicillin, ciprofloxacin, and norfloxacin. The least resistance was shown in amikacin, imipenem, and meropenem which shows their effectiveness to be used for the treatment of UTIs. MBL isolates in the study showed resistance to imipenem, meropenem, piperacillin-tazobactam, ampicillin, and cephalosporins. Prevalence and antibiotic-susceptibility studies should be undertaken on a regular basis to aid in the early diagnosis of antibiotic resistance and the formulation of guidelines for suitable and cost-effective UTI therapy.

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