Original Research Article

Study Of Multidrug Resistance And Extended Spectrum Beta Lactamases Producing Klebsiella Pneumoniae Isolated From Hospitalized Patients Of SKMCH, Muzaffarpur, Bihar, India

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Abstract

Introduction: The aim of this study was to determine the prevalence of ESBL-producing bacteria and their antibiotic susceptibility pattern among uropathogens isolated from patients. **Methodology:** In the current descriptive cross-sectional study, 1000 urine samples were aseptically collected from patients more than 18 years of age and suspected to have UTI attending Medical out Patient Department of Microbiology, Shri Krishna Medical College & Hospital, Muzaffarpur, and Bihar during a period of one year From May 2020 to April 2021. Detection of ESBL-producing organisms was performed by Double Disc Synergy Test (DDST) method following CLSI recommendations. In this method, first, a suspension was prepared for each pure bacterial isolate according to the 0.5 McFarland turbidity standards and cultured on Mueller–Hinton agar. Fifteen minutes after bacterial cultures, pairs of antibiotic disks containing Ceftazidime (30 µg) with Ceftazidime/Clavulanic acid (30/10 µg), and Cefotaxime (30 µg) with Cefotaxime/Clavulanic acid (30/10 µg) were placed on Mueller–Hinton agar medium center to center, at a distance of 20 mm apart from each other. **Results:** Out of 1000 participants surveyed in the present study, majority (74.8%) were female. Positive bacterial growth was detected in urine samples of 74 (7.4%) patients. Among uropathogens, *E. coli* (72.9%) was the most commonly isolated species, followed by *K. pneumoniae* (13.5%). **Conclusion:** The ESBL production was found in a significant amount of *Klebsiella* spp isolates. An intensifying level of resistance to various classes of antimicrobial agents was observed among ESBL producers compared with non-ESBLs.

Key Words: Multidrug Resistance, Extended Spectrum Beta Lactamases, Klebsiella Pneumoniae

Introduction

For decades, antibiotics have been used for the treatment of bacterial infections successfully; however, over the past few years, the abuse of antibiotics has led to the emergence of antimicrobial resistance around the world and has become a serious global threat to the public health[1, 2]. Recently, it has been reported that approximately 700,000 people worldwide die annually from antimicrobial resistance (AMR) infections and it has been predicted that this number would reach 10 million by 2050[3].At present, β-lactam drugs are a key factor in the treatment of bacterial infections worldwide and account for almost 65% of antibiotic usage[4]. They have been classified into six main groups based on the chemical structure of the β-lactam ring which includes Penicillins, Cephalosporins, Cephamycins, Carbapenems, Monobactams, and β-lactamase inhibitors. These drugs block cell wall synthesis by preventing accurate working of the Penicillin-binding protein (PBP), which has a principal role in the synthesis of the bacterial cell wall, and finally leads to cellular death. Nevertheless, it is unfortunate that, in recent years, resistance to this important class of antibiotics is also increasing globally[5]. Resistance to b-lactams can occur through different mechanisms such as the generation of efflux pumps, changes in the production of outer membrane porins, alterations of PBPs, and the production of β-lactamase for inactivating antibiotics. Of these mechanisms, the production of B-lactamases is the most prevalent source of resistance to β-lactam antibiotics which are produced by both Gram-positive (extracellularly) and Gram-negative (in the Periplasmic space) bacteria. These enzymes are able to make the β-lactam antibiotics inactive by binding covalently to their carbonyl section and hydrolyzing the b-lactam ring thus enabling β-lactam resistance[6-7].

To date, various β-lactamases have been reported to be generated by diverse microorganisms, including Penicillinases, Extended-spectrum β-lactamases (ESBLs), Cephalosporinases (AmpC), Metallo-β-lactamases (MBLs), and Carbapenemases (KPCs). Among these, ESBL-producing bacteria are very important and have attracted the attention of the scientific community[8]. ESBLs are β-lactamases enzymes with the capability to hydrolyze β-lactam antibiotics containing Penicillins, Aztreonam, as well as the first-, second-, third- and fourth-generation Cephalosporins, while sparing Cephamycins, Moxalactam, and Carbapenems. Further, they are inhibited by β-lactamase inhibitors, such as clavulanic acid, Tazobactam, and Sulbactam [8-10]. ESBLs producing organisms may also induce resistance to some of the non β-lactam antibiotics including Aminoglycosides, Quinolones, and Trimethoprim sulfamathoxazoles[11]. Today, the outbreak of infections caused by ESBL producing pathogens is becoming increasingly frequent and has become a world health threat[12]. The plasmid location of ESBL genes contributes to their spread through the horizontal gene transfer among the same and different species of bacteria[13]. The prevalence of ESBL-producing isolates depends on some factors including species, geographic region, hospital/ward, group of patients and type of infection, and extensive overuse of antibiotics[14, 15].ESBLs are mostly produced by Gram-negative bacilli, especially *Enterobacteriaceae* family[8].

ESBL-producing *Enterobacteriaceae* cause a variety of hospital and community-acquired infections such as bloodstream, wound

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infections, respiratory tract, and urinary tract infections[16]. Urinary tract infections (UTIs) are very common infectious diseases that occur in a high proportion of the population and are a serious concern in the healthcare system[17]. At present, Carbapenems are selective drugs for the effective treatment of infections caused by ESBL-producing organisms. However, increasing Carbapenem resistance bacteria has also been associated with the use of Carbapenems[18, 19].

Due to expanding antibiotic resistance among bacteria and the high distribution of ESBL producing isolates, the recognition of the prevalent species that produce this enzyme as well as their antibiotic susceptibility pattern is necessary for each community to select the most effective treatment options. Thus, the aim of this study was to determine the prevalence of ESBL-producing bacteria and their antibiotic susceptibility pattern among uropathogens isolated from patients.

Methodology

In the current descriptive cross-sectional study, 1000 urine samples were aseptically collected from patients more than 18 years of age and suspected to have UTI attending Medical out Patient Department of Microbiology, Shri Krishna Medical College & Hospital, Muzaffarpur, and Bihar during a period of one year From May 2020 to April 2021. Samples with positive bacterial growth were cultivated on Blood Agar and Eosin Methylene Blue Agar (EMB) medium (Merck, Germany) and incubated at 37 ℃ for 24 h. Initially, the colonies were counted. In cultures with bacterial counts of $> 10^4$ cfu/ml, the specimens were considered as positive, and gram-staining technique was performed. Then, bacterial genus and species were determined by standard biochemical tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was performed by the standard Kirby–Bauer disk diffusion method on the Mueller–Hinton agar media (Merck, Germany) using commercially available antibiotic disks (Mast, UK). The diameter of inhibition zone was measured for each antibiotic disk, and the results were defined in accordance with the CLSI guidelines[20].

Phenotypic identification of ESBL-producing strains

Detection of ESBL-producing organisms was performed by Double Disc Synergy Test (DDST) method following CLSI recommendations. In this method, first, a suspension was prepared for each pure bacterial isolate according to the 0.5 McFarland turbidity standard and cultured on Mueller–Hinton agar. Fifteen minutes after bacterial cultures, pairs of antibiotic disks containing Ceftazidime (30 µg) with Ceftazidime/Clavulanic acid (30/10 µg), and Cefotaxime (30 µg) with Cefotaxime/Clavulanic acid (30/10 µg) were placed on Mueller–Hinton agar medium center to center, at a distance of 20 mm apart from each other. The plates were incubated for 24 h at 37 ℃. Then, the diameter of inhibition zone was measured. According to CLSI guidelines, an increase of ≥ 5 mm in the zone diameter around the clavulanic acid combination disks versus the same disks alone confirmed the presence of ESBL producer strains[20].

Ethical considerations

All ethical aspects of this research have been completely observed by the authors. It was approved by the Institutional Ethics committee. Informed consent was obtained from all participants before the study. The patient's demographic characteristics were recorded in a questionnaire and their information remained confidential.

Data analysis

Data were analyzed using descriptive statistics (frequency and frequency percent, average and standard deviation) on SPSS software version 22.

Results

Of a total of 1000 participants surveyed in the present study, majority (74.8%) were female. The age of the patients ranged from 18-72 years with a mean age of32.7 ± 20.8 years.

Positive bacterial growth was detected in urine samples of 74 (7.4%) patients. Among uropathogens, *E. coli* (72.9%) was the most commonly isolated species, followed by *K. pneumoniae*(13.5%). Out of the 74 positive bacterial cultures, 86.5% cases were related to females. The patients mostly belonged to the age groups of > 45 years, which constitute a little more than one-third of the case load. In both genders, the main infectious strain was *E. coli*.

Antimicrobial susceptibility profile and ESBL production of K. pneumonia was studied as described in the methodology section. According to Table 1, *K. pneumoniae* showed the highest rate of sensitivity towards Imipenem (100%), followed by Amikacin (83.3%), Meropenem (83.3%), and Gentamycin (83.3%), and the least sensitivity to tetracycline (0%), Tobramycin (0%), and Ampicillin (16.7%). From 10*K. pneumoniae* isolates, 4 (40%) were confirmed as ESBL producer. The expression of ESBL resulted in developing resistance to the antibiotics Ceftazidime, Cefotaxime, Ceftriaxone, Gentamycin, Cefalexin, Nalidixic-acid, and Co-trimoxazole.

Table 1: Antibiogram report of K. pneumonia

Antibiotic	Sensitive ESBL producers	Sensitive Non-ESBL producers
Ciprofloxacin	0	2
Norfloxacin	0	2
Amikacin	1	5
Ceftazidime	0	2
Cefotaxime	0	2
Ceftriaxone	0	2
Gentamycin	0	5
Imipenam	2	6
Meropenam	2	5
Nitrofurantoin	0	4
Cefalexin	0	2
Ampicillin	1	4
Nalidixic-acid	0	4
Co-trimoxazole	0	2
Colistin	1	2
Chloramphenocaol	1	2
Piperacillin	1	3
Tetracycline	0	3
Tobramycin	0	3

Discussion

Urinary tract infection (UTI) is the second common infectious disease throughout the world caused by a wide range of microbial pathogens[21]. In the present work, from 1000 suspected UTI patients, 7.4% uropathogenic bacteria containing different species of Gram-negative and Gram-positive bacteria were isolated. The majority of the isolates were obtained from females. This finding is supported by other studies reporting a higher rate of UTI prevalence in female patients compared to males[14-22]. These studies suggest that females are more at risk of developing infection by uropathogens which is due to their anatomical structure[23]. In terms of age, it was found that the most frequently uropathogens were related to the patients with age> 45 years old. These outcomes agree with previous studies in which the incidence of UTIs was higher among elderly patients[24, 25].

Klebsiella sp has been identified as vital causative agents of UTIs as they possess a number of factors including adhesion, pilli, fimbrae, and P1 blood group genotype receptor, which contribute to the attachment of bacteria to the urothelium[26]. In this regard, here the predominant urinary isolates were *E. coli* followed by *K. pneumoniae*. These results are in line with the earlier studies conducted by other researchers[27-29].

Among the isolates of *Klebsiella* spp. (40%) were ESBL producers. This is in accordance with other studies in which among various ESBL isolates, *E. coli* species was the most prominent isolates followed by *Klebsiella* spp while minimum ESBL isolates were related to *Citrobacter* spp[30-32]. In contrast, several studies reported the highest ESBL production among *K. pneumoniae*[33-35].

The antibiotic susceptibility testing by commonly prescribed antibiotics was accomplished for the ESBL-producing *K. pneumoniae* presented a higher resistance rate to Ceftazidime, Cefotaxime, Ceftriaxone, Gentamycin, Cefalexin, Nalidixic-acid, and Co-trimoxazole. In line with these outcomes, a study carried out by Poovendran et al. indicated that resistance to Tetracycline, Amikacin, Ampicillin, Tobramycin, and Norfloxacin was comparatively higher among ESBL-producer than non-ESBL producer. Nevertheless, both groups of isolates were 100% sensitive to Imipenem[36]. Furthermore, Albu et al. found that ESBL-producing *K. pneumoniae* isolates were more resistant to most of the antibiotics tested compared to non-ESBLs, except for Imipenem for *K. pneumoniae*, which had lower resistance rates than non-ESBLs[37]. Further, another study by Abayneh et al. indicated that ESBL-positive bacteria had higher resistance to most of the antimicrobial agents tested, while in both ESBL-producing and non-ESBL-producing isolates, no resistance was observed toward Imipenem and resistance to Amikacin was low[38].

Conclusion

In conclusion, the current study indicated a significant rate of infection with ESBL-producing Gram-negative bacilli among UTI patients. The ESBL production was found in a significant amount of *Klebsiella* spp isolates. An intensifying level of resistance to various classes of antimicrobial agents was observed among ESBL producers compared with non-ESBLs.

Conflict of interest

None

Funding

None

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