Phenotypic Detection and Antibiotic Resistance Pattern of AmpC β-lactamase Producers among the Gram-Negative Uropathogens at A Tertiary Care Hospital

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Abstract

Background: An increase in the incidence of Urinary Tract Infections (UTI) leads to more consumption of antimicrobials and increases the chances of resistance among organisms. The objective of this study was to understand the prevalence of AmpC β-lactamase producing gram-negative uropathogens by using the inhibitor-based method and their antimicrobial susceptibility pattern.

Materials and methods: The cross-sectional study was conducted in 2015 and urine samples were collected from patients at a medical college hospital. Identification of gram-negative uropathogens was done by standard laboratory methods of identification and screened for AmpC beta-lactamase production by cefoxitin disc. AmpC β-lactamase producing organisms were confirmed by phenotypic inhibitor-based combined disc diffusion method.

Results: Out of 280 urine samples, 120 (42.9%) were culture positive, and among them, 118 (98.3%) organisms were gram-negative bacteria and 2 (1.7%) were gram-positive bacteria. Among the gram-negative bacteria, the majority were E. coli (40.7%), Klebsiella spp. (33.9%), Pseudomonas spp. (22.9%), followed by Proteus spp., Acinetobacter spp., and Serratia spp. (2.4%). Among the isolated bacteria, 48 (40.7%) were AmpC β-lactamase producers and they showed high degree of resistance to amoxiclav (100.0%) cefotaxime (95.8%) ceftazidime (87.5%) nalidixic acid (85.4%) ceftriaxone (81.3%) cefepime (75.0%) aztreonam (70.8%) co-trimoxazole (68.8%) nitrofurantoin (66.7%) ciprofloxacin (58.3%) etc.

Conclusion: Antimicrobial resistance among AmpC β-lactamase producing bacterial pathogens was considerably high which underscores the need of updating UTI guidelines and surveillance. The inhibitor-based combined disc diffusion method can be used as a simple, cheap and rapid phenotypic test for the detection of AmpC β-lactamase in clinical laboratories.

Key words: Antimicrobial resistance; AmpC β-lactamase; Phenotypic detection; Susceptibility; Uropathogens.

Introduction

Urinary Tract Infection (UTI) is considered one of the most common bacterial infections encountered in communities as well as hospitalized patients.[1] The gradual increase in the incidence of UTI leads to more consumption of antimicrobial drugs and drug resistance both on a higher scale. In the last few decades, there have been remarkable alterations in antimicrobial resistance patterns of uropathogens, especially in developing countries like Bangladesh due to uncontrolled and widespread use of antibiotics. Of the various resistance mechanisms prevailing among bacteria, the production of β-lactamases is the most widespread and effective mechanism through which bacteria become resistant to β-lactam drugs. Hence, the trend of antimicrobial resistance due to AmpC beta-lactamase-producing uropathogenic Escherichia coli (E. coli) and Klebsiella pneumoniae is flourishing with clinical importance accounting for 80% of community-acquired and nosocomial urinary tract infections[2].

AmpC β-lactamases are class C or group-1 cephalosporinases that confer resistance to a wide variety of β-lactam antibiotics including alpha methoxy β-lactams such as cefoxitin, narrow and broad-spectrum cephalosporins, aztreonam and poorly inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, tazobactam, etc. AmpC β-lactamase can be chromosomal or plasmid-mediated. Chromosomal AmpC enzymes

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are seen in organisms like Citrobacter freundii, Enterobacter cloacae, Morganella morganii, Serratia marcescens, Pseudomonas aeruginosa, and Burkholderia cepacia. These are inducible by β-lactam antibiotics such as cefoxitin and imipenem but poorly induced by the third or fourth-generation cephalosporins[3]. Plasmid-mediated AmpC β- lactamase has been found frequently in Klebsiella spp, E.coli, Proteus spp, Salmonella typhi, Citrobacter freundii, etc[4].

Studies on detection of AmpC production are much fewer comparing with Extended Spectrum β-Lactamase (ESBL) detection and there is no recommended guideline for the detection of this resistance mechanism. For clinical microbiologists, detection of AmpC-mediated resistance in gram-negative organisms creates a problem because ESBL and AmpC β-lactamases both may co-exist, and mask each other. In Bangladesh, the exact prevalence of AmpC β-lactamase is unknown due to lack of a simple and reliable method for detection in routine clinical laboratories. Screening with cefoxitin disc is recommended for initial detection but it does not reliably indicate AmpC production. There are some conventional methods for the detection of AmpC β-lactamase such as the three-dimensional test, AmpC disc test, modified disc diffusion test and inhibitor-based test (Cefoxitin-boronic acid disc) having advantages and disadvantages both.[5-8] So, this study was designed to determine the prevalence of AmpC β-lactamase producing gram-negative uropathogens and their antimicrobial susceptibility pattern which would guide clinicians and microbiologists for proper detection of these pathogens and prevent misuse of antibiotics.

Materials and methods

This cross-sectional study was carried out from January 2015 to December 2015 in the department of Microbiology, Chittagong Medical College, Chattogram, after due approval from the institutional ethical review committee.

Inclusion criteria: Patient with burning sensation during micturition having frequency and urgency, cloudy or bloody urine, fever, chills and pain in lower abdomen.[9]

Exclusion criteria: Patient received antibiotic for the recent infections.

Based on the following criteria, a total of 280 urine samples were collected from patients with suspected urinary tract infection from Out-patient Department as well as In-patient Department after receiving informed written consents.

All urine specimens were inoculated on Blood agar, MacConkeys agar media, Cysteine Lactose Electrolyte Deficient agar (CLED) media, and incubated at 37^0C for 18-24 hours. Identification of organisms were done as per standard laboratory methods of identification and antimicrobial sensitivity of the isolates were tested against different antibiotics according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).[10] Isolated gram-negative bacteria were screened for presumptive AmpC β-lactamase production by testing their susceptibility to 30 µg cefoxitin disc (Oxoid, UK) by Kirby Bauer disc diffusion method and isolates with an inhibition zone diameter of less than 18mm were considered as screen positive for AmpC β-lactamase producer. These screen positive isolates were selected for phenotypic confirmatory test by inhibitor-based method.

Inhibitor-based method described by Coudron was used as a standard phenotypic method to detect AmpC β -lactamase production[8]. In this method, discs containing boronic acid were prepared as follows: 120 mg of phenylboronic acid (Sigma-Aldrich, Milwaukee, Wis.) was dissolved in 3 ml of dimethylsulfoxide and three milliliters of sterile distilled water was added to this solution. Twenty

microliters of this stock solution was dispensed onto discs containing 30 µg of cefoxitin. Discs were allowed to dry for 30 min and used immediately or stored in airtight vials with desiccant at 4^0C and at -70^0C. The cefoxitin-boronic acid disc test was performed by inoculating Mueller-Hinton agar (Oxoid, UK) media with a 0.5 McFarland's turbidity adjusted suspension of the test strain and placing a disc containing 30µg of cefoxitin (Oxoid, UK) and a disc containing 30µg of cefoxitin with 400µg of boronic acid at a distance of 20 mm. Inoculated plates were incubated overnight at 35^0C. After overnight incubation, diameter of zone of inhibition was measured. Enhancement of zone of inhibition by 5 mm around a cefoxitin disc with phenylboronic acid in comparison to a cefoxitin disc alone was confirmed as AmpC β-lactamase producing organism.[8]

Results

A total of 280 urine samples were studied, of which 120 (42.9%) samples showed significant growth. Among the 120 isolates, 118 (98.3%) were gram-negative bacteria and 2 (1.7%) were gram-positive bacteria. Among the gram-negative bacteria, majority were E. coli (48, 40.7%), Klebsiella spp. (40, 33.9%) and Pseudomonas spp. (27, 22.9%) followed by Proteus spp., Acinetobacter spp., and Serratia spp. (3, 2.4%) (Fig 1). Gram-positive bacteria were Staphylococcus saprophyticus and Enterococci spp.

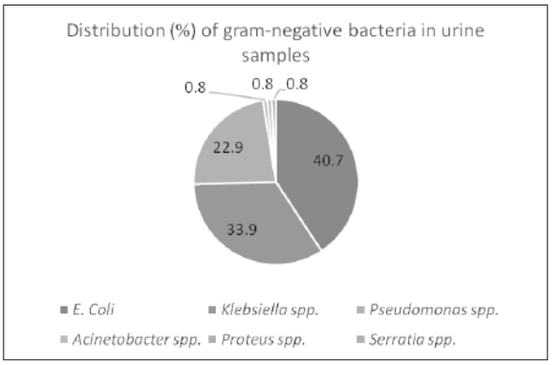

Figure 1 Distribution of gram-negative bacteria among isolates detected in urine samples

Among 118 gram-negative bacteria, 57 (48.3%) were cefoxitin resistant by screening test with cefoxitin disc (30µg), and 48 (40.7%) were confirmed as AmpC β-lactamase producer by cefoxitin-boronic acid disc (Table I). A total of 70 (59.3%) organisms were non-AmpC gram negative bacteria. Higher rate of AmpC β-lactamase was observed in Pseudomonas spp. (15, 55.6%), Klebsiella spp. (14, 35.0%), E. coli (16, 33.3%) followed by Acinetobacter spp, Proteus spp and Serratia spp. (3, 100%) (Table I).

Table I Distribution of AmpC β-lactamase producing gram-negative bacteria on the basis of screening test and phenotypic confirmatory test (n=118)

Name of gram negative bacteria	Total number of gram-negative bacteria, n (%)	Number of AmpC β-lactamase producers, by screening test n (%)	Number of AmpC β-lactamase producers by phenotypic confirmatory test, n (%)
E. coli	48 (40.7)	20 (41.7)	16 (33.3%)
Klebsiella spp.	40 (33.9)	16 (40.0)	14 (35.0%)
Pseudomonas spp.	27 (22.9)	18 (66.7)	15 (55.6%)
Acinetobacter spp.	1 (0.8)	1 (100.0)	1 (100.0%)
Proteus spp.	1 (0.8)	1(100.0)	1 (100.0%)
Serratia spp.	1 (0.8)	1 (100.0)	1 (100.0%)
Total	118 (100.0)	57 (48.3)	48 (40.7%)

*Two gram-positive bacteria were excluded from the total isolates (n=118).

Figure 2 Combined disc diffusion test (Cefoxitin-Boronic acid disc test) in Mueller Hinton agar inoculated with the test isolate showed increased zone of inhibition with cefoxitin disc (30 µg) supplemented with 400µg of phenylboronic acid in comparison to cefoxitin disc alone. The discs from left to right: Cefoxitin-Boronic acid disc and cefoxitin disc alone.

AmpC β-lactamase producing isolates showed high degree of resistance to amoxiclav (100.0%), cefotaxime (95.8%), followed by ceftazidime (87.5%) nalidixic acid (85.4%) ceftriaxone (81.3%) cefepime (75.0%) aztreonam (70.8%) co-trimoxazole (68.8%) nitrofurantoin (66.7%) ciprofloxacin (58.3%) piperacillin-tazobactam (45.8%) amikacin (41.7%) and netilmicin (35.4%) (Figure 3). The most effective antibiotic was imipenem which showed 25% of resistance to AmpC β-lactamase producing isolates.

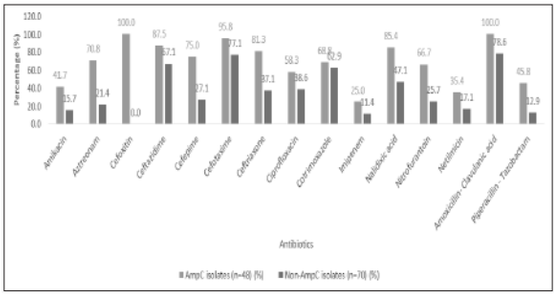

Figure 3 Resistant pattern of AmpC (n=48) and Non-AmpC (n=70) gram negative bacteria

*Non-AmpC (n= 70) =Cefoxitin sensitive (63) + Non AmpC cefoxitin resistant (9).

Discussion

In the present study, 42.9% bacteria were isolated from clinically suspected UTI patients. The finding is comparable with the other studies conducted by Haque et al. in Bangladesh (42.7%) and Pardeshi in India (33.5%)[11,12]. Demonstration of previous studies showed that gram-positive

bacteria had a low contribution in causing UTIs which was also observed in our study. Among the gram-negative bacteria, E. coli was the most prevalent bacterial species followed by Klebsiella spp. and Pseudomonas spp. This finding correlated well with the result of Akram et al. in India and Islam et al. in Bangladesh.[1,13]

Uropathogens with antimicrobial resistance continues to be a major health problem in different parts of the world.[11] Antibiotic abuse and practice of incomplete antibiotic dosage have considerably promoted the distribution of multidrug resistant bacteria.[14] As of now, no countrywide study has been conducted for the detection of the prevalence of AmpC β-lactamase production in Bangladesh, rather individual studies were done in different parts of the country showed a varying prevalence, based on various risk factors. In our study, 48.3% gram-negative bacteria were detected as cefoxitin resistant by screening whereas 42.3% was found by Mol et al in India [15]. We found 40.7% of AmpC β-lactamase producers detected by inhibitor based phenotypic confirmatory method. Our finding is closely related to Mol et al in India and Hassan et al in Pakistan reported respectively 53.4% and 52.3% of AmpC producers by inhibitor-based method (Cefoxitin-boronic acid disc).[15-17] On the contrary, in Bangladesh, 31.6% isolates were found cefoxitin resistant (Screening positive) and 25.5% were confirmed as AmpC β–lactamase producers.[18] In the study conducted by Mol et al showed that out of 58 cefoxitin resistant isolates, 42 (72.4%) were AmpC genes detected by multiplex PCR and 25 (59.5%) by inhibitor based test while 9 (21.4%) were detected by disc approximation test.[15] In a study conducted in 2005, Coudron also found that boronic acid disc test detected 98.2% of isolates out of 55 AmpC PCR positive isolates.[8] In our study, we observed that 7.6% (Table I) of cefoxitin resistant isolates were not true AmpC β-lactamase producers which is also supported by Helmy et al and Mol et al.[19,15] These might be due to other antimicrobial resistance mechanisms like extended spectrum beta lactamases, metallo-beta-lactamases, mutation of porin channels or increase in efflux pump expression. In our study, among the AmpC β-lactamase producers, predominant organisms were Pseudomonas spp., Klebsiella spp and E.coli. which is supported by the findings of Sasirekha and Mol et al in India and Ghonaim et al. in Egypt[20,15,21]. The variations in AmpC β-lactamase positivity in different studies might be due to the number of isolates studied, variation in institutions, and geographic locations.[22]

The antibiogram of isolated uropathogens demonstrated that E. coli isolates were mostly resistant to penicillin and it's derivatives, and also to quinolone group. On the other hand, imipenem, amikacin, and netilmicin had good sensitivity against E. coli. Similar result of sensitivity pattern of uropathogens except imipenem was found 99% and 100% respectively against E.coli.[1,23]

In the present study, Klebsiella spp. and Pseudomonas spp. showed higher susceptibility to imipenem followed by netilmicin, amikacin, and piperacillin-tazobactam while resistance was found to amoxiclav, cefotaxime, nalidixic acid, ceftazidime, carbenicillin, nitrofurantoin, ceftriaxone, cefepime, and aztreonam. The result is also supported by Akram et al and Sharmin et al.[1,23]

The clinical isolates were found to coexist with resistance to two or more antimicrobial drugs and the most effective antibiotic was imipenem showed 75% sensitivity to AmpC β-lactamase producing isolates. In this study, highest rate of susceptibility among gram-negative isolates was observed to imipenem followed by amikacin and netilmicin. Similarly, Noor et al. reported highest rate of susceptibility to imipenem followed by amikacin among uropathogens.[24] Neupane et al also found the highest rate of susceptibility toward amikacin and nitrofurantoin.[25] Almost similar antimicrobial resistance pattern had been observed by Mol et al and least resistance was found with amikacin (1.1%) and piperacillin-tazobactam (2.2%).[15]

AmpC β-lactamase producing bacterial pathogens may cause a major therapeutic failure if not detected properly and reported in time. Because AmpC β-lactamases can act as hidden reservoir for ESBL and high-level expression of AmpC β-lactamases may mask recognition of ESBL.

Limitation

Genotypic study was not performed due to lack of unavailability of institutional multiplex pcr machine though it is a gold standard method.

Conclusion

The widespread and inappropriate use of antibiotics has resulted a antibiotic resistant microbial ecosystem gradually. So, development of regional surveillance program is necessary to provide information which will enable us to update UTI treatment guidelines and reduce antimicrobial resistance.

Recommendation

Combined disc diffusion (Cefoxitin-Boronic acid disc) test can be used as a simple, convenient and rapid phenotypic test for detection of AmpC β-lactamase in clinical laboratories.

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Contribution of authors

DA-Conception, acquisition of data, manuscript writing & final approval.
MMR-Data analysis, critical revision & final approval.
SA-Interpretation of data, critical revision & final approval.
SB-Data analysis, manuscrip writing & final approval.
AHMSKC-Acquisition of data, manuscript writing & final approval.
MSA-Design, interpretation of data, critical revision & final approval.

Discloser

All the authors declared no conflict of interest.

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