ORIGINAL ARTICLE

SUSCEPTIBILITY PATTERN OF AMP-C PRODUCING GRAM NEGATIVE RODS

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ABSTRACT

Objectives: To determine the frequency of Amp-C producing Gram negative rods and susceptibility pattern of isolates from various clinical specimens at a tertiary care setting.

Material and Methods: It was a Descriptive cross sectional study, conducted in the Department of Microbiology, Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi from February 2014 to February 2015. Gram negative rods isolated from various specimens were processed and identified according to standard procedures. The isolates were screened for production of Amp-C with cefoxitin disc (30μg) and antimicrobial sensitivity against first and second line antibiotics was tested by Kirby-Bauer disc diffusion method. The isolates resistant to cefoxitin were confirmed for Amp-C by cefotetan/cloxacillin E-strip method.

Results: Out of total 342 isolates of Gram negative rods, 135 were resistant to cefoxitin disc, and 120 were confirmed as Amp-C producers through cefotetan/cloxacillin E-strip method. Susceptibility pattern of 120 isolates yielding growth of Amp-C producing isolates was determined. The age of patients ranged from 2 to 93 years with male predominance. The most frequent isolation of Amp-C producing organisms was from urine 50 (42%) followed by pus and pus swab (33%) and blood (8%). Escherichia coli (42.5%) was the most common organism isolated followed by Klebseilla pneumoniae (25%) and Enterobacter cloacae (15%). Sensitivity of isolates was highest to imipenem (82.5%) and lowest to cefoperazone-sulbactam (3.4%).

Conclusion: Frequency of Amp-C producing bacteria was high in our setup and carbapenems are being used to treat infections caused by multidrug resistant Gram negative rods. Sensitivity of the Amp-C producing isolates was higher to Carbapenems and Aminoglycosides.

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INTRODUCTION

Multidrug resistant (MDR) Gram negative rods (GNRs) are responsible for increasing number of serious nosocomial and community acquired infections. Among the enzymes responsible for MDR, extended spectrum β-lactamases (ESBL), metallo β-lactamases and AmpC β-lactamases are associated with nosocomial infections with significant mortality and morbidity[1].

Amp-C β-lactamases are chromosomal, plasmid and transposon encoded; produced in constitutive or inducible manner[2]. Most class C enzymes are resistant to inhibition by clavulanate, sulbactam and tazobactam. Chromosomally mediated Amp-C enzymes are particularly important in Citrobacter freundii, Enterobacter aerogenes, Enteorbacter cloacae, Morganella morganii, Pseudomonas aeruginosa and Serratia marcescens. Host strains harbouring plasmid mediated Amp-C β-lactamases include Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Morganella morganii and Klebsiella oxytoca. Amp-C beta lactamase production is also induced by certain antibiotics like cefoxitin, clavulanic acid and imipenem. Isolates producing Amp-C type of β-lactamases are resistant to penicillins, cephalosporins, monobactams, beta lactam / beta lactamase inhibitor combinations and cephamycins[3].

This study was conducted to determine the frequency of Amp-C producing bacteria from various clinical specimens along with their susceptibility pattern.

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MATERIAL AND METHODS

This cross-sectional study was carried out at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan from February 2014 to February 2015. Permission was taken from institutional ethical committee. GNRs isolated from various clinical specimens were included in the study; repeat samples and contaminated specimens were excluded. GNRs isolated from various clinical specimens were collected and processed according to standard protocols. The specimens were cultured on Blood agar (Oxoid, UK), MacConkey's agar (Oxoid, UK) plates and isolates were identified on the basis of colony morphology, Gram stain, motility and biochemical reactions. (API biomeurix, France).

The isolates were initially screened for production of Amp-C with a cefoxitin disc 30μg (Oxoid UK). Isolates with a zone size less than 18 mm[4] were considered resistant to cefoxitin and were confirmed later for Amp-C production by inoculating (0.5 McFarland standard) on Mueller Hinton agar alogwith E-strips (Oxoid UK) containing cefotetan on one side and cefotetan with cloxacillin at the other end and incubated for 16-20 hours at 37˚C. Isolates showing reduction in cefotetan MIC by ≥3 two fold dilutions in the presence of cloxacillin which is an Amp-C inhibitor were confirmed as Amp-C producers. Antimicrobial discs were used for antimicrobial testing according to the CLSI criteria.

The data were analyzed by SPSS (version 20) software. Descriptive statistics were calculated for both qualitative & quantitative variables. For quantitative variables, mean ± SD were calculated. For qualitative variables like, frequency of Amp-C producing bacteria and drugs sensitivity, frequency and percentages were calculated.

RESULTS

A total of 342 isolates of GNRs were isolated during the study period. Age of the patients ranged from 2 to 93 years; mean 50 years, 74 (62%) were isolated from male patients while remaining 46 (38%) specimens from female patients with a ratio of 1.6:1. On screening with cefoxitin disc, 135 out of 342 were resistant and 120 were confirmed as Amp-C producers by cefoteten/ cloxacillin E-strip method.

Amp-C producing isolates were isolated from urine 50 (42%) followed by pus/ pus swab 39 (33%) and blood 6 (5%). Other specimens containing Amp-C producing isolates included catheter tips (3.4%), body tissues (6.6), sputum (5%), endobronchial washings and throat swabs (3.4%). Antimicrobial discs and the zone sizes are shown in Table 1.

Escherichia coli 52 (42.5%) was the most common organism isolated followed by Klebsiella pneumoniae 30 (25%) and Enterobacter cloacae 20 (15%). Other pathogens included Proteus mirabilis (6.6%), Citrobacter freundii (6.6%), Acinetobacter baumanii (8.3%), Klebsiella oxytoca (3.3%) and Providencia rettgeri (1.6%). 99 (82.5%) isolates were sensitive to imipenem, 80 (66.7%) to amikacin, 4 (3.34%) to cefoperazone-sulbactam, 8 (6.67%) to piperacillin-tazobactam and 22 (18.3%) to ciprofloxacin 21 (17.5%) isolates were resistant to imipenem, 40 (33%) to amikacin, 116 (96.6%) to cefoperazone-sulbactam, 112 (93%) to piperacillin-tazobactam and 98 (82%) to ciprofloxacin. Out of 50 Amp-C positive urinary isolates, 22 (44%) were resistant to nitrofurantoin. Susceptibility pattern of various GNRs (E.coli, Klebsiella pneumoniae and Enterobacter) to the antibiotics is shown in Table 2.

DISCUSSION

Globally there has been an increase in the reporting of Amp-C beta lactamases with considerable variation in prevalence from different geographic locations. The presence of Amp-C beta lactamases in GNR has led to widespread resistance and ineffectiveness of a variety

Table 1: Antimicrobial discs and the zone sizes

Name of Antimicrobial	Disk Potency	Susceptible Zone	Intermediate Zone	Resistant Zone
Amikacin	30μg	≥17	15-16	≤14
Imipenem	10μg	≥23	21-22	≤20
Cefoperazone-sulbactam	15μg	≥21	16-20	≤15
Piperacillin-tazobactam	15μg	≥21	18-20	≤17
Ciprofloxacin	15μg	≥20	14-20	≤13
Nitrofurantoin	300 μg	≥17	18-19	≤20

Table 2: Sensitivity pattern of most commonly isolated organisms

Organism	Imipenem	Amikacin	Piperacillin-tazobctam	Cefoperazone-sulbactam
E.coli	77%	61.5%	5.7%	3.8%
K.pneumonaie	40%	20%	—	—
Enterobacter cloacae	70%	40%	10%	5%

of antibiotics empirically used in hospitals. The various methods of detection of Amp-C have been evaluated in many studies including the phenotypic methods and PCR[6]. The susceptibility pattern of isolates producing Amp-C beta lactamases has also been analyzed in various studies and there has been considerable variation in resistance patterns of Amp-C producing isolates from various parts of the world.[7]

In our study; frequency of Amp-C was 33.6%. A study conducted at Zurich Institute on E. coli isolates showed a frequency of 35%[8] and another study on K. pneumoniae isolates in Kolkata showed 32% of isolates were Amp-C producers[9]. The high frequency of Amp-C in our setup can be attributed to the injudicious use of antibiotics in particular third generation cephalosporins.

In our study, there was a male predominance. The reason could be the male predominance in Army setup as most of the patients visiting the Army institutes are male. Urine was the most common specimen dealt in our study as most of the Amp-C producing isolates were recovered from urine. This can be attributed to the fact that the majority of isolates were from indoor patients who are from medical and surgical units and are catheterized. They get their urine routine examination done as per protocols and are prone to developing urinary tract infections.

The most frequent pathogen was E.coli (42.5%) followed by K. pneumoniae[10], Enterobacter spp., Proteus spp. and others. This finding was similar to results of a regional study[11]. E.coli has been the most common pathogen of urinary tract infections followed by K. pneumoniae in both studies[12,13] and as discussed earlier urine was the most common specimen.

Sensitivity of Amp-C producing isolates to imipenem was low (82%) in comparison to 100% sensitivity reported in most of the studies[14,15]. The sensitivity to imipenem in Amp-C producers in Nigeria was about 84%[15]. The regional studies also have not reported any resistance to carbapenems[16]. Carbapenems are the only beta- lactams that are not hydrolyzed by Amp-C beta lactamases. With the steady rise in carbapenem resistance, the choice of antibiotics for treating such infections would be further limited.

The sensitivity to flouroquinolones like ciprofloxacin/levofloxacin was considerably low (18%) in our study as compared to that reported in various regional studies[17]. This is not due to the presence od Amp-C beta lactamases as quinolones are not hydrolyzed by Amp-C. In vitro susceptibility to piperacillin/tazobactam, cefoperazone/sulbactam, amoxicillin/clavulanic acid in our study (6.7%, 3.4%,0) is in contrast to the results from a study in India reporting sensitivity of 63%, 80%, 9% respectively[11]. All the Amp-C producing isolates are resistant to the beta lactamase inhibitors. Combinations of beta lactam/ beta lactamase cannot be used to treat infections caused by these bacteria. They may show in vitro susceptibility to these antibiotics but are not susceptible to them[14] in vivo . In our study, these antibiotics were not susceptible in vitro as well. The susceptibility to amikacin in our study (66.7%) is slightly higher than the sensitivity of Amp-C producers (60%)[11] reported in a similar study from India. However similar study from Pakistan has shown that 35% of their isolates that were Amp-C producers are sensitive to amikacin[13]. It is the second most effective antibiotic after imipenem according to our results.

The frequency of Amp-C producing E.coli in our study (43%) is higher than regional studies[18] and in concordance with a Swiss study[15]. The resistance to imipenem in our isolates was higher than that reported in regional study while the pattern for aminoglycoside resistance was similar to other studies. The in vitro resistance to cefoperazone-sulbactam was much higher than the regional study[11] although these drugs even if susceptible in vitro are not effective in vivo[12]. The frequency of Klebsiella pneumoniae isolates producing Amp-C is 25% in our study while in regional studies its 32%[7] and 22%. The resistance to imipenem in our isolates was 60% while the isolates in regional study showed no resistance to imipenem. The resistance to amikacin was 80% in our isolates which is higher compared to that in a regional study[18].

Amp-C can be inducible as well as constitutive, inducible resistance is a matter of concern while treating the isolates who have the ability to develop the resistance during the course of treatment with certain Beta- Lactam antibiotics[19]. Isolates which are known to develop the inducible resistance are Serratia, Pseudomonas, Acinetobacter, Citrobacter and Enterobacter spp[16]. The genotypic studies for the detection of cefoxitin resistant non-Amp-C producers were not done. Multiplex PCR can be done for the detection of plasmid mediated Amp-C beta lactamases and has been supported in studies[20].

CONCLUSION

Frequency of Amp-C producing bacteria was high in our setup. Carbapenems can be used to treat infections caused by MDR GNRs, however there is a steady increase in carbapenem resistance.

RECOMMENDATIONS

Local antibiotic policies must be made and followed to control spread of resistance among isolates and resistant isolates.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Afzal A: Idea, data collection.

Rasool A: Statistics.

Rafiq MY: Literature Review.

Arif S: Bibliography.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.