PHENOTYPIC IDENTIFICATION OF AMPC BETA-LACTAMASE PRODUCING GRAM NEGATIVE RODS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN AMONG CLINICAL SAMPLES OF INTENSIVE CARE UNIT PATIENTS

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ABSTRACT

Objective: To determine the frequency of AmpC β-lactamase producing GNRs and their antibiotic susceptibility pattern, isolated from clinical samples of Intensive Care Unit (ICU) patients from a tertiary care hospital of Rawalpindi.

Material & Methods: The current study was conducted in Department of Microbiology, AFIP; Rawalpindi from April to October 2018. Gram Negative Rods (GNRs) were isolated and identified by standard microbiological methods (Colony Morphology, Gram's stain, Catalase Test, Oxidase Test, Motility Test and API 20 E). Screening of isolates for AmpC β-lactamases was done by cefoxitin disc (30 µg). The double disc synergy test was duplicated with ceftazidime disc (30 µg) and cefotaxime disc (30 µg) using cloxacillin disc (250 µg) as an inhibitor.

Results: Out of 196 total isolates, 100 (51.02 %) were screened positive AmpC producers then out of screened positive AmpC producing isolates 21 isolates were confirmed as AmpC producers (10.7 %). Among AmpC producers 100% resistance was observed for ampillicin and co-amoxiclave, 40 % for ceftriaxone and imipenim, 15 % cefepime, 20 % doxycycline, 30 % ciprofloxacin, 40 % amikacin and gentamicin, 20 % tazobactam-pipracillin and 50 % for co- trimoxazole. However, no resistance was seen with meropenem.

Conclusion: The present study highlights low burden of AmpC β Lactimase producing GNRs in our setup. Double Disc Synergy test is suggested to be used for detection in areas with high AmpC burden, as Cefoxitin disc screening alone is not reliable method.

Key Words: AmpC β-lactamase, GNR, Antibiotic susceptibility.

This article can be cited as: Bushra R, Hussain W, Zaman G, Ahmed A, Khursheed U, Zahid U, Khadim MT. Phenotypic identification of AMPc beta-lactamase producing gram negative rods and their antibiotic susceptibility pattern among clinical samples 0f intensive care unit patients. Pak J Pathol. 2019: 30(2): 45-48.

INTRODUCTION

With the continuing spread and emergence of cases of antibiotic resistance, it is evident that there is scarcity of new treatment agents for multidrug-resistant organisms (MDROs). This global health crisis has been increasing over the past few years [1]. Amongst the MDROs, MDR-GNRs (Multidrug-Resistant Gram-Negative Rods) have been considered as one of the most rampant agents of infections in individuals and their frequency has been increasing worldwide [2]. The most significant mechanism of resistance in GNR is the production of certain enzymes termed β-lactamases. There are various types of β-lactamases. Nonetheless, AmpC-type beta-lactamases has been given little attention [3]. Though, several international research studies have investigated the occurrence of AmpC [4,5,6,7]; however, in Pakistan it is less investigated. Highly resistant isolates include those that are resistant to at least one member from three or more groups of antibiotics [8]. AmpC-type beta-lactamases are also found to be associated with "C Class" β-lactamases; thereby, it has been reported that there is an increase in the prevalence of Plasmid-encoded AmpC enzymes (pAmpC) [9,10,11].

AmpC beta lactamases are associated with resistance to all beta lactam drugs except carbapenems and cefipime. They are cephalosporinases which are either chromosomal encoded or plasmid encoded and it is a great challenge to detect them in laboratories as Clinical and Laboratory Standards Institute (CLSI) has no approved guide lines for their identification [12,13,14].

As there is a scarcity of valid and simple detection methods; particularly in *Enterobacteriaceae* (mediated by plasmids), the accurate prevalence of AmpC is largely unknown (13). Shafiq *et al.* (2013) determined in their study the prevalence of ESBLs and AmpC β lactamases in *K. pneumoniae* and *E. coli* as 47% (n = 5) and 29.24% (n = 8) respectively

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Received: ***17 Jan 2019****; Revised:* ***21 Feb 2019****; Accepted:* ***24 Mar 2019***

[15]. For epidemiological/surveillance studies and clinical purposes, the detection of the prevalence of AmpC β lactamases is very significant; because these genes not only spread readily in the hospital settings but organisms with inducible AmpC β-lactamases are found susceptible to third generation cephalosporins *in vitro* and may become resistant upon therapy with these agents resulting in clinical failure. Thereby, this study aims to detect frequency of AmpC producing isolates through accurate and easy methods like double disk synergy method and inhibition-based method to incorporate these detection methods in routine laboratory work or otherwise.

MATERIAL AND METHODS

All consecutive non-repeated clinical samples including pus, body fluids, urine, blood, sputum, NBL and tissue, received at department of Microbiology AFIP Rawalpindi were processed as per standard protocols. Sample size 196 has been calculated by WHO calculator. Non-probability convenience sampling technique was used. Gram negative rods were identified by Colony Morphology, Gram's stain, Catalase Test, Oxidase Test, Motility Test and by using API 20 E (Biomerieux, France). Standard disc diffusion methods were used for screening and confirmation of AmpC production. Screening of isolates for AmpC β-lactasmases was done by cefoxitin 30 µg disc. Isolates with zone diameter of ≤18 mm was considered as probable AmpC β-lactamase producer. All screened positive isolates were subjected to confirmatory double disc synergy test (DDST). There are three discs in DDST; Ceftazidime (30 µg), Cefotaxime (30 µg) with cloxacillin (250 µg) in the centre, applied to Muller Hinton agar at a distance of 10mm. A synergism (ghost zone or keyhole) between any of CAZ, cloxacillin and CTX confirms the occurrence of AmpC β lactamase as shown in Figure-1.

The antibiotic susceptibility of AmpC producing isolates is done as per CLSI guidelines using Modified Kirby-Bauer disc diffusion method for following discs; amikacin, ampicillin, cefepime, ceftriaxone, ciprofloxacin, co-amoxiclav, co-trimoxazole, gentamicin, imipenem, meropenem, piperacillin-tazobactam and doxycycline.

The statistical package SPSS version 21.0 was used for calculating the results of this study. Descriptive statistics were used to summarize the data.

RESULTS

Out of total 196 clinical samples yielding the growth of GNRs, 100 were found screened positive for AmpC production. Out of 100 screened positive isolates, 21 were found confirmed positive and 75 Negative for AmpC production by DDST method.

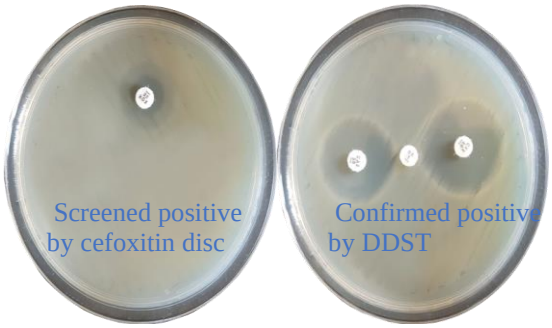

Figure-1: Detection of AmpC β lactamase by cefoxitin screening and DDST confirmatory method.

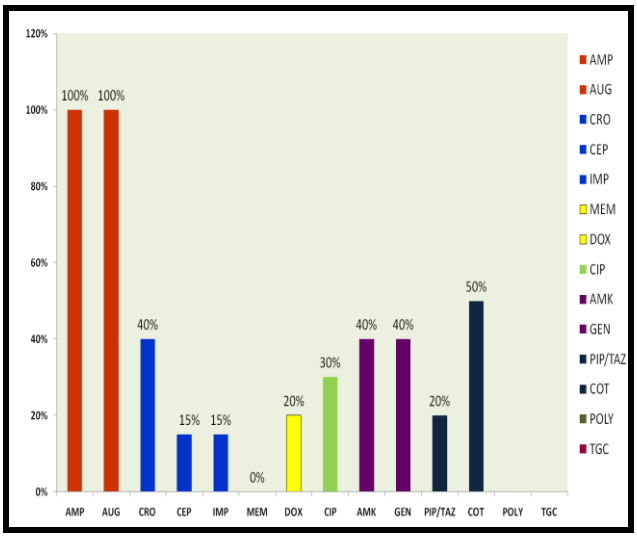

Figure-2: Antibiogram of AmpC producing isolates.

Out of 196 collected isolates, 116 were yielded from male and 80 from female patients. Out of 21 confirmed AmpC producers, 16 were isolated from male and 5 from female patients.

The sample sites for all 196 collected samples were as follows; 46 were yielded from pus, 22 from NBL, 12 body fluids, 76 from urine, 25 from blood, 11 from sputum and 4 from tissue specimens.

Out of 21 confirmed AmpC producing isolates 100 % resistance was seen with Ampicillin and Co-amoxiclav, 50 % for co-trimoxazole, 40 % for ceftriaxone, amikacin and gentamicin, 30 % for ciprofloxacin, 20 % for doxycycline and tazobactum-piperacillin (TZP), 15 % for cefepime and imipenem;

while, no resistance was seen with meropenem as shown in Figure-2.

DISCUSSION

For clinical microbiologists it is a great challenge to detect AmpC β-lactamases, as CLSI has not given accurate guidelines for its detection and isolates with inducible AmpC β-lactamases may appear sensitive invtro to 3rd generation cephalosporins but may become resistant *in-vivo* upon therapy with these agents causing clinical failure [16,17]. In order to improve the clinical management of infection and for sound collection of epidemiological data it is important to accurately detect AmpC beta lactamases [18]. In our study we determined the frequency of AmpC β-lactamase producing Gram Negative Rods. Out of 196 isolates, cefoxitin screening method detected 100 (51 %) as potential AmpC producers and majority (94.9 %) were identified as *Enterobacter* isolates. Likewise, a study by Saad *et al.* (2014) included 120 Gram negative rods and found 82 (68.33 %) isolates as potential AmpC producers by cefoxitin resistance method [19]. Our study detected less AmpC producers by screening method as comparable to above study. Apart from AmpC β-lactamase production, Cefoxitin resistance can also be due to reduced outer membrane permeability of the isolates resulting in false positive results for cefoxitin screening method [11].

The confirmatory Double Disc Synergy test detected 21 (10.7 %) of the isolates as confirmed AmpC producers. Another study conducted by Pal *et al.* (2016), also determined very low frequency (4 %) of AmpC β-lactamases producing Gram Negative Rods, resembling results of our study [20]. Similarly, study by Noor-ul-Ain Jameel *et al.* (2012) also found out similar results as in our study with low frequency (12%) of AmpC β-lactamase in Gram Negative Rods [16].

On the contrary to our results; Saad *et al*. (2014), showed that AmpC beta-lactamase producing bacteria were 52.4 % and were more sensitive to carbapenems (meropenem) and tigecycline. Their results showed marked resistance to aminoglycosides, fluoroquinolones, co-trimoxazole and tetracycline [19]. In our study antimicrobial sensitivity testing showed carbapenems (meropenem) to be better therapeutic options and these results are comparable to a study by Hassan *et al.* showing carbapenems to be 100 % sensitive against these resistant pathogens [21].

In our study 100 % resistance was detected with ampicillin and co-amoxiclave, 50 % resistance was seen with co-trimoxazole, 40 % with ceftriaxone, amikacin and gentamicin, 20 % with doxycycline and tazobactum piperacillin, 15 % resistance was seen with cefepime and imipenem; however, none of the isolates were resistant to meropenem. Resistance to ceftriaxone and cefipime in our study by AmpC producers is probably due to presence of some other genes, as AmpC β-lactamases producers may appear sensitive invtro to 3rd generation cephalosporin. Proteus species are most common isolates in our study, showing resistance to different classes of drugs including; amikacin, ceftriaxone, amoxicillin-clavulanic acid and gentamicin. High antimicrobial resistance to many classes of drugs by AmpC β-lactamase producing isolates has also been reported in many studies [22, 23, 24].

CONCLUSION

The present study emphasizes the low frequency of AmpC β-lactamases in Gram-negative bacteria in our setup. Double Disc Synergy method is suggested to be used for detection in areas with high AmpC burden.

AUTHORS CONTRIBUTION

Riffat Bushra: Paper writing, statistical analysis and data collection.

Wajid Hussain: Design and results analysis.

Gohar Zaman: Final review and approval of manuscript.

Abeera Ahmed and Umer Khursheed: Literature review.

Muhammad Tahir Khadim: Director incharge of project.

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