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Extended Spectrum Beta Lactamases Producing *Pseudomonas aeruginosa* Expressing Amp C Beta-Lactamase Enzyme

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Abstract: This study determined the presence of Amp C beta lactamase producing *Pseudomonas aeruginosa* among the seventy four uropathogens studied using standard techniques. Results obtained revealed that the studied isolates show an alarming rate of resistance to the extended spectrum beta lactam antibiotics and the penicillin derivatives tested. The combination of piperacillin and tazobactam was however found to exert reasonable antibacterial activity as reflected in their relatively higher percentage susceptibility pattern (77%). 62.2% of the isolates were also sensitive to gentamicin. Of the 74 *Pseudomonas aeruginosa* tested in this study, 30 were Amp C Beta lactamase producers, 7 and 23 produced inducible and non inducible Amp C beta lactamases respectively. Five of the 30 Amp C beta lactamases producers produced extended spectrum beta lactamases while 21 of the these isolates produced metallo beta lactamase enzymes in addition to the Amp C enzymes. In conclusion, it can be said that there is an upsurge of Amp C beta lactamase producing *Pseudomonas aeruginosa* and treatment should only be commenced only prior to knowing the sensitivity pattern of Amp C isolates

Key words: ESBL · *Pseudomonas aeruginosa* · AmpC

INTRODUCTION

Infections caused by *Pseudomonas aeruginosa* are difficult to treat as majority of isolates exhibit varying degrees of innate resistance. Most of these resistances are acquired and may be caused by production of plasmid mediated Amp C beta (β)-lactamase, extended spectrum β-lactamase and metallo β-lactamase (MBL) enzymes [1]. β-lactamases are the most widespread and effective mechanism through which bacteria can become resistant to β-lactam drugs. With the increasing use of β-lactam drugs and introduction of various inhibitor combinations such as amoxicillin-clavulanic acid or sulbactam, Ambler class C and Bush group I β-lactamase enzyme, known as AmpC β-lactamases have emerged and are being reported worldwide with varying prevalence rates [2-4].

Amp C beta lactamases inactivate the effect of broad-spectrum cephalosporins and penicillins while production of these enzymes in clinically significant *Enterobacteriaceae* represents an increasing problem resulting in higher patient morbidity and mortality [5]. For example, a recent study described a mortality rate of as high as 60% in patients with bloodstream infections due to ESBL-positive *Enterobacteriaceae* when adequate antibiotic therapy was not administered [6]. ESBL-positive *Enterobacteriaceae* which produce ESBLs and AmpC broad-spectrum beta-lactamases have been studied for more than two decades [7, 8]. Similarly, articles describing their prevalence in food animals and foods of animal origin have been published in recent years [9-14].

In many bacteria, Amp C enzymes are inducible and can be expressed at high levels by mutation. Overexpression confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime and ceftriaxone and is a problem [15] especially in infections due to *Pseudomonas aeruginosa*, where an isolate

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initially susceptible to these agents may become resistant upon therapy. This resistance may be located on transmissible plasmids. Transmissible plasmids have acquired genes for Amp C enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal Amp C gene, such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis.* Resistance due to plasmid mediated Amp C enzymes is less common than ESBL production in most parts of the world but may be both harder to detect and broader in spectrum [5].

In Nigeria, indiscriminate use of antibiotics, poor hygienic practices in hospitals and lack of monitoring of microbial drug resistance have created suitable conditions for the emergency and uncontrollable spread of the ESBLs and Amp C enzymes [16] and thus make their detection complicated due to the variable affinity of these enzymes for different substrates and inoculum effect. Although, for more than a decade, since plasmid-mediated Amp C beta-lactamases were discovered, most clinical laboratories remain ignorant of their clinical consequences [17]. Consequently, microorganisms producing these enzymes are concealed and are primarily liable for various nosocomial infections in hospitals. The objective of this study was to determine the presence of Amp C beta-lactamases in the strains of *Pseudomonas aeruginosa* studied.

MATERIALS AND METHODS

Bacterial Strains: The 74 bacterial strains used in this study were supplied by Mrs O.D.Popoola of the Department of Microbiology (Medical Microbiology unit), Nnamdi Azikwe University, Awka, Anambra, Nigeria.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility was performed using the CLSI method [18] for various antibiotics, namely: Ampicillin (30µg), Amikacin (30µg), Co-trimoxazole (25µg), Ciprofloxacin (5µg), Ceftizoxime (30µg), Cefuroxime (30µg), Kanamycin (30µg), Piperacillin (100µg), Gentamicin (10µg), Piperacillin/ tazobactam (100µg/10µg) and Carbenicillin (100µg) (Abtek, U.K). The results were interpreted based on the CLSI interpretative standard chart [18].

AmpC Disc Test: Screening for Amp C β-lactamase production was performed by Cefoxitin disk test [17]. Isolates that yielded a zone diameter less than 18 mm (screen positive) were further subjected to confirmatory testing. The disk antagonism test was used for detection of inducible Amp C β-lactamase in all the isolates of *P. aeruginosa*. A test isolate (with a turbidity equivalent to that of 0.5 McFarland standards) was spread over a Mueller Hinton agar (Oxoid, U.K) plate. Cefotaxime (30µg) and cefoxitin (30µg) (Abtek, U.K) disks were placed 20 mm apart from center to center. Isolates showing blunting of the cefotaxime zone of inhibition adjacent to the cefoxitin disk were screened as positive for Amp C β-lactamase. Further confirmation of Amp C production was tested by a modified three-dimensional test [17]. This method was particularly helpful in detecting non inducible Amp C B-lactamases. The extended spectrum beta-lactamase (ESBL) status of these strains was established by combined disk diffusion method per CLSI recommendations [18] using Cefotaxime (30µg) and Ceftazidime (30µg) disks alone and in combination with clavulanic acid. Metallo β-lactamase production was detected by Imipenem-EDTA disk test. Two 10 µg imipenem disks were placed on the plate and appropriate amounts of 10 µl of 0.5M EDTA solution were added to one of them to obtain the described concentration (750 µg). The inhibition zones of imipenem and imipenem-EDTA disks were compared after 18 hours of aerobic incubation at 37°C. If the increase in inhibition zone with imipenem and EDTA disk was = 7 mm, then the imipenem disk alone was considered to be the MBL producer [19].

RESULTS

Table 1 represents the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* studied. studied isolates showed an alarming rate of resistance to the extended spectrum beta lactam antibiotics and the penicillin derivatives tested. The combination of piperacillin and tazobactam was however found to exert reasonable antibacterial activity as reflected in their relatively higher percentage susceptibility pattern (77%). 62.2% of the isolates were also sensitive to gentamicin. Of the 74 *Pseudomonas aeruginosa* used in this study, 30 were Amp C Beta lactamase producers, 7 and 23 produced inducible and non inducible Amp C beta lactamases respectively (Table 2). Table 3 represents the beta lactamase mediated resistance mechanism in Amp C producing *Pseudomonas aeruginosa.* Five of the 30 Amp C beta lactamases produced extended spectrum beta lactamases while 21 of the same isolates produced metallo beta lactamase enzymes in addition to the Amp C enzymes.

Table 1: Antibiotic susceptibility pattern of the studied *Pseudomonas aeruginosa* isolates

Antibiotics	Ampicillin
Sensitivity	2(2.7%)
Amikacin	16 (21.6%)
Carbenicillin	8(10.8%)
Cefoxitin	3(4.1%)
Ceftazidime	7(9.5%)
Ceftizoxime	6(8.2%)
Cefuroxime	14(19%)
Co-trimoxazole	32(43.2%)
Ciprofloxacin	32(43.2%)
Gentamicin	46(62.2%)
Imipenem	21(28.4%)
Piperacillin +Tazobactam	57(77%)
Kanamycin	8(10.8%)

Table 2: Prevalence of Amp C Beta lactamase producing *Pseudomonas aeruginosa*

N	EP	IA	INA
74	30	7	23

N = Total number of isolates, EP = Amp C producers, IA = inducible Amp C, INA = non inducible Amp C.

Table 3: β-lactamase mediated resistance mechanism in Amp C producing *Pseudomonas aeruginosa* (n = 30)

AMP C	AMPC + ESBL	AmpC +MBL
30	5	21

DISCUSSION AND CONCLUSION

Isolates producing Amp C β-lactamases raise special concerns as these isolates have been responsible for several nosocomial outbreaks and high rate of clinical failure among infected patients [20-22]. In this study, we found a relatively lower prevalence of Amp C β-lactamase producing *P. aeruginosa* (40.5%). This prevalence rate is lower than that reported by Livermore and Woodford [23]. Production of multiple β-lactamases by *P. aeruginosa* has tremendous therapeutic consequences and poses a significant clinical challenge if it remains undetected. Since these organisms also carry other drug-resistant genes and the only viable treatment option remains the potentially toxic polymyxin B and colistin [24], early identification of the infections due to these organisms is necessary as the appropriate treatment might reduce the spread of these resistant strains as well as reduce the mortality in hospitalized patients. This emphasizes the need for the detection of isolates that produce these enzymes to avoid therapeutic failures and nosocomial outbreaks. Since there is no standard guideline for detection of most of these β-lactamase enzymes in *P. aeruginosa*, the comparison between studies becomes difficult as the patient population in a particular center and the method of study differs.

The presence Amp C producing isolates may be indicative of the ominous trend of more and more isolates acquiring resistance mechanisms thereby rendering the antimicrobials ineffective [25]. The comparison of our results with the above-mentioned studies clearly shows that *Pseudomonas aeruginosa* strains producing ESBL and AmpC enzymes are less prevalent. This observation is not surprising as Amp C producers are susceptible to fourth generation cephalosporins like cefepime while ESBL producers are variably resistant to fourth-generation cephalosporins [26]. Both ESBL producers and non-producers showed high level resistance to cefepime. A high inoculum effect has been reported with cefepime for ESBL-producing and Amp C-producing isolates of Enterobacteriaceae [27]. The fact that 21 of the total 30 Amp C producers also produced metallo beta lactamases is very frightening. This is because MBLs also represent a clinical threat due to their unrivalled spectrum of activity and their resistance to therapeutic serine beta-lactamase inhibitors [25-27]. In conclusion, routine screening for ESBL and AmpC production need to be done for all multi drug resistant isolates prior to treatment.

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