Occurrence of Extended-Spectrum Beta-Lactamase-Producing Pseudomonas aeruginosa Strains in South-West Nigeria

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Abstract: Extended-spectrum beta-lactamase production has been reported as one of the mechanisms that contribute to acquired beta-lactam resistance in Pseudomonas aeruginosa. This study investigated ESBL production among clinical isolates of P. aeruginosa in the institution and their susceptibility to antimicrobials. Ninety clinical isolates of P. aeruginosa were screened for ESBL production using the double-disc synergy test at the Department of Medical Microbiology Laboratory of University College Hospital, Ibadan, Nigeria between June 2010 and July 2011. The susceptibilities of the ESBL producers were determined against various classes of antibiotics. Rate of ESBL production was found to be 22.2%. ESBL production was found only in isolates from sputum (40%) and urine (60%). Susceptibility of the ESBL-producing P. aeruginosa strains to imipenem, meropenem and amikacin was 100% each while susceptibility to ciprofloxacin and gentamicin were 50 and 30%, respectively. Activities ofceftriaxone, ceftazidine and cefepime against P. aeruginosa were 0, 50 and 40%, respectively. In conclusion, this study has demonstrated ESBL-producing P. aeruginosa strains in this environment. Efforts should therefore be made to detect them. Clinicians should consider ESBL production as a possibility in treatment failure with beta-lactam antibiotics.

Key words: Pseudomonas aeruginosa, ESBL, susceptibility, antibiotic, treatment, Nigeria

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen and a leading cause of nosocomial infections which include pneumonia, urinary tract infections and bacteremia (Shahid and Sheeba, 2003). Infections by P. aeruginosa are often difficult to treat due to the natural resistance of the species as well as their remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents. Often, these mechanisms exist simultaneously thus conferring combined resistance to many strains (McGowan, 2006).

P. aeruginosa is intrinsically resistant to many structurally unrelated antimicrobial agents because of the low permeability of its efflux pumps with wide substrate specificity and the naturally occurring chromosomal AmpC beta-lactamases (also known as cephalosporinase) (Mesaros et al., 2007; Livermore, 2001; Nordmann and Gilbert, 1998). The pseudomonal beta-lactams (such as ticarcillin, piperacillin, ceftazidime, cefepime, aztreonam and the carbapenems) represent a major weapon against pseudomonas infections either for monotherapy or for combination therapy for which beta-lactams almost invariably represent one of the components (Giamarello, 2002; Pollack, 2000). However, extended-spectrum beta-lactamases such as TEM, SHV, PER, VEB, GES and more recently, CTX-M variants have been reported to be increasingly found in P. aeruginosa in various areas (Aktas et al., 2005; Al Naiemi et al., 2006; Celenza et al., 2006). In addition to the innate resistance in P. aeruginosa, acquired additional resistance due to plasmids is also a problem. Plasmid-mediated resistance involving modifying enzymes is particularly associated with indiscriminate antibiotic use and with sites where high levels of antibiotics are achieved (Shahid and Sheeba, 2003). This study set out to determine whether there are ESBL producers among the clinical isolates of P. aeruginosa in the environment and to also evaluate their susceptibility pattern.

MATERIALS AND METHODS

A total of 90 clinical isolates of P. aeruginosa recovered from various clinical specimens brought to the Medical Microbiology Laboratory of University College Hospital, Ibadan, Nigeria were included in this study which spanned 10 months (July 2010 to May 2011). The clinical specimens included sputum, wound swabs, urine
and ear swabs. *P. aeruginosa* isolates were identified from the clinical specimens by their colonial morphology and biochemical reactions. ESBL production was detected by the double disc synergy test. A susceptibility disc containing amoxiclav (20 μg amoxicillin and 10 μg clavulanic acid) was placed in the center of each Mueller-Hinton agar plate inoculated with a test organism. Cefotaxime (30 μg) and cefazidime (30 μg) discs were placed 20 mm (center to center) from the amoxicillin-clavulunate disc and incubated at 37°C overnight. Enhancement of the diameter of the zone of inhibition of the oxymino-B-lactam caused by the synergy of the clavulane in the amoxicillin-clavulanate disc was considered as evidence of ESBL production (Jarlier et al., 1988).

The positive strains were tested for susceptibility according to the CLSI guidelines (CLSI, 2007) using the following antibiotic discs: imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), gentamycin (30 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), cefazidime (30 μg) and ceftizime (30 μg). The relevant demographic data of patients were obtained from their clinical records and these were their age and sex.

**RESULTS AND DISCUSSION**

Ninety isolates of *P. aeruginosa* were analysed during the study period. They were identified from various clinical specimens which included sputum 10 (11.1%), wound swabs 20 (22.2%), urine 10 (11.1%), wound biopsy 10 (11.1%) and ear swabs 40 (44.4%). The clinical specimens were obtained from patients who consisted of 80 (88.9%) males and 10 (11.1%) females. The age range of patients were between 5 days and 42 years. Out of the 90 isolates of *P. aeruginosa* included in this study, only 20 (22.2%) were found to be positive for ESBL production while 70 (77.8%) were negative for the Phenotypic Presumptive test (Table 1). Total 10 (50%) positive cases were from inpatients while 10 (50%) were from inpatients. ESBL production was found only in isolates from sputum 8 (40%) and urine 12 (60%) (Table 2). ESBL production was not detected in isolates from wound swabs, wound biopsy and ear swabs.

Susceptibility of the ESBL producers to imipenem, meropenem and amikacin was 100% each while susceptibility to ciprofloxacin and gentamycin was 50 and 30%, respectively. Susceptibility to ceftriaxone, cefazidime and ceftizime were 0, 50 and 40%, respectively (Table 3). Extended-spectrum beta-lactamases-producing organisms pose unique challenges to clinical microbiologists, clinicians, infection control professionals and scientists engaged in finding new antibacterial agents.

These resistant organisms are clinically important because they result in increased morbidity and mortality. In addition, the incidence of ESBLs varies with geographic location and time (Tavajohi et al., 2011). In this study, 22.2% ESBL production was recorded among *P. aeruginosa* isolates analysed. This result is similar to the report of a study conducted by Aggarwal et al. (2008) where a prevalence rate of 20.27% was reported. However, it is contrary to the results of some other researchers which depicted rates as low as 3.7 and 4.2%, respectively (Woodford et al., 2008; Lim et al., 2009). ESBL production was found only in isolates from sputum (40%) and urine (60%). There was no ESBL production in isolates from wound swabs, wound biopsy and ear swabs. In contrast, Aibinu et al. (2007), reported maximum ESBL production in isolates from wound swabs (55.6%) followed by catheter tips (33.3%) and pus (11.1%) (Aibinu et al., 2007).

*P. aeruginosa* is naturally susceptible to carbapenem, cefazidime and aztreonam however, it can acquire resistance to third-generation cephalosporins. The most frequent mechanism by which this occurs is through the constitutive hyperproduction of AmpC beta-lactamase (Bagge et al., 2002). ESBL-producing bacteria are also frequently resistant to many other classes of antibiotics which include fluoroquinolones and aminoglycosides. This is due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL (Nathisuwon et al., 2001). In
this study, the activities of the carbapenems (imipenem and meropenem) and amikacin against ESBL-producing strains of *P. aeruginosa* were high with 100% susceptibility for each. Therefore, these can be considered as therapeutic options for ESBL-producing strains of *P. aeruginosa* infections in this environment.

However, there was a relatively low activities of the quinolone (ciprofloxacin) and gentamycin recorded against the ESBL-producing *P. aeruginosa* and this suggests a possible existence of co-resistance to the quinolones and aminoglycosides on the gene responsible for ESBL production (Thomson et al., 1999). A report from a study conducted by some researchers has stated that *P. aeruginosa* isolates that are resistant to multiple antibiotics are of particular concern and pose a significant clinical challenge (Hauser and Sriman, 2005). The results of this study also imply that quinolone or gentamycin alone cannot be depended upon as an antisupemoidal antimicrobial in this environment. Another study by Okesola also lends credence to this statement as it demonstrated a rather low activity of gentamycin against clinical isolates of *P. aeruginosa* in the same environment (Okesola and Oni, 2011). The high activities demonstrated by the carbapenems against ESBL-producing strains of *P. aeruginosa* in this study have also been documented by other researchers (Aibinu et al., 2007; Aggarwal et al., 2008).

**CONCLUSION**

This study has demonstrated the presence of ESBL-producing *P. aeruginosa* strains in this environment. Therefore, improved methods for antimicrobial susceptibility are needed to detect these. In order to combat the problem of resistance caused by ESBL production in these isolates, a nationwide antibiotic policy should be instituted. In addition, inappropriate use of antibiotics which is rampant in this study location should be curtailed. Clinicians should also consider ESBL production as a possibility in treatment failure with beta-lactam antibiotics.

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**REFERENCES**


