Research Journal of Medical Sciences 6 (3): 93-96, 2012
ISSN: 1815-9346

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

Abiola Olukemi Okesola and Anthony Alaba Oni
Department of Medical Microbiology and Parasitology, College of Medicine,
University of Ibadan, University College Hospital, Ibadan, Nigeria, West Africa

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 gntamycin were 50 and 30%, respectively. Activities of ceftriaxone, ceftazidime 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 antipseudomonal 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 (Giamarellou, 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

Corresponding Author: A.O. Okesola, Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, University College Hospital, Ibadan, Nigeria, West Africa

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 ceftazidime (30 μg) discs were placed 20 mm (center to center) from the amoxicillin-clavulanate 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 clavulanate 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), ceftazidime (30 μg) and cefepime (30 μg). The relevant demodraphic 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 outpatients 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, ceftazidime and cefepime 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.

Table 1: Frequency of ESBL production among clinical isolates of *P. aeruginosa*

Isolates	Frequency	Percentage
Positive	20	22.2
Negative	70	77.8
Total	90	100.0

Table 2: ESBL production in *P.aeruginosa* strains from various clinical specimens

Clinical specimens	Number	Percentage
Sputum	8	40.0
Wound swabs	0	0.0
Wound biopsy	0	0.0
Ear swabs	0	0.0
Urine	12	60.0
Total	20	100.0

Table 3: Antimicrobial susceptibility of ESBL-producing strains of *P. aeruginosa*

	Susceptibility pattern (N = 10)	
Antibiotics	No. of susceptible isolates	Percentage
Imipenem	20	100.00
Ciprofloxacin	10	50.00
Cefepime	8	40.00
Amikacin	20	100.00
Ceftriaxone	0	0.00
Ceftazidime	10	50.00
Gentamycin	6	30.00
Meropenem	20	100.00

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 (Tavajjohi *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 carboxypenicillins, ceftazidime 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 (Nathisuwan *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 Sriram, 2005). The results of this study also imply that quinolone or gentamycin alone cannot be depended upon as an antipseudomonal 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.

ACKNOWLEDGEMENTS

The researchers appreciate Mrs. Agboola for her technical support and Mr. Oladejo for his secretarial assistance.

REFERENCES

Aggarwal, R., U. Chaudhary and K. Bala, 2008. Detection of extended-spectrum beta-lactamase in *Pseudomonas aeruginosa*. Ind. Pathol. Microbiol., 51: 222-224.

Aibinu. I., T. Nwanneka and T. Odugbemi, 2007. Occurrence of ESBL and MBL in clinical isolates of *Pseudomonas aeruginosa* from Lagos, Nigeria. J. Am. Sc., 3: 81-85.

Aktas, Z., L. Poirel, M. Salcioglu, P.E. Ozcan, K. Midilli, C. Bal, O. Ang and P. Nordmann, 2005. PER-1-and OXA-10-like beta-lactamases in ceftazidime-resistant *Pseudomonas aeruginosa* isolates from intensive care unit patients in Istanbul, Turkey. Clin Microbiol Infect., 11: 193-198.

Al Naiemi, N., Duim and B., A. Bart, 2006. A CTX-M extended-spectrum beta-lactamase in *Pseudomonas aeruginosa* and *Stenoprophomonas maltophilia*. J. Med. Microbiol., 55: 1607-1608.

Bagge, N., O. Clofu, M. Hentzer, J.I. Campbell, M. Givskov and N. Holby, 2002. Constitutive high expression of chromosomal beta-lactamase in *Pseudomonas aeruginosa* caused by a new insertion sequence (IS1669) located in Amp D. Antimicrob. Agents Chemother., 46: 3406-3411.

CLSI, 2007. Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA., USA., ISBN-13: 9781562386252, Pages: 177.

Celenza, G., C. Pellegrini, M. Caccamo, B. Segatore, G. Amicosante and M. Perilli, 2006. Spread of bla CTX-M-type and bla PER-2 beta-lactamase genes in clinical isolates from BoSpread of bla CTX-M-type and bla PER-2 beta-lactamase genes in clinical isolates from Bolivian hospitals.livian hospitals. J. Antimicrob. Chemother., 57: 975-978.

Giamarellou H., 2002. Prescribing guidelines for severe Pseudomonas infections. J. Antimicrob. Chemother., 49: 229-233.

Hauser, A.R. and P. Sriram, 2005. Severe *Pseudomonas aeruginosa* infections: Tackling the conundrum of drug resistance. Postgrad Med., 117: 41-48.

Jarlier, V., M. Nicolas, G. Fournier and A. Philippon, 1988. Extended-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis., 10: 867-878.

Lim, K.T., R.M. Yasin, C.C. Yeo, S.D. Puthucheary and G. Balan *et al*., 2009. Genetic fingerprinting and antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* hospital isolates in Malaysia. J. Microbiol. Immunol. Infect., 42: 197-209.

Livermore, D.M., 2001. Of Pseudomonas, porins, pumps and carbapenems. J. Antimicrob. Chemother., 47: 247-250.

McGowan Jr, J.E., 2006. Resistance in non-fermenting Gram- negative bacteria: Multidrug resistance to the maximum. Am. J. Infect. Control., 34: 529-537.

Mesaros, N., P. Nordmann, P. Plesiat, M. Roussel-Delvallez and J. van Eldere *et al*., 2007. *Pseudomonas aeruginosa*: Resistance and therapeutic options at the turn of the new millennium. Clin. Microbiol. Infect., 13: 560-578.

Nathisuwan, S., D.S. Burgess and J.S. Lewis, 2001. Extended spectrum beta-lactamases, epidemiology, detection and treatment. Pharmacotherapy, 21: 920-928.

Nordmann, P. and M. Gilbert, 1998. Extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*.. J. Antimicrob. Chemother., 42: 128-131.

Okesola, A.O. and A.A. Oni, 2011. Susceptibility profile of clinical isolates of *Pseudomonas aeruginosa* to recommended antipseudomonal antibiotics in Ibadan, Nigeria. Ann. Trop. Path., 2: 37-43.

Pollack, M., 2000. *Pseudomonas aeruginosa*. In: Principles and Practice of Infectious Diseases, Mandell, G.L., J.E. Bennett and R. Dolin (Eds.). 5th Edn. Churchill Livingstone, Edinburgh, Scotland, pp: 02310-2335.

Shahid, M., Malik A. Sheeba, 2003. Multidrug resistant *Pseudomonas aeruginosa* strains harbouring R-plasmids and AmpC beta-lactamases isolated from hospitalized burn patients in tertiary care hospital of North India. FEMS Microbiol. Lett., 228: 181-186.

Tavajjohi, Z., R. Moniri and A Khorshidi, 2011. Detection and characterization of multidrug resistance and extended-spectrum-beta-lactamase-producing ($ESBL_S$) *Pseudomonas aeruginosa* isolates in teaching hospital. Afr. J. Microbiol. Res., 20: 3223-3228.

Thomson, K.S., C.C. Saunders and E.S. Moland, 1999. Use of microdilution panels with and without beta-lactamase inhibitors as a phenotypic test for beta-lactamase production among *Escherichia coli*, *Klebsiella spp*., *Enterobacter spp*., *Citrobacter freundii* and *Serratia marcescens*. Antimicrob. Agents. Chemother., 43: 1393-1400.

Woodford, N., J. Zhang, M.E. Kaufmann, S. Yarde and M.M. Tomas *et al*., 2008. Detection of *Pseudomonas aeruginosa* isolates producing VEB-type extended-spectrum beta-lactamases in the United Kingdom. J. Antimicrob. Chemother., 62: 1265-1268.