



ANTIBIOGRAM OF ESBL-PRODUCING *PSEUDOMONAS AERUGINOSA* ISOLATES OF NOSOCOMIAL ORIGIN

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ABSTRACT

The emergence of newer beta-lactamases such as extended spectrum beta-lactamases (ESBLs) is an important mechanism by which pathogenic bacteria develop resistance to some commonly available drugs; and this trend is of global concern due to the multidrug resistant nature of such microbes. ESBLs are plasmid-mediated beta-lactamases capable of hydrolyzing oxyimino 3rd-generation cephalosporins and monobactams but are yet inhibited by clavulanic

acid (a beta-lactamase inhibitor). Fifty (50) clinical isolates of *Pseudomonas aeruginosa* was tested phenotypically by the double disk synergy test (DDST) method for ESBL production. Only 11 *P. aeruginosa* isolates (22 %) produced ESBLs phenotypically. The ESBL-producing *P. aeruginosa* isolates in this study were completely resistant to sulphamethoxazole-trimethoprim (100 %). Higher levels of resistance amongst the ESBL-positive *P. aeruginosa* were also recorded for ceftazidime (90.1 %), cefotaxime (90.1 %), gentamicin (72.7 %), ciprofloxacin (54.5) and ofloxacin (81.8 %). Nonetheless, the ESBL-positive *P. aeruginosa* isolates were highly susceptible to imipenem and meropenem which are both carbapenems; and are the drug of choice for treating infections caused by ESBL-positive bacteria. The high level of resistance of ESBL-positive bacteria to some commonly available antibiotics (as obtainable in this study) gibes impetus to the antibiotic degrading capability of microbes expressing this enzyme. It is critical for hospitals (especially in this

part of the globe) to always be on the lookout for multidrug resistant pathogens inclusive of those expressing ESBLs in order to contain any outbreak due to them.

KEYWORDS: *Pseudomonas aeruginosa*, ESBLs, Nosocomial, Antibiogram.

INTRODUCTION

Increasing resistance to beta-lactam drugs especially the 3rd-generation cephalosporins in *Pseudomonas aeruginosa* is predominantly due to the production of multidrug resistant enzymes (in particular, extended spectrum beta-lactamases, ESBLs) which degrade some of these potent antibiotics and thus render them almost inefficacious for the treatment of some bacterial-related diseases.^[1] ESBLs are newer beta-lactamases that confer resistance some of the latest beta-lactam antibiotics especially the cephalosporins.^[2,3] ESBLs are often encoded by genes located on bacterial plasmids which also carry genes responsible for resistance to other antimicrobial agents such as aminoglycosides, tetracyclines and sulphonamides.^[4] They are derived from the earlier beta-lactamases such as the TEM enzymes, SHV and OXA-beta-lactamases with a narrower-spectrum of activity in terms of the antibiotics they degrade; and ESBLs are largely responsible for the multidrug resistance amongst Gram negative bacteria.^[1,2,5] ESBLs arise by mutation in the active site of the earlier beta-lactamases as aforementioned, and this allow the bacterial pathogen producing or harbouring them to confer a broader range of activity by allowing attack on oxyimino cephalosporins e.g. ceftazidime and cefotaxime.^[6,7,8] Since their discovery in the early 1980's (i.e. shortly after the discovery and introduction of the cephalosporins into clinical medicine), ESBL-producing bacteria have turn out to be a worldwide public health problem and the emergence of thee microbes in the community including those that produce the CTX-M enzymes has added to this problem.^[4,9,10] *P. aeruginosa* is a gram negative, non-enteric bacillus that colonize moist environment in the hospital, and it is also an important opportunistic nosocomial pathogen and causes a variety of infections such as wound and burn infections in humans.^[11] ESBL production in *P. aeruginosa* has been previously reported; and the organism is among the Gram negative bacilli that show remarkable resistance to some available antibiotics including but not limited to the penicillins and cephalosporins.^[7,12] Failure to detect pathogenic bacteria that produces multidrug resistant enzymes such as the ESBLs amongst others as a routine in the clinical microbiology laboratory portends numerous health problems. Possible treatment failure (especially with the cephalosporins and other beta-lactams), prolonged illness, prolonged hospitalization, inappropriate antibiotic prescription and limited therapeutic

options for ESBL infections are some of the consequences of not promptly and accurately detecting bacterial pathogens that produce ESBLs. Most hospitals in Nigeria especially in the Southeastern part of the country are not fully aware of the clinical significance of ESBLs; and their microbiology laboratories rarely lookout for these multidrug resistant enzymes from clinical specimens and pathogens. This study set out to detect and evaluate by phenotypic technique the antibiogram of ESBL-producing *P. aeruginosa* from wound infections in a tertiary hospital in Southeast Nigeria.

MATERIALS AND METHODS

Microorganisms: A total of 50 *Pseudomonas aeruginosa* isolates were recovered from the culture collection unit of the tertiary hospital under study. The *P. aeruginosa* isolates were recovered from wound swab specimens of patients with burns and wounds, and who attended the hospital under study for medical treatment during the period of the study.

Re-characterization of the bacterial isolates: All the *P. aeruginosa* isolates were transported from the point of collection to the laboratory; and each of the isolates were re-characterized by culturing them in 5 ml nutrient broth for 18-24 hrs. A loopful of the turbid growth in the nutrient broth tubes were subcultured onto nutrient agar plates, MacConkey agar and cystein lactose electrolyte deficient (CLED) medium (Oxoid, UK), and incubated at 37°C overnight. Suspect colonies were subcultured onto nutrient agar plates; and pure cultures of the *P. aeruginosa* isolates were identified biochemically using conventional identification techniques including oxidase test, Gram staining, and colonial morphology such as pigmentation.^[13,14]

Screening and detection of ESBL production: *P. aeruginosa* isolates that were resistant to any of the third generation cephalosporins including ceftazidime and cefotaxime according to the breakpoints of the Clinical Laboratory Standard Institute, CLSI were tested for ESBL production phenotypically.^[15,16] ESBL production in the *P. aeruginosa* isolates were detected phenotypically using the double disk synergy test (DDST) method as was previously described.^[1,12] Briefly, amoxicillin-clavulanic acid disk (30 µg) was aseptically placed at the center of a Mueller-Hinton (MH) agar already inoculated with the test bacterium. Ceftazidime (30 µg) and cefotaxime (30 µg) single antibiotic disks were each placed adjacent to the central disk (amoxicillin-clavulanic acid) at a distance of 15 mm. The plates were incubated at 37°C for 18-24 hrs. A ≥ 5 mm increase or difference in the inhibition zone diameter for either of the cephalosporins (ceftazidime and cefotaxime) tested in combination

with amoxicillin-clavulanic acid versus its zone when tested alone confirms ESBL production phenotypically.

Antimicrobial susceptibility testing (AST): AST was carried out by the Kirby-Bauer disk diffusion method in line with the CLSI criteria.^[15] All the *P. aeruginosa* isolates were evaluated for susceptibility to single antibiotic disks of sulphamethoxazole-trimethoprim (SXT), ofloxacin (OFX), gentamicin (CN), ciprofloxacin (CIP), ceftazidime (CAZ), cefotaxime (CTX), imipenem (IMP) and meropenem (MEM) (Oxoid, UK). Suspensions of the test organism equivalent to 0.5 McFarland turbidity standards were aseptically swabbed on MH agar plates; and the antibiotic disks were each placed aseptically on the inoculated plate using laboratory forceps and at a distance of 20 mm apart. The plates were incubated overnight at 37°C; and the inhibition zone diameter was recorded in millimeters as per the CLSI criteria.^[1,12,15]

RESULTS AND DISCUSSION

The results of the detection of ESBL production in the *P. aeruginosa* isolates are shown in Table 1. Out of the fifty (50) clinical isolates of *P. aeruginosa* recruited for this study, only 11 *P. aeruginosa* isolates (22 %) were confirmed to be ESBL producers by the DDST method. *P. aeruginosa* isolates that did not express ESBL phenotypically according to the DDST method used in this study were only 33 isolates (78 %). Figure 1 shows a keyhole effect produced by a *P. aeruginosa* isolate expressing ESBL; and this pattern is characteristic for ESBL-producing bacteria (including members of the *Enterobacteriaceae*) due to the synergistic effect produced between amoxicillin-clavulanic acid (a beta-lactamase inhibitor) and the third generation cephalosporins such as ceftazidime and cefotaxime. The results of the antimicrobial susceptibility test of the ESBL-producing *P. aeruginosa* isolates to some commonly used antibiotics are shown in Table 2. The result showed that imipenem and meropenem were the most effective drugs against the *P. aeruginosa* isolates. Highest levels of resistance were recorded for ceftazidime and cefotaxime. All the *P. aeruginosa* isolates were resistant to sulphamethoxazole-trimethoprim (SXT). A higher level of resistance by the *P. aeruginosa* isolates was also recorded for cefotaxime, ofloxacin, gentamicin and ceftazidime (Table 2). It is no doubt that antimicrobial agents since their discovery have to a large extent reduced the threat posed by pathogenic bacteria. However, these very important options (i.e. antibiotics) for the treatment of microbial-related diseases in both humans and animals have in recent time experienced some level of low activity due to the emergence and

spread of drug-resistant microbes in both the hospital and community settings. The expression of ESBLs by *P. aeruginosa* and other Gram-negative bacteria has been previously reported both within and outside Nigeria; and this important opportunistic bacterial pathogen is notorious for harbouring antibiotic resistance genes that are either chromosomally or plasmid-mediated ^[7,12].

Table 1. Occurrence of ESBL production in clinical isolates of *P. aeruginosa*

Sample	Organism	ESBL positive n(%)	ESBL negative n(%)
Wounds swabs	<i>P. aeruginosa</i>	11 (22)	39 (78)

Table 2. Result of antibiogram

Antibiotics (μg)	Resistant	Susceptible
Ofloxacin (10)	9 (81.8)	2 (18.1)
Gentamicin (10)	8 (72.7)	3 (27.3)
*SXT (25)	11 (100)	0 (0)
Imipenem (10)	1 (9.1)	10 (90.9)
Meropenem (10)	0 (0)	11 (100)
Cefotaxime (30)	10 (90.1)	1 (9.1)
Ceftazidime (30)	10 (90.1)	1 (9.1)
Ciprofloxacin (10)	6 (54.5)	5 (45.5)
*SXT-sulphamethoxazole-trimethoprim		



Figure 1. Mueller-Hinton agar plate showing phenotypic detection of ESBL production using the double disk synergy test (DDST) method. The bacterium is *P. aeruginosa*; the antibiotic disk on the left is ceftazidime (30 μg) while the disk on the right is cefotaxime (30 μg). The central disk is amoxicillin-clavulanic acid, a beta-lactamase inhibitor.

In this present study, the antibiogram of ESBL-producing *P. aeruginosa* isolates recovered from wound samples were studied with a view to elucidating their susceptibility patterns so that therapy can be properly guided in the course of treatment. The frequency of ESBL production amongst the clinical isolates of *P. aeruginosa* was 22 %; and this shows that only 11 out of the 50 isolates of *P. aeruginosa* used in this study produced ESBL by the DDST method. The implication of this result is that ESBL production amongst *P. aeruginosa* isolates is high in this region; and this has the tendency of spreading to other nosocomial pathogens within the hospital environment. Such a scenario portends grave danger to patient care and infectious disease control in the hospital community. The results of ESBL production in *P. aeruginosa* isolates obtained in this study is in accordance to similar studies conducted in Lagos, Southwest Nigeria ^[12]. Similar prevalence rates of ESBL-producing *P. aeruginosa* were recently reported in Southeast Nigeria ^[17]. Pathogenic bacteria harbouring ESBLs have the ability to be resistant to third-generation cephalosporins, other beta-lactams and some non-beta-lactam agents ^[2,9]. And due to their multidrug resistant nature especially to the beta-lactams, bacterial pathogens found to be ESBL producers should be assumed and reported as resistant to all extended-spectrum cephalosporins irrespective of their antibiogram result. Knowing this fact especially in clinical medicine will help guide the physician on the course and choice of therapy for the affected patient. The susceptibility test result showed that the ESBL-producing *P. aeruginosa* isolates were highly resistant to sulphamethoxazole-trimethoprim, cefotaxime, ceftazidime, and gentamicin. The organism was also resistant to ofloxacin and ciprofloxacin at different rates. High levels of resistance of *P. aeruginosa* isolates to both beta-lactam and non-beta-lactam drugs as reported in this study has been previously reported in some parts of Nigeria and elsewhere ^[12,17,18]. Surveillance, monitoring and the accurate detection of multidrug resistant pathogens especially those producing ESBLs from clinically relevant samples and even from the community is critical particularly now that drug-resistance is fast becoming a global phenomenon that is putting in jeopardy the clinical efficacy of some available antimicrobial agents. This study has presumptively shown the need to continuously detect ESBL production from bacterial pathogens as well as to properly ascertain their antibiogram to avoid wrong drug prescription.

CONCLUSION

From our results, it could be deduced that clinical isolates of *P. aeruginosa* harbour or express ESBLs; and they are resistant to some commonly used antibiotics inclusive of some beta-lactams and non-beta-lactam antibiotics. There is therefore need for proper monitoring

and detection of this all important enzymes from clinically important bacterial pathogens so as to guide therapy and to contain their nefarious effect on antibiotics. Further molecular studies are required to type by molecular techniques the genes responsible for ESBL production in the *P. aeruginosa* isolates from this region.

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REFERENCES

1. Ejikeugwu C, Iroha I, Adikwu M and Esimone C. Susceptibility and Detection of Extended Spectrum β -Lactamase Enzymes from Otitis Media Pathogens. *American Journal of Infectious Diseases*. 2013; 9(1): 24-29.
2. Jacoby GA and Munoz-Price LS. Mechanisms of Disease: The New β – Lactamases. *N Engl J Med*. 2005; 352: 380-91.
3. Paterson DL and Bonomo RA. Extended – Spectrum β – lactamases: a clinical update. *Clinical Microbiology Review*. 2005; 18(4): 657-686.
4. Pitout JDD, Nordmann P, Laupland KB and Piorel L. Emergence of *Enterobacteriaceae* producing extended – spectrum β – lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy*. 2005; 1-8.
5. Bradford PA. Extended – Spectrum β – Lactamases in the 21st century: Characterization, Epidemiology, and Detection of this Important Resistance Threat. *Clinical Microbiology Reviews*. 2001; 14(4): 933-951.
6. Arlet G, Roveau M and Philippon A. Substitution of alanine for aspartate at position 179 in the SHV-6 extended spectrum β -lactamase. *FEM Microbiol Lett*. 1997; 152: 163-167.
7. Weldhagen GF, Poirel L and Nordmann P. Ambler Class A extended spectrum β -lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. *Antimicrobial Agents Chemother*. 2003; 47: 2385-2392.
8. Livermore DM and Woodford N. Laboratory Detection and Reporting of Bacteria with Extended – Spectrum β – Lactamases. Health Protection Agency: Guidelines to Diagnostic Laboratories. 2004.
9. Bonnet R. Growing group of Extended – Spectrum β – Lactamases: the CTX-M Enzymes. *Antimicrobial Agents Chemotherapy*. 2004; 48(1): 1-14.

10. Radice M, Power P, Di Conza J, Gutkind G. Early dissemination of CTX-M- derived enzymes in South America. *Antimicrob Chemother.* 2002; 54: 735-743.
11. Brooks GF, Butel JS and Morse SA. Medical Microbiology, 23rd edition. McGraw Hill Publishers. USA. 2004.
12. Aibinu I, Nwanneka T and Odugbemi T. Occurrence of ESBL and MBL in clinical isolates of *Pseudomonas aeruginosa* from Lagos, Nigeria. *Journal of American Sciences.* 2007; 3(4): 81-85.
13. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd edition. Cambridge University Press, UK. 2000.
14. Vandepitte, J, Verhaegen J, Engbaek K, Rohner P, Piot P, and Heuck C.C. Bacteriological investigations. In: World Health Organization Basic Laboratory Procedures in Clinical Bacteriology. 2nd edition. WHO publications, Geneva, Switzerland, ISBN: 9241545453, 2003.
15. Clinical Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S15. 2005; CLSI, Wayne, PA. <http://www.fsis.usda.gov>
16. Livermore DM. ESBLs: Global aspects and laboratory detection: Supplement to *SCIEH* Weekly Report. 2004; 38(50): 4.
17. Afunwa RA, Odimegwu DC, Iroha RI and Esimone CO. Antimicrobial resistance status and prevalence rates of extended spectrum beta-lactamase producers isolated from a mixed human population. *Bosnian Journal of Basic Medical Sciences.* 2011; 11(2): 91-96.
18. Ramazanzadeh R. Etiologic agents and extended-spectrum beta-lactamase production in urinary tract infections in Sanandaj, Iran. *Eastern Journal of Medicine.* 2010; 15: 57-62.