Occurrence of ESBL and MBL in Clinical Isolates of *Pseudomonas aeruginosa* From Lagos, Nigeria

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ABSTRACT: Background: Widespread use and misuse of anti-infective agents have resulted in problems of drug resistance linked to treatment of infectious diseases caused by worrisome pathogens such as *Pseudomonas aeruginosa*. Infections caused by *Pseudomonas aeruginosa* with resistance to extended spectrum β-lactams and the carbapenems is emerging and requires the identification of effective alternative antimicrobial therapy and improved surveillance of the emergence of such resistance. A total of non-duplicate 97 strains of *P. aeruginosa* recovered from various clinical specimens and collected between March and August, 2006 were analysed for their resistance pattern. **Methods:** Antimicrobial susceptibility, Extended-Spectrum β-Lactamase (ESBL)- and Metallo- β-Lactamase-Production (MBL) were determined using disk diffusion method, double disk synergy test and combined disk test respectively. **Results:** The Carbapenems had the highest activity against the strains tested (95.9%). This was followed by ceftazidime (79.4%). Amongst the 20 ceftazidime-resistant strains, 9 were found to be ESBL-producers. While the 4 strains resistant to the carbapenems were detected to be MBL-producers and were observed to be multi-resistant. Amikacin was the most potent amongst the aminoglycosides (78.4%) while there was high resistance to the quinolones. **Discussion:** This study highlights needs to establish antimicrobial resistance surveillance networks for *P. aeruginosa* to determine the appropriate empirical treatment regimen. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate uses of drugs are curtailed and continuous education of infection control practices maintained. [The Journal Of American Science. 2007;3(4):81-85]. (ISSN: 1545-1003).

Keywords: Emergence, *Pseudomonas aeruginosa,* ESBL, MBL, Multidrug-resistance

Introduction

Resistance to anti-infective agents is worldwide, both in developed and developing countries. Antimicrobials have been used successfully for over 6 decades, but genes expressing resistance to them have emerged in strains of bacteria and have disseminated through the global ecosystem to reach infecting microorganisms, produce disease, and seriously interfere with therapy, allowing infections to progress and kill, despite antibiotic administration. (Isturiz and Carbon, 2000).

Pseudomonas aeruginosa has been reported to be an opportunistic and a worrisome nosocomial pathogen (Gales *et al*., 2001). It is reported to be a leading cause of nosocomial infections, including pneumonia, urinary tract infections, burn infection, meningitis and bacteremia.(Pollack, 2000). Its ability to survive on inert materials, live on minimal nutritional requirement, with its tolerance to a wide variety of physical conditions and antiseptics; has contributed enormously to its ecological success and its role as an effective opportunistic pathogen (Gales *et al*., 2001). It is known to exhibit intrinsic resistance to several antimicrobial agents.

With the occurrence of ESBLs and MBL-producing *Pseudomonas aeruginosa* being increasingly reported worldwide (Lee *et al*., 2005, Walsh *et al*., 2005, Pagani *et al*., 2004, Gibb *et al*., 2002, Toleman *et al*., 2002), this study evaluated the current resistance pattern of *Pseudomonas aeruginosa* strains from 2 tertiary hospitals in Lagos, Nigeria.

MATERIALS AND METHODS

Bacterial Isolates

A total of non-duplicate 97 isolates of *P. aeruginosa* recovered from various clinical specimens of patients treated at the Lagos University Teaching Hospital (LUTH), and the National Orthopaedic Hospital Igbobi (NOHI) Lagos were collected between March and August, 2006.

Isolation and Identification

Pseudomonas aeruginosa strains were previously isolated and identified from the various specimens at the microbiology laboratory of these 2 tertiary hospitals. The isolates were obtained from these laboratories and their identity reconfirmed using a combination of colonial morphology, positive oxidase test, pigment

formation, growth at 42^0C on nutrient agar, Gram's reaction, motility, growth on *Pseudomonas* agar base medium (Oxoid, UK) to which C- N supplement SR – 102 (Oxoid, UK) has been added using *Proteus vulgaris* ATCC 13315 and *Pseudomonas aeruginosa* ATCC 27853 as controls. The isolates were maintained on nutrient agar slant. (Oxoid, UK).

Antibiotic Susceptibility Testing

Antibiotic susceptibilities were determined on Mueller-Hinton agar (Oxoid, UK) by standard disk diffusion procedures of Bauer *et al* (1966) which conforms to the recommended standard of National Committee for Clinical Laboratory Standards now known as CLSI (NCCLS, 2000). The following antibiotics were used: Levofloxacin (5µg), Gentamicin (30µg), Aztreonam (30µg), Meropenem (10µg), Amikacin (30µg), Tobramycin (30µg), Piperacillin (30µg), Imipenem (10µg), Ceftazidime (30µg), Piperacillin/Tazobactam (110µg) [Oxoid, UK];:Ciprofloxacin (5µg), Ofloxacin (5µg), (Ranbaxy, Nigeria) and Pefloxacin (5µg) (May and Baker, Nigeria). *Pseudomonas aeruginosa* ATCC 27853 was run simultaneously with the test organisms. Results were interpreted according to the NCCLS/CLSI standard (NCCLS, 2000).

Beta-Lactamase Detection

(a) **Nitrocefin Test**

All the isolates were tested for the production of beta-lactamase with the nitrocefin beta-lactamase test strip (Oxoid, UK).

(b) Extended Spectrum Beta-Lactamase Detection

Double Disk Synergy Test (DDST) were performed on ceftazidime resistant strains by placing disks of ceftazidime and aztreonam (30µg each) at a distance of 20 mm (center to center) from a disk containing augmentin (amoxicillin, 20µg, and clavulanic acid, 10µg) (Pagani *et al.*, 2004)). ESBL production was inferred when the cephalosporin and monobactam zones were expanded by the clavulanate.

© Detection Of Metallo – Beta-Lactamase (MBL) Producing Strains

Meropenem and Imipenem- non susceptible isolates were tested for MBL production according to Pitout *et al*'s method (2005). This method recommended the use of both imipenem and meropenem in testing for MBL. In this method, an increase of ≥7mm in zone diameter in the presence of 930µg EDTA compared to those with both IPM and MEM tested alone was considered to be a positive test for the presence of an MBL .

RESULT

A total of 97 *Pseudomonas aeruginosa* strains from different clinical specimens were tested for their antimicrobial sensitivity pattern between March and August 2006.

Antimicrobial Susceptibility Testing

Table 1 shows the distribution of *Pseudomonas aeruginosa* strains by site of isolation and table 2 shows the antimicrobial susceptibility pattern of *P. aeruginosa* strains.

Meropenem and Imipenem were the most active of all the antimicrobial agents used with only four (4.1%) of the 97 strains of *Pseudomonas aeruginosa* resistant to these carbapenems. This was followed by ceftazidime with the strains showing 79.4% susceptibility. Aztreonam, a monobactam, had activity against 63.9% of the strains. Only 30.9% were susceptible to piperacillin while combination of piperacillin with tazobactam (a beta-lactam/beta-lactamase inhibitor) was highly active with 76.3% susceptible to it when compared to piperacillin alone. Activity of the quinolones against the *Pseudomonas aeruginosa* strains in this study was poor. The most active was ofloxacin with 42.3% susceptibility. This was closely followed by ciprofloxacin (40.2%) and then Levofloxacin (30.9%). Imipenem, meropenem and Ceftazidime had good activity against all the quinolone resistant strains. While for the aminoglycosides, susceptibility was highest in amikacin (78.4%), followed by tobramycin (46.4%) and Gentamicin (42.3%).

Beta-Lactamase Production

Beta-lactamase production by the nitrocephin test was observed in 32 (33%) of the *Pseudomonas aeruginosa* strains. All the ESBL and MBL producers were among those positive for beta-lactamase production by the nitrocephin test.

ESBL Production

Of the 20 strains resistant to ceftazidime, 9 were found to be ESBL-producers. Six of the ESBL-producers were from LUTH while 3 were from NOHI. The ESBL-producers were spread across the various sites of isolation. Five were from wound swabs, 3 from Catheter-tips and 1 was from the pus of an abcess. Susceptibility to piperacillin tazobactam amongst the ESBL-producers varied. All the ESBL-producers were found to be highly susceptible to imipenem, meropenem and amikacin Two (22.2%) of the ESBL-producers sensitive to amikacin and gentamicin were found to be susceptible to ofloxacin. There was resistance to the other quinolones by the ESBL-producers.

Detection of MBL

Of the 4 carbapenem resistant strains of *Pseudomonas aeruginosa*, the Pitout *et al*. (2005) method detected all as MBL-producers with meropenem while Imipenem plus EDTA detected 2 as MBL-producer. The 4 carbapenem resistant strains were observed to be multi-resistant to all the antimicrobials tested in this study The four strains were recovered from LUTH. and were obtained from wound sites. ESBL was not detected in any of the 4 carbapenem resistant strains phenotypically.

Table 1. Distribution of *Pseudomonas aeruginosa* by site of Isolation

Source/Sites	**No of Isolates**	**Percentage (%)**
Wounds	65	67
Semen	9	9.3
Gastric Aspirate	7	7.2
Eye Swab	4	4.1
Ear Swab	4	4.1
Pus from Abcess	3	3.1
Catheter-tip	3	3.1
High Vaginal Swab	2	2.1
Total	**97**	**100**

Table 2. Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Strains

Antimicrobials	**No**	**(%) S**	**No**	**(%) R**
Gentamicin	41	(42.3)	56	(57.7)
Amikacin	76	(78.4)	21	(21.6)
Tobramycin	45	(46.4)	52	(53.6)
Ofloxacin	41	(42.3)	56	(57.7)
Ciprofloxacin	39	(40.2)	58	(59.8)
Pefloxacin	7	(7.2)	90	(92.8)
Levofloxacin	30	(30.9)	67	(69.1)
Ceftazidime	77	(79.4)	20	(20.6)
Imipenem	93	(95.9)	4	(4.1)
Meropenem	93	(95.9)	4	(4.1)
Aztreonam	62	(63.9)	35	(36.1)
Piperacillin/Tazobactam	74	(76.3)	23	(23.7)
Piperacillin	30	(30.9)	67	(69.1)

Key: S=sensitive; R=Resistant; No=Number

DISCUSSION

Pseudomonas aeruginosa is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen (Todar, 2002). Its' infections are a frequent cause of morbidity and mortality in hospitalized patients. Treatment of these infections is further complicated by the intrinsic and acquired resistance of this bacterium to many commonly used antimicrobial agents (Hauser and Sriram, 2005).

The result of this study showed that there is an increase in resistance to the quinolones by strains of *Pseudomonas aeruginosa*. In previous studies by Oduyebo *et al* (1997) and Kesah *et al*.(1999) in this environment, *Pseudomonas aeruginosa* strains were found to be highly susceptible to the quinolones (96%); but from this study, our data shows increase in resistance to this family of antibacterial agents by the current clinical strains of *Pseudomonas aeruginosa*. Ofloxacin was observed to have the highest activity (41%) and was closely followed by ciprofloxacin (39%). This result implies that quinolones alone, cannot be depended upon as an antipseudomonal antimicrobial in this environment. They will have to be used in combination or replaced with another antimicrobial preferably the broad-spectrum beta-lactams/penicillins. The activities of carbapenems, ceftazidime, piperacillin/tazobactam and amikacin against *Pseudomonas aeruginosa* strains is still high and they can be considered as therapeutic options available for *Pseudomonas aeruginosa* infection treatment in this region.

Of the 20 strains resistant to ceftazidime, 9 were found to be ESBL-producers by the double-disk diffusion test. Amongst the ESBL-producers, there was high resistance to the quinolones. This suggests a possible existence of co-resistance to the quinolones on the gene responsible for ESBL-production (Thomson *et al*., 1999). The 4 strains resistant to the carbapenems were also detected phenotypically to be MBL-producers. These MBL-producers were resistant to all other antibiotics including the aminoglycosides and the quinolones used in this study. In a report by Hauser and Sriram (2005), it was stated that *P aeruginosa* isolates that are resistant to multiple antibiotics are of particular concern and pose a significant clinical challenge.

Multidrug-resistant isolates of *Pseudomonas* spp (defined as being resistant to piperacillin, ceftazidime, imipenem, and gentamicin or ciprofloxacin) have become an increasingly frequent problem, especially in Europe and Latin America, and now constitute from 3.6% to 7.7% of all *P aeruginosa* isolates (Gales *et al*., 2001, Harris *et al*., 1999). As a consequence, strains are now being identified that are resistant to all commonly used antibiotics (Stein *et al*., 2002). Few therapeutic options are hence available for the treatment of patients infected with these strains.

The 4 MBL-producing strains were from LUTH. This is a tertiary hospital where various types of patients who have moved from one hospital to the other both locally and internationally are referred, for management or continuation of therapy. The implication of this is that most of the patient would have been on one form of treatment or the other before getting to this hospital, thus the selective pressure of use, overuse and misuse of antibiotics cannot be ignored in the emergence of these resistance phenotypes in this health-care centre. Not to be underestimated also is the likelihood of importation of resistance genes from other health-care centers around the world.

Data on the detection of ESBL- and MBL- producing *Pseudomonas aeruginosa* strains from clinical samples in Lagos, is scarce and the result of this work shows the presence of these enzymes in the already notorious pathogen, *Pseudomona aeruginosa,* in this environment. Thus, the emergence and existence of metallo-beta-lactamase and extended-spectrum beta-lactamase-enzyme production with multidrug resistance, in strains of *Pseudomonas aeruginosa* in this environment, will greatly complicate the clinical management of patients infected with such strains if utmost care is not taken. Further molecular studies need to be carried out to confirm the MBL- and ESBL- type present in *Pseudomonas aeruginosa* strains in Lagos, Nigeria and their association with resistance to other classes of antibiotics.

In conclusion, this study highlights the need to establish an antimicrobial resistance surveillance network for *P. aeruginosa* to monitor the trends and new types of resistance mechanism emerging. Resistance to different kinds of antimicrobial agents among *P. aeruginosa* is clearly on the increase. Results from this study with regards to high quinolone resistance, ESBL and MBL production suggests that resistance gene carried on an epidemic plasmid which has the ability to travel freely may be present in this environment. Thus optimum caution must be taken; good antibiotic prescription policies and infection control practices must be put in place to prevent spread of the gene, responsible for these resistances, which could result in return to the pre-antibiotic era.

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References

1. Bauer AW, Kirby MM, Sharis JL and Turck M. Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol. 1966; **45:**493–496.
2. Gales AC, Jones RN, Turnidge J, *et al*. Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis;, 2001; 32 Suppl 2:S146-55.
3. Gibb AP, Tribuddharat C, Moore RA, *et al.* Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new *bla*IMP allele, *bla*IMP-7. Antimicrob Agents Chemother, 2002; 46:255–258.
4. Harris A, Torres-Viera C, Venkataraman L, *et al*. Epidemiology and clinical outcomes of patients with multiresistant *Pseudomonas aeruginosa*. Clin Infect Dis, 1999; 28(5):1128-33.
5. Hauser AR, Sriram P. Severe *Pseudomonas aeruginosa* infections: tackling the conundrum of drug resistance. Postgrad Med, 2005; 117(1):41-8.
6. Istúriz RE, Carbon C. Antibiotic use in developing countries. Infection Control and Hospital Epidemiology, 2000; 21:394–397.
7. Kesah CN, Egri-Okwaji MTC, Odugbemi TO, Iroha EO. Resistance of Nosocomial Bacterial Pathogens to Commonly Used Antimicrobial Agents. The Nigerian Postgraduate Medical Journal, 1999; 6(2): 1-6
8. Lee S, Park YJ, Kim M, *et al*. Prevalence of Ambler class A and D beta-lactamase among clinical isolates of *Pseudomonas aeruginosa* in Korea. J Antimicrob Chemother, 2005; 56:122–127.
9. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.Approved standard M7-A5 and informational supplement M100-S10. 2000. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. Oduyebo, O, Ogunsola FT, Odugbemi Tolu. Prevalence of Multi-Resistant Strains of Pseudomonas aeruginosa Isolated at The Lagos University Teaching Hospital Laboratory from 1994 to 1996. Nig. Qt J Hosp Med 1997; 7(4): 373-376
11. Pagani L, Mantengoli E, Migliavacca R, *et al.* Multifocal detection of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended spectrum beta-lactamase in northern Italy. J Clin Microbiol, 2004; 39**:**1865–1870.
12. Pitout JDD, Gregson DB, Poirel L, McClure J-A, Le P, Church DL. Detection of *Pseudomonas aeruginosa* producing metallo-beta-lactamases in a large centralized laboratory. J Clin Microbiol. 43:3129–3135.
13. Pollack M **.** *Pseudomonas aeruginosa*. In: Principles and practice of infectious diseases 5th ed. Mandell GL, Bennett JE and Dolin R (ed.), Edinburgh,Churchill Livingstone , Scotland , 2000; p. 2310–2335.
14. Stein A, Raoult D. Colistin: An antimicrobial for the 21st century? Clin Infect Dis. 2002; 35(7):901-2
15. Thomson KS, Sanders CC, Moland ES. Use of microdilution panels with and without β-lactamase inhibitors as a phenotypic test for β-lactamase production among *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, and *Serratia marcescens*. Antimicrob Agents Chemother.1999; 43:1393-1400.
16. Todar K. *Pseudomonas aeruginosa*. Todar's Online Textbook of Bacteriology. Availabvle from: URL:http://www.testbookofbacteriology.net/ pseudomonas.html, 2002.
17. Toleman MA, Simm AM, Murphy TA *et al*. Molecular characterization of SPM-1, a novel metallo-beta-lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme. J Antimicrob Chemother, 2002; 50:673–679.
18. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clin Microbiol Rev. 2005; 18:306–325.