

Incidence of cephalosporin resistance among clinical isolates of *Pseudomonas aeruginosa* in Ibadan, South-Western Nigeria



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Received: 17.09.15; Accepted: 04.12.15; Published: 12.12.15

ABSTRACT

Background: The emergence of beta-lactam resistance in *Pseudomonas aeruginosa* is a major global challenge, particularly, the rise in the resistance to 3rd and 4th generation cephalosporins. **Aim:** This study was carried out to determine the resistance pattern of *Pseudomonas aeruginosa* to different generations of cephalosporins. **Methods:** A total number of one hundred clinical isolates of *Pseudomonas aeruginosa* were collected from June to November 2014 at University Teaching Hospital Ibadan, Oyo State. These were tested for their sensitivity to antibiotics by means of disc diffusion method using prepared antibiotics disc containing different μ of antibiotics; Cefotaxime (30 μ), Cefaclor (30 μ), Cefamandole (30 μ), Cefixime (5 μ), Cefepime (30 μ), Cefpodoxime (30 μ) and Ceftazidime (30 μ). **Results:** *Pseudomonas aeruginosa* showed absolute resistance to all antibiotics used except Ceftazidime, and Cefepime which are third and fourth generation of cephalosporin respectively. Ceftazidime had minimal resistant of 21% and higher susceptibility rate of 76%, Cefepime had the highest susceptibility rate of 90% and minimal resistance of 6%. Cefotaxime and Cefpodoxime had minimal intermediate of 1%, Ceftazidime of 3% and Cefepime of 4%. **Conclusion:** The result from this study provided more evidence that among third generation of cephalosporins used, some are more active than the other while fourth generation is still the most effective of all other generations. Knowledge on the distribution of cephalosporin-resistant organisms is of ultimate importance as a guide in empirical therapy, taking note of preventive strategies as well as control measures against the spread of resistant microorganisms.

Key words: Cephalosporins, resistance, susceptibility, *Pseudomonas aeruginosa*, antibiotics, organism

INTRODUCTION

Resistance of pathogenic organisms has become a worldwide problem with serious consequence on the treatment of infectious

diseases. Emergence and dissemination of β -lactam resistance in nosocomial *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have pose a serious problem worldwide, especially the increasing resistance to different



generations of Cephalosporins.^[1] Infections due to gram-negative organisms, *Pseudomonas aeruginosa* being the most common, continue to be one of the leading causes of health care-acquired infections as well as morbidity and mortality in the healthcare system.^[2,3] *Pseudomonas aeruginosa*, a gram-negative bacterium, is found in healthcare-associated infections especially in patients who have been hospitalized more than a week and is reported in different outbreaks of infections such as pneumonia, bacteremia, urinary tract infection, and endocarditis, which are often complicated and potentially life-threatening.^[4,5] Wound infections have been the leading reservoir for the bacterium as well as burn patients.^[6] Cephalosporins are used to treat a wide variety of bacterial infections caused by *Pseudomonas aeruginosa* not limited to respiratory tract infections, otitis media, gonorrhoea, skin infections, urinary tract infections and sexually transmitted diseases.^[7] According to Oladipo *et al.*,^[2,3] Eyo *et al.*,^[1] and Ramalingam *et al.*,^[8] the fundamental mechanism of *Pseudomonas aeruginosa* resistance to cephalosporin involves the production of enzymes, extended spectrum β -lactamases (ESBLs) that aids in the bacterium resistance, that is, bacterial resistance to beta-lactam antibiotics is due to the enzymatic inactivation of antibiotics, that is, the production of enzyme β -lactamase which hydrolyses the β -lactam ring of the antibiotics rendering them inactive. Extensive use and misuse of antibiotics of the public alongside inappropriate practices of unskilled health workers and practitioners have led to the increasing resistance of nosocomial microorganisms.

Different generations of cephalosporin, first, second, third and fourth generations, are broad spectrum antimicrobial agents with enhanced activity against both gram-positive and gram-negative organisms.^[3] They have been assured successful in the treatment of severe and multi-drug resistant infections, however, increased prevalence of nosocomial strains of *Pseudomonas aeruginosa* resistance to cephalosporin among clinical isolates has been reported.^[9] Knowledge on the distribution of cephalosporin-resistant organisms is important for guide in empirical therapy, taking note of preventive strategies as well as control measures against the spread of resistant microorganisms. This study therefore is designed to determine the antimicrobial susceptibility pattern of selected clinical

isolates to different generations of cephalosporin.

METHODOLOGY

Study area

This research was carried out in Ibadan, Oyo State, South Western part of Nigeria, in order to determine the resistance pattern of clinical isolates of *Pseudomonas aeruginosa* to different generations of cephalosporin among the patients at University Teaching Hospital Ibadan, Oyo state, Nigeria over a period of five months (June to November 2014).

Bacterial isolates

A total number of one hundred (100) clinical isolates of *Pseudomonas aeruginosa* from different clinical sites including burns, wounds and ear were used for this study. These samples were obtained from University Teaching Hospital Ibadan.

Processing of samples

Media were prepared according to the manufacturer's instructions and sterilized at 121°C for 15min at 15 lb pressure. The inoculum were standardized using the MacFarland standard.^[2,3]

Susceptibility test

The susceptibility test was conducted using the Kirby-Bauer method of sensitivity determination.^[2,3] Petri – dishes of Mueller Hinton agar were prepared according to the manufacturer's instruction. 0.1ml of *Pseudomonas aeruginosa* equivalent to 0.5 McFarland standard was seeded into each of the Petri-dishes containing Mueller-Hinton agar using sterile swabs.^[2] These were allowed to stand for 45min to enable the inoculated organisms to pre-diffusion.^[3] The antibiotics discs of Cefadroxil (30 μ g; Oxoid, UK), Cefotaxim (30 μ g; Oxoid, UK), Cefamandole (30 μ g; Oxoid, UK), Cefador (30 μ g; Oxoid, UK), Cefpodoxime (10 μ g; Oxoid, UK), Cefixime (5 μ g; Oxoid, UK) and Cefepime (30 μ g; Oxoid, UK) were aseptically placed on the surfaces of the sensitivity agar plates.^[2,3] These were incubated aerobically for 18–24 hours at 37°C and the radial zone of inhibitions were taken.^[2,3] The results were expressed as susceptible, intermediate or resistant according to criteria developed by Clinical Laboratory Standard Institute.^[10,11]

Statistical analysis

Susceptibility rates were analyzed using ANOVA at significant level of $P \leq 0.05$. All interval estimates are 95% confidence

intervals. SPSS program for Windows (version 16.0; SPSS, Inc., Chicago, IL) was used.

RESULTS

Distribution of *Pseudomonas aeruginosa* from different sites

Figure 1 shows the distribution of the clinical isolates of *Pseudomonas aeruginosa* from the different sites. Ear swab was 23, burns was 30 while wound swabs was 47.

Resistance pattern of *Pseudomonas aeruginosa* to different generations of Cephalosporin

From table 1, out of 23 *Pseudomonas aeruginosa* isolated from ear swab, 17(73.9%) were susceptible to Ceftazidime while the remaining 6(26.1%) were resistant. 19(89.2%) of the isolates were susceptible to Cefepime while 2(8.9%) showed intermediate effect. All the isolates were resistant to Cefotaxime, Cefamandole, Cefador, Cefpodoxime and Cefixime.

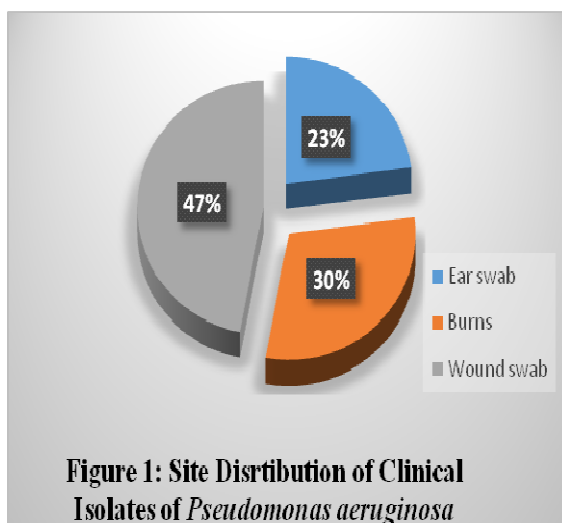


Figure 1: Site Distribution of Clinical Isolates of *Pseudomonas aeruginosa*

Among 30 isolate obtained from burns, 1(3.3%) showed intermediate effect to Cefotaxime and 29 (63.3%) were resistant. 24(80%) of the isolate were susceptible to Ceftazidime, 1(3.3%) showed intermediate and the remaining 5(6.7%) were resistant to the antibiotic. 29(96%) of the isolate were susceptible to Cefepime and the remaining 1(3.3%) was resistant. All the isolate from this site were resistant to Cefamandole, Cefador, Cefpodoxime and Cefixime.

From the 47 isolates from wound, 1(2.1%) showed intermediate effect to Cefpodoxime while the remaining 46(97.9%) were resistant. 35(74.5%) were susceptible to ceftazidime, 2(4.3%) showed intermediate effect and the

remaining 10(21.3%) were resistant. 42(89.4%) of the isolate were susceptible to Cefepime, 2(4.3%) showed intermediate effect and 3(6.4%) were resistant. All the *Pseudomonas aeruginosa* isolates from this site were resistant to Cefotaxime, Cefamandole, Cefador and Cefixime.

Summarily, the susceptibility rate of *Pseudomonas aeruginosa* to Cefotaxime was 0% sensitive, 1% intermediate and 99.0% resistant. Cefamandole was 0% sensitive, 0% intermediate and 100% resistant. Cefador was 0% sensitive, 0% intermediate and 100% resistant. Cefpodoxime was 0% sensitive, 1% intermediate and 99.0% resistant. Cefixime was 0% sensitive, 0% intermediate and 100% resistant. Ceftazidime was 76% sensitive, 3.0% intermediate and 21% resistant. Cefepime was 90% sensitive, 4% intermediate and 6.0% resistant. The overall resistant pattern of *Pseudomonas aeruginosa* to different generations of cephalosporin was 23% sensitive, 1.3% intermediate and 75.0% resistant.

ANOVA for resistant *Pseudomonas aeruginosa* isolates from different sites to cephalosporin

From table 2, no significant difference was recorded in the mean effect of the second (Cefotaxime, Cefamandole and Cefador) and third (Cefpodoxime and cefixime) generations on the ear swab isolates of *Pseudomonas aeruginosa*. However, significant difference was recorded on Ceftazidime and Cefepime with mean values of 1.52 and 1.26 respectively.

From the isolates got from burn a slight difference was recorded on Cefotaxime with a mean value of 2.97. No significant difference was recorded on Cefamandole and Cefador (second generation) as well as Cefpodoxime and Cefixime (third generation). There was significant difference in the mean effect of Ceftazidime and Cefepime (third and fourth generations) with mean values of 1.37 and 1.07, respectively.

From the isolates on wound swabs, no significant difference was recorded in the mean effect of the second generation cephalosporin. On the third generation, Cefpodoxime, Cefixime and Ceftazidime showed significant differences with mean values of 2.96 and 15.67 and 9.94, respectively. Cefepime, a fourth generation, also showed a significant difference with a mean value of 1.17.

Table 1: Susceptibility pattern of *pseudomonas aeruginosa* to different generations of Cephalosporin using CLSI [10,11] Criteria

Isolation Sites	Second Generation									Third Generation									Fourth Generation			Total				
	CTX			MA			CEC			CPD			CFM			CAZ			FEP							
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
Ear swab	0	0	23	0	0	23	0	0	23	0	0	23	0	0	23	17	0	6	19	2	2	36	2	123		
Burns	0	1	29	0	0	30	0	0	30	0	0	30	0	0	30	24	1	5	29	0	1	53	2	155		
Wound swab	0	0	47	0	0	47	0	0	47	0	1	46	0	0	47	35	2	10	42	2	3	77	5	247		
Total	0	1	99	0	0	100	0	0	100	0	1	99	0	0	100	76	3	21	90	4	6	16	9	525		
Susceptibility (%)	0	1	99	0	0	100	0	0	100	0	1	99	0	0	100	76	3	21	90	94	6	23	1.3	75.0		

CTX = Cefotaxime, MA = Cefamandole, CEC = Cefador, CPD= Cefpodoxime, CFM = Cefixime, FEP = Cefepime. S = Sensitive, I = Intermediate, R = Resistant. CLSI criteria; For Cefotaxime, S is ≥ 23, I is ≤ 15-22 and R is ≤ 14, Cefamandole, S is ≥ 18, I is ≤ 15-17, and R is ≤ 14; for Cefadroxil, S is ≥ 18, I is ≤ 15-17, and R is ≤ 14; for Cefpodoxime, S is ≥ 21, I is ≤ 18-20 and R is ≤ 17, for Cefador, S is ≥ 18, I is ≤ 15-17 and R is ≤ 14; for Cefixime, S is ≥ 20, I is ≤ 17-19, and R is ≤ 16, for Cefepime, S is ≥ 18, I is ≤ 15-17 and R is ≤ 14.

Table 2: ANOVA for the resistance of *Pseudomonas aeruginosa* isolated from different sites to different generations of cephalosporin

Isolate site	Generation of Cephalosporins						
	Second			Third			Fourth
	CTX	MA	CEC	CPD	CFM	CAZ	FEP
Ear swab	3.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	1.52±0.00 ^a	1.26±0.00 ^a
Burns	2.97±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	1.37±0.00 ^a	1.07±0.00 ^a
Wound swab	3.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a	2.96±0.00 ^a	3.00±0.00 ^a	15.67±0.00 ^a	1.17±0.00 ^a

P=0.05; value=mean±standard mean error; values followed by the same alphabet are not significantly different according to Duncan's multiple range test. CTX=Cefotaxime, MA=Cefamandole, CEC=Cefador, CPD= Cefpodoxime, CFM= Cefixime, CAZ = Ceftazidime, FEP = Cefepime.

DISCUSSION

Cephalosporin is one of the most widely used antibiotics in the treatment of both gram-positive and gram-negative organisms.^[2,3] This study was conducted to evaluate the resistance activity of *Pseudomonas aeruginosa* obtained from clinical specimens against different generations of cephalosporin. In vitro activities of cephalosporins (Cefamandole, Cefaclor, Cefpodoxime, Cefixime, Ceftazidime and Cefepime) were tested using 100 isolates of *Pseudomonas aeruginosa*. This bacteria has been reported to develop resistance to antimicrobial agents both through accumulation of resistance genes on extra chromosomal genetic elements as well as through mutational processes.^[12,13,14,8] According to previous studies, emerging resistant strains of *Pseudomonas aeruginosa* are potentially linked to injudicious use of drugs, failure to complete therapeutic regimens and variation in the doses administered leading to ineffective empirical therapy which in turn leads to the appearance of even more resistant strains of the bacterium.^[5,9]

Pseudomonas aeruginosa isolates from different sites; wounds (n=47), ear swabs (n=30) and burns (n=23) were found to be similar to those obtained by Masaad^[15] and Rajat *et al.*^[16] The majority of the isolates (n = 47) were recovered from infected wounds which agrees with the reports of Masaad,^[15] Anuradha *et al.*,^[17] Oladipo *et al.*,^[2,5] Eyo *et al.*,^[1] Zafer *et al.*,^[7] and Shah *et al.*,^[9] It has been observed that *Pseudomonas aeruginosa* is more associated with wound infections when compared with other clinical sites.

In this study, *Pseudomonas aeruginosa* had high resistance to Cefotaxime (99%), Cefamandole (100%) and Cefaclor (100%) which are second generation cephalosporin. This study is comparable with a study by Smith *et al.*,^[18] and Tassios *et al.*,^[19] with 96% and 92% multidrug resistant isolates respectively. The 99% resistance of *Pseudomonas aeruginosa* to Cefotaxime does not agree with the reports of Manchi *et al.*,^[14] who recorded a resistance of 45% and 55% sensitivity; Anjum *et al.*,^[20] who recorded a resistance of 73%, 18% intermediate and 9% sensitivity and Prakash *et al.*,^[4] who recorded a resistance of 60.47% this could be as a result of access and usage of antibiotics in this community. However, the intermediate activity against *Pseudomonas aeruginosa* agrees with the comparative study of Ahmad *et al.*^[21]

Also, the resistance (6.0%) and sensitivity (90%) of *Pseudomonas aeruginosa* to Cefepime in this study does not correlate with the study of Manchi *et al.*,^[14] who recorded equal sensitivity and resistance of 50%. This suggests that the organism have developed resistance with the passage of time as explained by Chaudhary and Payasi.^[22] This statement explains the disagreement between this present study and the reports of Manchi *et al.*,^[14] and Shah *et al.*,^[9] about the resistance of *Pseudomonas aeruginosa* to Ceftazidime which is 21% to 45% and 45.5% respectively.

In a study conducted by Jazani *et al.*^[23] on antibiotic resistance of Cefepime, it has shown 75.4% resistance, 22.4% intermediate resistance and 2.1% sensitivity. However, in this present study, the observations were comparatively less similar as 6.0% resistance, 4.0% intermediate resistance and 90% sensitivity was seen. This result also disagrees with the reports of Prakash *et al.*^[4] who recorded a resistance of 65.26%, Oladipo *et al.*,^[2] who reported a 34.38% sensitivity, 6.25% intermediate and 59.37% resistance as well as Shah *et al.*,^[9] who gave a 63.9% resistance. This suggests that the organism have developed resistance with the passage of time as well as inappropriate use of the antibiotics.

Of the tested isolates of *Pseudomonas aeruginosa* against third generation cephalosporin, Cefpodoxime and Cefixime showed high resistance of 99% and 100% respectively, Ceftazidime showed significant sensitivity with 76%. This result confirmed the findings of Masaad,^[15] Kechrid and Hassen,^[24] and Ramalingam *et al.*^[8] who found the activity of third generation cephalosporin on *Pseudomonas aeruginosa* to be 97%. The resistance to second generation cephalosporin could be as a result of drug abuse since they are cheaper as compared to the third and fourth generations. According to Manchi *et al.*,^[14] third and fourth generation cephalosporins have better sensitivity when compared to first and second generation as shown from the study carried out in India which agrees with this study. The result also revealed a high sensitivity to Cefepime, a fourth generation, with 90%. This agrees with the result obtained from the studies of Pichichero^[25] and Eyo *et al.*,^[1] that newer generations of cephalosporin have significant antimicrobial properties than the preceding generations. From the result obtained from this study, Cefepime is considered as empirical therapy of first choice for the treatment of

infections caused by *Pseudomonas aeruginosa* as less resistance was shown.

Analysis from this study showed that a significant difference was observed in the mean effect of fourth generation cephalosporin (Cefepime) on *Pseudomonas aeruginosa* on the isolates from ear swab, burn and wound, with mean values of 1.26, 1.07 and 1.17 respectively. This may be due to the ability of *Pseudomonas aeruginosa* obtained from different sites to produce beta-lactamase that reduces the activity of cephalosporin. This result agrees with the report of Pichichero.^[25] However, it disagrees with the report of Oladipo *et al.*,^[2,3] with mean effect of the antibiotics on the isolate from ear swab and wound to be 2.32 and 2.45 respectively. No significant difference was observed in the mean effects of both second and third generations cephalosporin used on the clinical isolates of *Pseudomonas aeruginosa*. This may be due to mutation in the genetic makeup of the bacteria and thus resistance.

CONCLUSION

Antibiotic resistance is threatening the accomplishment of health services and it has since been considered as a universal risk. Third and fourth generation cephalosporins could still be used if found appropriate based on culture and sensitivity despite high incidence of resistance of *Pseudomonas aeruginosa* to all the different generation of cephalosporins; however, indiscriminate use may lead to increased resistance. Diagnostic procedures should not only aim at identifying the pathogen but also determining its susceptibility to antibiotics before prescription. Furthermore, there should be a continued search for new diagnostic methods due to the complexity of multiresistant phenotypes and emergence of new resistance mechanisms. Also, essential prophylactic measures such as proper hygiene, reducing of the risk factors of nosocomial infections and evidence-based treatment, should be encouraged.

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doi: <http://dx.doi.org/10.14194/ijmbr.4.3.4>

How to cite this article: Oladipo E.K, Oloke J.K, Omomowo I.O., Oyeniran A.O, Awoyelu E.H, Ogundele S.O. Incidence of cephalosporin resistance among clinical isolates of *Pseudomonas aeruginosa* in Ibadan, South-Western Nigeria. *Int J Med Biomed Res* 2015;4(3):135-141

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