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Full Length Research Paper

Molecular characterization of multiple antibiotic-resistant *Pseudomonas aeruginosa* isolated from selected hospital fomites and hands of health care workers in Ondo, Nigeria

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The study reported the molecular characterization, antibiotic susceptibility profile and the nature of resistance genes in the multiple antibiotic resistant Pseudomonas aeruginosa isolated from selected hospital fomites and hands of health care workers in Ondo, Nigeria. Various fomites and hands of health care workers were swabbed for the detection of Pseudomonas aeruginosa. Each sample was cultured separately on MacConkey and Centrimide agar plates and incubated for 18-24h at 37oC. Pure isolates were obtained using Analytical Profile Index (API) 20E kit. Kirby Bauer's disc diffusion technique was used to decide the susceptibility of the pure isolates to known antibiotics. Resistant genes to 3 or more antibiotics were determined by Polymerase Chain Reaction (PCR) using appropriate primers. Two hundred Gram-negative bacterial isolates were recovered from 480 swab-stick samples analyzed out of which 54 were Pseudomonas aeruginosa. All the P. aeruginosa isolates showed total resistance to augmentin, cefixime and cefuroxime meanwhile 30 were resistant to nitrofuratoin, gentamycin 11, ceftazidime 7, ofloxacin 2 and ciprofloxacin 1. 31 (57.4%) were resistant to three or more classes of antibiotics. Out of the 12 representative isolates, 6 harboured blaCTX-M (585 bp) gene and were not susceptible to beta lactam antibiotics while 4 of the 7 Aminoglycoside (gentamycin) resistant isolates harboured aac-3-iv (286 bp) gene. In conclusion, different hospital fomites might be possible sources of nosocomial infections.

Key words: *P. aeruginosa*, antibiotic-resistance, hospital workers, Ondo, Nigeria.

INTRODUCTION

Nosocomial infections are hospital acquired infections and its severity is due to the level contamination of the hospital environment and the intrinsic characteristics of the organism. They are due to infections with organisms such as *Klebsiella* spp., *Shigella* spp., *Escherichia* coli, *Acinetobacter* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Enterococcus* spp., *Proteus* spp., *Salmonella* spp. and *Pseudomonas* spp (Moti et al., 2018).

A major cause of public health concern is the worldwide dissemination of multi- or extensively drug resistant 'high

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risk clones' of *P. aeruginosa*, which need urgent attention (Lopez-Causape et al., 2018; Horcajada et al., 2019). The routes of transmission of the pathogen include environmental and patient-to-patient contamination, hands of healthcare workers (after contact with contaminated fomites, and infected patient amongst others. It can also survive on dry fomites from 6 h to 6 month due to its rugged adaptability and survival ability (Pachori et al., 2019; Centre for Disease Control, 2020).

An emerging global health threat are infections caused by *P. aeruginosa*, which are life-threatening because of their mechanisms for survival, adaptation and resistance to multiple antibiotics classes (Moradali et al., 2017). Also, the organism's pathogenicity is related to the complexity of its genome and various virulence factors (Maurice et al., 2018).

Its survival mechanisms include quorum sensing, viable but not culturable state, biofilm formation and antibiotic resistance mechanism (Dey et al., 2019; Verderosa et al., 2019).

The ability of *P. aeruginosa* to colonize a lot of habitats, persistence and prevalence in health care settings and antimicrobial resistance is due to its ability to use diverse responsive mechanisms such as reduced permeability, degrading enzymes, active efflux and modification of the antimicrobial targets (Moradali et al., 2017). It is also innately resistant to a lot of anti-microbials because of its ability to prevent membrane penetration by antimicrobial molecules or to release them if penetration occurs. Some active antimicrobials include: some b-lactams (e.g. ceftolozane-tazobactam, piperacillin tazobactam, ceftazidime-avibactam, ceftazidime, imipenem, meropenem, cefepime and doripenem), fluoroquinolones (e.g. levofloxacin and ciprofloxacin), aminoglycosides (e.g. tobramycin, amikacin and gentamicin) and so on (European Centre for Disease Prevention and Control, 2018).

MATERIALS AND METHODS

Selection and collection of samples

Swab stick samples of fomites and hands of health care workers were collected from Mother and Child Hospital, Ondo and State Specialist Hospital, Ondo, Ondo State, Nigeria after approval of ethical clearance with a reference number (MCHO/06/15/002) by the research committee of the institutions.

Identification and characterization of bacterial isolates

Fomites samples were collected using sterile swap sticks and were immediately transported to the Laboratory for identification. Samples collected were streaked on Cetrimide agar for the isolation of *Pseudomonas aeruginosa*

Presumptive identification of isolates was done using colony morphology and Gram staining reaction. Pure isolates were furthered identified by biochemical tests, such as catalase, citrate, Methyl Red-Voges Proskaeur, motility and sugar fermentation test (Olutiola et al., 2000).

Antibiotic susceptibility test

The *P. aeruginosa* isolates that were susceptible to commonly used antibiotics were identified using disc diffusion method and the susceptibility test was interpreted following Clinical and Laboratory Standard Institute (CLSI, 2013) guidelines. Discs immersed into concentrations of different antibiotics (gentamycin, augmentin, ceftazidime, nitrofurantoin, ofloxacin, ciprofloxacin, cefixime and cefuroxime (Oxoid Ltd, UK) were carefully inserted on the inoculated Mueller –Hinton agar plate with the aid of sterile forceps and incubated for 18 to 24 h at 37^{o}C. The dimensions of inhibition were taken with a transparent calibrated ruler.

Amplication and detection of PCR products

Twelve representative multiple antibiotic resistant *P. aeruginosa* isolates were further examined to detect resistance genes Cefotaximase- Munich (*blaCTX-M)* and aminoglycoside 3-N-acetyltransferase *(aac3*-IV) using Polymerase Chain Reaction (PCR). Isolates were selected on the basis of their reaction to antibiotics (Colom et al., 2003; Van et al., 2008).

RESULTS

Occurrence of *Pseudomonas aeruginosa* cultured from hospital fomites and hands of health workers in Ondo

Table 1 shows the occurrence of *P. aeruginosa* cultured from hospital fomites and hands of medical personnel. Of the 54 *P. aeruginosa* isolates cultured, 19 (35%) were cultured from bed, trolley 15 (28%), door handles 5 (9%), wash hand basin 8 (15%), mattress 5 (9%), bed sheet 1 (2%) and health care worker 1 (2%). However, *P. aeruginosa* was not recovered from other hospital fomites such as incubator, drip stand, cupboard and kidney dish.

Antibiotic susceptibility profile of *Pseudomonas aeruginosa* cultured from hospital fomites and hands of health care workers in Ondo

The antibiotic susceptibility profile of *P. aeruginosa* to the various antibiotics tested (augmentin, ceftazidime, cefixime, cefuroxime, ciprofloxacin, gentamycin, nitrofuratoin and ofloxacin) is shown in Table 2.

Multiple antibiotic resistance patterns of *Pseudomonas aeruginosa* cultured from hospital fomites in Ondo

Multiple antibiotic resistance patterns of *P. aeruginosa* are represented in Table 3. Multiple antibiotic resistances were defined as resistance to at least 3 or more different classes of antibiotics. The classes of antibiotics used include Beta-lactams (augmentin, cefixime, ceftazidime and cefuroxime), Fluoroquinolones (ciprofloxacin and

Table 1. Occurrence of *P. aeruginosa* from Hospital Fomites and Hand of Health Care Worker in Ondo.

Sample	Number of Isolates (n)	Percentage of Isolates (n)%
Bed	19	35
Trolley	15	28
Wash hand basin	8	15
Mattress	5	9
Door handle	5	9
Bed sheet	1	2
Hand of health care worker	1	2
Incubator	-	-
Drip stand	-	-
Cupboard	-	-
Kidney dish	-	-
Total	54	100

Source: Authors

Table 2. Antibiotic susceptibility profile of *P. aeruginosa* cultured from Hospital Fomites and Hand of Health Care worker in Ondo.

Antibiotic (µg)	Abbreviation	No. of Isolates	Number of isolate occurrence (%)		
			Susceptibility	Intermediate	Resistant
Augmentin (30 µg)	AUG	31	0	0	31 (100)
Ceftazidime (30 µg)	CAZ	31	22 (71)	2 (6)	7 (23)
Cefixime (5 µg)	CXM	31	0	0	31 (100)
Cefuroxime (30 µg)	CRX	31	0	0	31 (100)
Ciprofloxacin (5 µg)	CPR	31	30 (97)	0	1 (3)
Gentamycin (10 µg)	GEN	31	20 (65)	0	11 (35)
Nitrofurantoin (300 µg)	NIT	31	1 (3)	0	30 (97)
Ofloxacin (5 µg)	OFL	31	29 (94)	0	2 (6)

Source: Authors

Table 3. Multiple antibiotic resistance pattern of *P. aeruginosa* cultured from hospital fomites and hand of health care worker in Ondo.

Isolate	No. of antibiotic class	Multiple antibiotic resistance pattern	Frequency
P. aeruginosa	5	AUG, CAZ, CRX, CXM, CPR, GEN, NIT, OFL	1
	5	AUG, CRX, CXM, GEN, NIT, OFL	1
	4	AUG, CRX, CXM, GEN, NIT,	2
	4	AUG, CAZ, CRX, CXM, GEN, NIT	6
	3	AUG, CAZ, CRX, CXM, GEN,	1
	3	AUG, CRX, CXM, NIT	20
		Total	31(100%)

Source: Authors

ofloxacin), Aminoglycosides (gentamycin) and Nitrofurans (nitrofurantoin). Thirty-one (57.4%) of the fifty-four *P. aeruginosa* isolates obtained in this study showed multiple resistance to at least three different classes of antibiotics. All the thirty one *P. aeruginosa* exhibited multiple antibiotic resistances, ranging from three to five

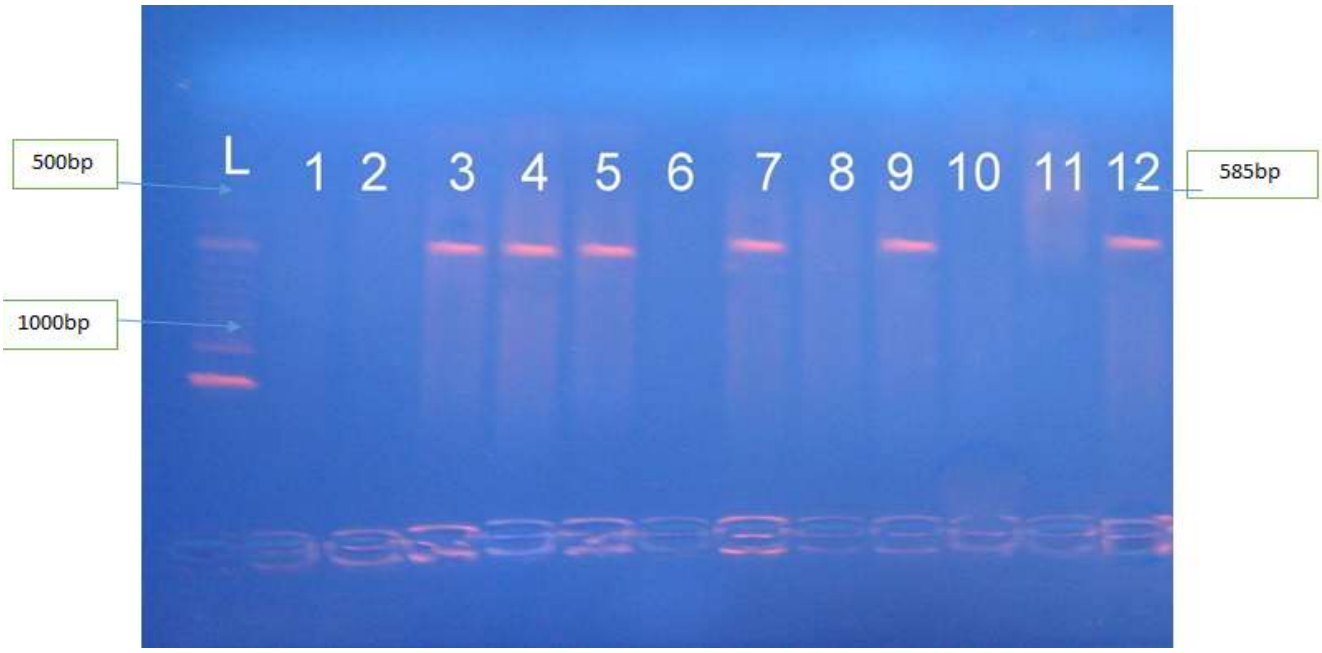

Plate 1. Agarose gel electrophoresis of the amplification product coding blaCTX-M (585 bp) gene in selected multiple antibiotic resistant *P. aeruginosa.* Lane L= DNA marker (100 bp), 3 = SPB1, 4 = SPB4, 5 = MmT17b, 7 = MND2, 9 = MCD5, 12=SPT10bp. Source: Authors

different classes. The *P. aeruginosa* isolates exhibited 6 different patterns with “GEN, CXM, AUG” appearing the most frequent.

Molecular Detection of *bla*CTX Resistance gene in *Pseudomonas aeruginosa*

Plate 1 shows the agarose gel electrophoresis of *bla* CTX-M (585 bp) gene in the 12 multiple antibiotic resistant (MAR) *P. aeruginosa* selected. Six of the beta-lactam antibiotics resistant isolates are depicted by Lanes 3, 4, 5, 7 and 9.

Molecular detection of *aac-3-iv* resistance gene in *Pseudomonas aeruginosa*

Plate 2 presents the MAR *P. aeruginosa* that harbor *aac-3-iv* (286 bp) gene. Four of the 7 representative isolates that were resistant to gentamycin antibiotics are depicted by Lanes 3, 4, 5 and 7 harboured *aac-3-iv* resistance gene of molecular weight of 286 bp.

DISCUSSION

The data obtained from this study revealed that some of the hospital fomites with which body have contact were contaminated with potential pathogens. The recovery of *P. aeruginosa* is an indication of gross contamination which calls for great concern considering the health risks of those that often have body contact with the fomites especially the immunocompromised patients. This is in line with some researchers (Hayden et al., 2006; Carling et al., 2008) who reported various disease causing organisms or nonpathogenic organisms can contaminate different surfaces and medical equipment often used in hospitals.

Thirty one (57.4%) of the 54 isolates of *P. aeruginosa* was multiple antibiotics resistant. Resistance was seen in three or more different classes of antibiotics. The trend of decreased antibiotic resistance observed in the study agrees with Lewis et al. (2012); Messadi et al. (2008) and Joseph et al. (2013) who earlier reported decreased resistance of *P. aeruginosa* to Ceftazidime and Ciprofloxacin but different from Senthamarai (2014), Mohanasundaram (2011) and Ibukun et al. (2007) where high resistance against Ceftazidime in their studies was reported.

Antibiotic resistance developed by pathogenic organisms is a global menace and has escalated over the years by the emergence of multi-drug resistant strains among these pathogens (Aslam et al., 2018). Development of resistance to antimicrobial agents by pathogens is a fitness trait acquired to survive in whatever environment they find themselves (Koskella et al., 2011). *P. aeruginosa* is known to exploit high level of intrinsic and acquired resistance mechanism to bombard quite a lot of antibiotics (Wilkie et al., 2021). A study suggests that the resistance of *P. aeruginosa* increases

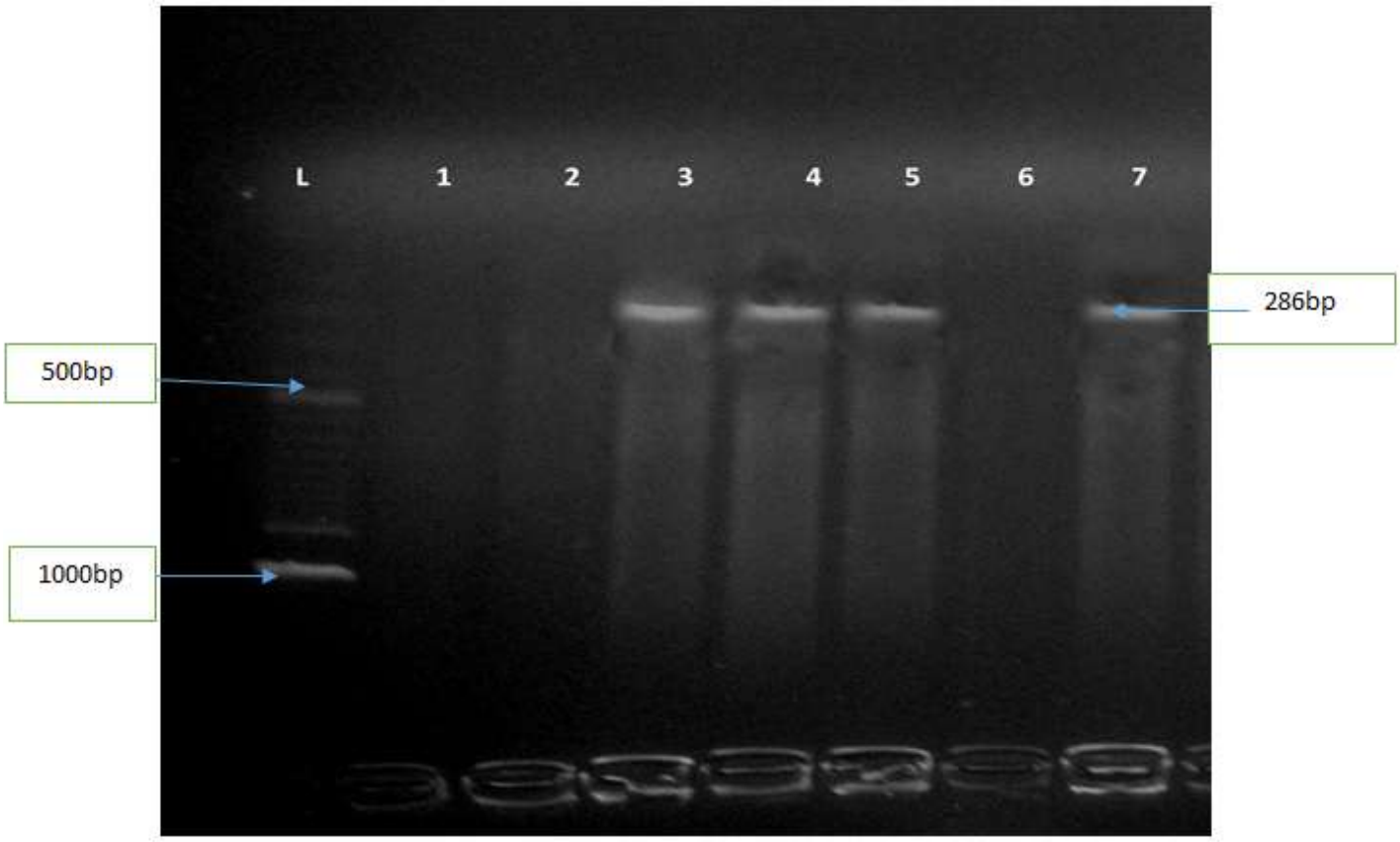

Plate 2. Agarose gel electrophoresis of the amplification product coding *acc-3-iv* (286 bp) gene in selected multiple antibiotic resistant *P. aeruginosa*. Lane L= DNA marker (100 bp), 3 = SPB1, 4 = SPB4, 5 = MmT17b, 7 =MND2.
Source: Authors

due to the uncontrolled usage and disposing of antibiotics in the environment. Treatment may fail to recover by constant contact of resistance isolates (Nasreen et al., 2015).

This study also revealed the detection of *bla*CTX-M (585 bp) and *aac-3-iv* (286 bp) resistance genes in *P. aeruginosa* isolates cultured from door handles of children and neo-natal wards in Mother and Child hospital and from beddings and trollies of post-natal ward in State Specialist hospital. The detection of *bla*CTX-M (585 bp) and *aac-3-iv (*286 bp) resistance genes, account for the resistance observed against beta-lactam group of antibiotics and gentamycin used, respectively. This agrees with Polotto et al. (2012) where *bla*CTX was detected in *P. aeruginosa* in his study.

Studies have revealed that unlike some exceptions, the CTX-M enzymes have nearly displaced other extended-spectrum Beta lactamase (ESBLs) enzymes in Enterobacteriaceae, including TEM and SHV ESBL variants (Cantón, 2008; Hawkey and Jones, 2009; Rodriguez-Villalobos et al., 2011). This displacement might have occurred not only as a consequence of the extraordinary dissemination of the corresponding *bla*CTX-M genes in highly mobilizable genetic platforms, including plasmids and transposons, but also because of these platforms within successful clones (Cantón and Coque, 2006; Rogers et al., 2011; Woodford et al., 2011). Another reason for this increase is the co-resistant phenomenon in CTX-M producing organisms, particularly to aminoglycosides and fluoroquinolones, which might facilitate co-selection processes (Morosini et al., 2006; Cantón and Ruiz-Garbajosa, 2011).

Apart from this general overview, within the CTX-M enzymes, the CTX-M-15 and CTX-M-14 are by far the most important ones, virtually invading all human and animal compartments as well as the environment all over the world (Cantón, 2008; Hawkey and Jones, 2009; Dolejska et al., 2011; Hiroi et al., 2012). Nevertheless, temporal emergence and penetration of these enzymes in different epidemiological scenarios might also explain the current epidemiology of CTX-M enzymes. Antibiotic consumption and dissimilar risk factors in different geographic areas and groups of patients and particularities of different compartments might have also contributed to the current CTX-M scenario (Carattoli, 2008; Rodríguez-Baño and Navarro, 2008; Rodríguez-

Baño and Pascual, 2008; Oteo et al., 2010a; Naseer and Sundsfjord, 2011).

Conclusion

This study showed that the different hospital fomites in the study location may be possible sources of nosocomial infections. It also revealed the presence of resistance genes (*bla*CTX-M, 585 bp and *aac-3-iv,* 286 bp) in the multiple antibiotic resistant *P. aeruginosa* isolates which accounted for the multiple antibiotic resistance observed. The susceptibility pattern of *P. aeruginosa* to ciprofloxacin (97%), ofloxacin (94%) and ceftazidime (71%) in this study showed the effectiveness of these drugs in the treatment of infections caused by *P. aeruginosa.*

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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