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RESEARCH ARTICLE **Open Access**

Prevalence of qacE AND qacEΔ1 resistance genes in pseudomonas species isolates from hospitals in Benin City, Nigeria

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Abstract

Background: The multi-drug efflux pump systems play a significant role in the mechanism of resistance to biocides in Pseudomonas species. This bacterium has been shown to harbor multi-drug transporter efflux systems genes involving QacE and QacEΔl, as do other gram negative bacteria. In Nigeria, the use of antimicrobial agents is unregulated, particularly the use of biocidal formulations both in health care facilities and for domestic purposes. Due to paucity of data on the distribution of qac genes in the health-care environment in Nigeria, this study aimed to determine the distribution of qacE and qacEΔ1 genes among Pseudomonads–both aeruginosa and non-aeruginosa isolates, from clinical and environmental sources within hospitals in Benin City.

Method: A total of 1200 specimens consisting of 500 clinical (wound, urine, ear swabs, high vaginal and endo-cervical swabs, eye swabs, aspirates, catheter tips, sputum and throat swabs) and 700 environmental (sinks, floors and bench tops) were used for this study. All specimens were processed to recover Pseudomonads using cetrimide agar. The isolates were identified with biochemical tests and 16S rRNA. The presence of qacE and qacEΔ1 genes was detected by PCR (polymerase chain reaction).

Result: Pseudomonas aeruginosa and non-aeruginosa isolates were recovered mostly from sinks and wounds. The prevalence of qacE and qacEΔ1 genes did not differ significantly ($p>0.05$) between P. aeruginosa and their non-aeruginosa counterparts in both clinical and environmental isolates. In conclusion, we highlight the prevalence of qacE and qacEΔ1 resistance genes in varying proportions among clinical and environmental pseudomonas species isolates in hospitals in Benin city , Nigeria

Keyword: QacE; QacEΔl genes; biocides; resistance; pseudomonas species.

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Introduction

Disinfectants used in eliminating microorganisms from inanimate surfaces, play an important role in infection control practices (Nona *et al.,* 2016). In healthcare setting, pathogenic bacteria with reduced susceptibility to disinfectants have been reported to exhibit increased resistance to antibiotics (Wales and Davies, 2015). The inappropriate use of disinfectants have been shown to result in resistance to both disinfectants and antibiotics(Gnanadhas, *et al* 2013).

The genus *Pseudomonas* is a metabolically versatile Gram negative bacterium ubiquitous in the environment (Davane, 2014). *Pseudomonas aeruginosa*: one of the most frequently isolated species of *Pseudomonas* accounting for over 80% of infections among hospitalized individuals (El Zowalaty *et al.,* 2015; Reynolds and Kollef, 2021), and a prominent causative agent of hospital acquired infections (Mohammadi *et al* 2020), with a high mortality rate reaching about 60% (Druge *et*

al., 2019; Mehri *et al,* 2013) especially among the immune-compromised individuals (Hernández-Jiménez, *et al* 2022).

Salman *et al*. (2024) have both reported the presence of the multidrug transporter efflux system genes qacE and qacEΔ1 in *Pseudomonas aeruginosa*, as well as other gram negative bacteria. Helaland khan (2015) have explained that *qacE*Δ1 is a defective form of *qacE;* a gene said to encode resistance to quaternary ammonium compounds (QAC). It is shown to be located in class 1 integrons, of Gram-negative bacteria (Rajabnia, 2013). *qacE* have been demonstrated as part of the 3′-CS in some integrons in Gram-negative bacteria (Helal and khan 2015). Bacteria harboring qac genes are resistant to disinfectants (Mahzounieh *et al*., 2014).

Infection control practice in Nigeria hospitals is very poor, which is thought to result to rise in nosocomial infections (Okonofua *et al*, 2015; Garba *et al.,* 2019). Poorly trained contract staffs that do not adhere to proper use of disinfectants are usually involved in disinfection practices (Olayinka *et al.,* 2014; Angus *et al.*, 2016).

In Nigeria, the use of antibacterial agents is unregulated (Ogbolu, 2013), and it may be considered a potent contributor in bacteria resistant to disinfectants. To our knowledge, no study has reported the prevalence of qac genes (*qacE* and *qacEΔ1*) in Nigeria. Due to paucity of data on the distribution of qac genes in the health-care environment in Nigeria, this study aims to demonstrate the prevalence of *qacE* and *qacEΔ1* resistance genes among *Pseudomonas* species in hospitals in Benin City, Nigeria.

Materials and Methods

For this study, a total of 1,200 swab specimens comprising of 700 environmental and 500 clinical specimens, respectively was used. Environmental samples were collected from designated sites (floors, table-tops and sinks) of the laboratory and wards of 3 health institutions (Central Hospital, Stela Obasanjo Women and Children Hospital, and University of Benin Teaching Hospital) in Benin City, Nigeria. With the help of sterile swabs that had been moistened with sterile peptone water, floor samples measuring 30 cm2 were taken. Likewise, swab samples were taken from sinks, washbasins, and tabletops; the samples were then brought to the microbiology laboratory for analysis. Similarly, clinical samples comprising of urine, sputum, aspirates, wound, and other swab specimen, taken from various anatomical sites was sent to the laboratory for processing.

Specimens were processed by standard techniques as reported by Koneman *et al*. (2006). Briefly clinical specimens were cultured on 0.03% cetrimide agar and incubated at 37^{o}C for 18 - 24 hours. The environmental specimens were inoculated onto sterile nutrient broth and incubated at 37^{o}C overnight. The overnight broth culture was inoculated onto 0.03% cetrimide agar and incubated at 37^{o}C for 24 – 48 hours.

All molecular analysis were carried out at the Nigeria Institute for Medical Research, Yaba, Lagos, Nigeria.

Bacterial identification

Bacterial DNA extraction was carried out according to method previously explained by Vingataramin and Frost, (2015).

The identity of all *Pseudomona*s isolates were confirmed by analysis of 16S rRNA following a modification of the method described by Spilker *et al* (2004). Identification of *Pseudomona*s isolates was carried out by polymerase chain reaction (PCR), using *Pseudomonas* genus specific primers PA-GS-F- (5'- GACGGGTGAGTAATGCCTA-3') and PA-GS-R (5′-CACTGGTGTTCCTTCCTATA-3'); and *Pseudomonas aeruginosa* specie specific (PA-SS) primers ,forward-(5'-GGGGGATCTTCGGACCTCA-3'), and Reverse (5'-TCCTTAGAGTGCCCACCCG-3'). Briefly, PCR reaction was carried out using the Solis Biodyne (Solis Biodyne, Estonia) 5X FIRE Pol Master Mix. The reaction concentration was reduced from 5x concentration to 1x concentration Master Mix buffer. PCR was performed in 25 µl reaction mixtures, containing 1.5 mM $MgCl_2$ (Solis Biodyne, Estonia), 200 µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne, Estonia), 25 pMol of each primer (Stab Vida, Portugal), 2.0 units of Hot FIRE Pol DNA polymerase (Solis Biodyne, Estonia), Proof reading Enzyme, 5 µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in a Techne Prime thermal cycler (Cole-Parmer, USA) for an initial denaturation for 2 minutes at 95°C, 35 cycles were completed, each consisting of 30 seconds at 94^{0}c , 30 seconds at 56°C, and 40 seconds at 72^{0}C. This was followed by a final extension step of 5 minutes at 72°C. Negative control PCR with all reaction mixture components excluding template DNA were included for every assay.

Agarose gel electrophoresis

The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100 bp DNA ladder (Solis Biodyne, Estonia) was used as DNA molecular weight standard.(Lee *et al*., 2012)

Detection of disinfectant resistance genes (qacE and qacEΔ1)

The NIMR Biotech DNA extraction kit, Lagos, Nigeria, was used to extract DNA using a spin column method, as directed by the manufacturer. Wang, *et al*. (2007) Description of the amplification of qac genes was followed. In summary, qacE resistance genes were identified through polymerase chain reaction (PCR) employing 10 pmol of specific qacE primers (Invitrogen, Grand Island, NY) for the forward (F-(5~-CCCGAATTCATGAAAGGCTGGCTT-3) and reverse (R-(5-TAAGCTTTCACCATGGCGTCGG-3) amplification of 350 bp fragments. With the exception of 10 pmol of the forward F-(5-TAGCGAGGGCTTTACTAAGC-3) and reverse specific R-(5-ATTCAGAATGCCGAACACCG-3) specific qacEΔ1 primers, which amplify 300 bp fragments, the qacE and qacEΔ1 genes were detected using the same PCR conditions. To perform the PCR, GoTaq Green Master Mix (Promega, Madison, WI) was used in the PCR assay. DNA was amplified according to the following conditions;120s of denaturation at 93°C followed by 35 cycles each of 30s at 93°C; 30s at 55°C; 60s at 72°C; and

finally, 5 min at 72°C. The PCR products were separated through a 1.5% agarose gel by electrophoresis.

Antibiotic Susceptibility Test: Antibiotic susceptibility test was carried out using the Kirby-Bauer diffusion test protocol. Zones of inhibition were read using the clinical Laboratory Standards Institute guidelines (CLSI, 2010). Briefly, isolates were tested against a panel of anti-pseudomonal antibiotics (Oxoid, UK) single discs. (Piperacillin/ Tazobactam110µg/10µg, Ciprofloxacin 30µg, Levofloxacin 30µg, Gentamycin10 µg, Amikacin 10 µg, Cefotaxime 30µg, Ceftaxidime 30µg and Imipenem 10µg) .

Discreet colonies of the respective isolates were emulsified in 3mls of U.V652nm physiological saline and turbidity matched with 0.5 McFarland's standard ($1.5x10^8$ cfu/ml), and was validated with Spectrophotometrically at 625nm wavelength

Previously prepared and dried Mueller – Hinton agar plates were seeded with each isolate per plate with the aid of a sterile swab stick.

Seeded plates were allowed to stand for 10 minutes; Antibiotic discs were aseptically placed onto the surface of the Agar plate following CLSI , (2010) protocol. Plates were allowed to stand for 25 minutes and were incubated at 37^0c for 18-24hrs 0

The zones of inhibition were measured in millimeters (mm), based on the zones of inhibition, a standard chart was employed to characterize bacterial isolates into Susceptible, and Resistance isolates.

Data analysis

Data obtained were analyzed with Chi square (X2) test using the statistical software INSTAT® (GraphPad Software Inc, La Jolla, CA, USA).

Results and Discussion

Out of the 700 environmental specimens that were processed, 99 (14.14%) different *Pseudomonas* species and 65 (9.29%) isolates of *Pseudomonas aeruginosa* were recovered (Table 1). When comparing sinks to other environmental sources, the recovery rate of *P. aeruginosa* ($p=0.0118$) and other *Pseudomonas* species ($p<0.0001$) was significantly higher (Table 1). Among clinical specimens, *P. aeruginosa* ($p<0.0001$) and other *Pseudomonas* species ($p=0.0002$) were mostly recovered from wound specimens (Table 2).

Plates 1A, 1B and 1C shows the gel electrophoresis for the detection of qacE (Plates 1A) and qacEΔ1 (Plate 1B), while Table 1C show the gel electrophoresis for *Pseudomonas aeruginosa*

Among clinical isolates, *Pseudomonas aeruginosa* have higher prevalence of *qacE* genes than other *Pseudomonas* species ($p<0.0001$). The reverse was the case for environmental isolates where other *Pseudomonas* species had significantly higher prevalence of *qacEΔ1* genes compared to *Pseudomonas aeruginosa* ($p=0.0106$). However, the prevalence of *qacE, qacEΔ1* and *qacE+qacEΔ1* did not differ significantly ($p>0.05$) between other *Pseudomonas* species and *P. aeruginosa* from both clinical and environmental settings (Table 3 and Table 4).

Table 1: Prevalence of *aeruginosa* and non-*aeruginosa* *Pseudomonas* isolates from Various environmental sources

Sources	No Tested	No with Pseudomonas aeruginosa (%)	No with Pseudomonas species (%)
Environmental (source)†			
Sink	220	31 (14.09)	50 (22.73)
Floor	350	24 (6.86)	32 (9.14)
Bench top	130	10 (7.69)	17 (13.08)
Subtotal	700	65(9.29)	99 (14.14)

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Table 2: Prevalence of *aeruginosa* and non-*aeruginosa* *Pseudomonas* isolates from various clinical specimen

Sources	No Tested	No with Pseudomonas aeruginosa (%)	No with Pseudomonas Species (%)
Wound swab	60	21 (35.00)	18 (30.00)
Urine	100	21 (20.00)	15 (15.00)
Ear swab	60	16 (28.33)	13 (23.33)
High vaginal swab	40	0(0.00)*	1 (2.50)
Endocervical swab	40	1 (2.50)	1 (2.50)*
Eye swab	40	2 (5.00)	0 (0.00)*
Aspirates	40	1(2.50)	0 (0.00)*
Catheter tip	40	4 (10.00)	2 (5.00)
Sputum	40	0 (0.00)*	0 (0.00)*
Throat swab	40	0 (0.00)*	2 (5.00)*
Subtotal	500	66 (13.20)	52 (10.40)

*= not included in analysis; †Source vs *Pseudomonas aeruginosa*: p=0.0118; Source vs other *Pseudomonas* species: p<0.0001; ‡Specimen vs Pseudomonas *aeruginosa*: p<0.0001; Specimen vs other *Pseudomonas* species:

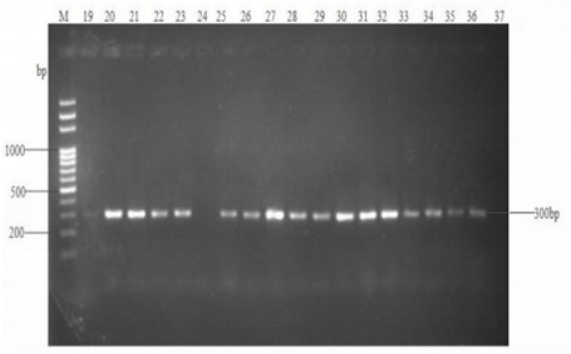

Plate 1A: Gel electrophoresis of PCR products for *qacE* gene detection

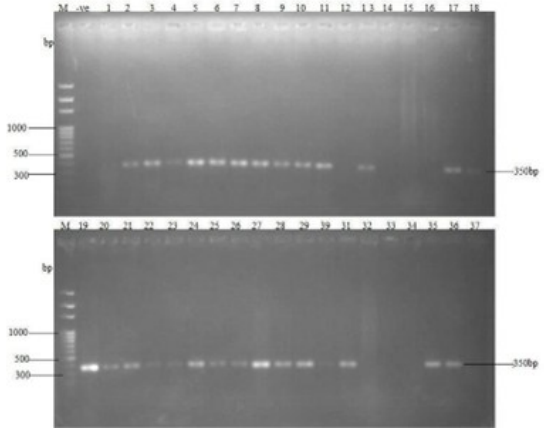

Plate 1B: Gel electrophoresis of PCR product of qacE delta1 gene detection in *Pseudomonas aeruginosa* clinical isolates on agarose gel electrophoresis (1.5%) showing the Lane (M) DNA marker (1000 bp), and negative control Lane (1-37).

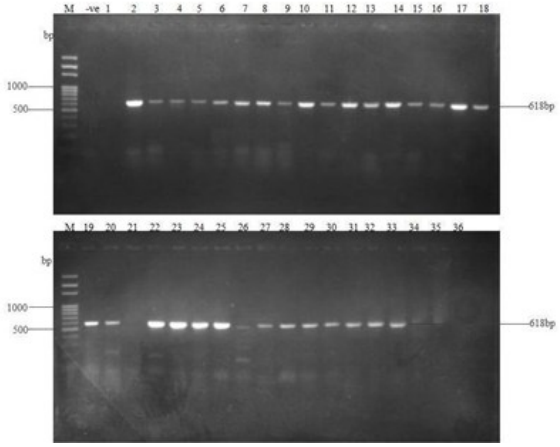

Plate. 1C Typical amplicon of the gene 16S rRNA product of *Pseudomonas aeruginosa* clinical isolates on agarose gel electrophoresis (1.5%) showing the Lane (M) DNA marker (1000 bp), Lane (1-36) represent positive isolates

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Table 3: Distribution of *qac* genes among *Pseudomonas* isolates

Type of qac genes	Pseudomonas aeruginosa	Pseudomonas species	OR	95%CI	P value
Clinical	(n=66)	(n= 52)			
QacE	22 (33.33)	10 (19.23)	2.100	0.890, 4.958	0.1330
QacEΔ1	10(15.15)	3 (5.77)	2.917	0.759. 11.209	0.1869
QacE+QacEΔ1	22 (33.33)	10 (19.23)	2.100	0.890, 4.958	0.1330
Sub-total	54 (81.82)	23 (44.23)	5.674	2.471, 13.029	<0.0001

n= number tested; OR= odd rati CI=confidencinterval

Table 4: Distribution of *qac* genes among *Pseudomonas* isolates

Type of qac genes	Pseudomonas aeruginosa	Pseudomonas species	OR	95%CI	P-Value
Environmental	n= 65	n= 99			
QacE	8 (12.31)	13 (13.13)	0.929	0.362, 2.383	0.8773
QacEΔ1	18 (27.69)	39 (39.39)	0.589	0.300, 1.159	0.1702
QacE+QacEΔ1	10 (15.38)	18 (18.18)	0.818	0.3512, 1.906	0.7999
Sub-total	36 (55.38)	70 (70.71)	0.390	0.197, 0.774	0.0106

n= number tested; OR= odd ratio; CI=confidencinterval

Discussion

Disinfectants used in eliminating microorganisms from inanimate surfaces, play an important role in infection control practices (Nona *et al.,* 2016). Infection control practice in Nigeria is very poor, which has led to rise in nosocomial infections (Okonofua *et al*, 2015; Garba *et al.,* 2019). In most Nigerian hospitals, poorly trained contract staffs that do not adhere to proper use of disinfectants are usually involved in disinfection practices (Olayinka *et al.,* 2014; Angus *et al.*, 2016). This inappropriate use of disinfectants may result in resistance to disinfectants.

A significant risk to the spread of health-care associated illnesses is posed by bacterial contamination of inanimate object and equipment (Rutala and Weber, 2019). Hospital acquired infections are frequently caused by bacterial pathogen that contaminates hospital equipment (Otter *et al.*, 2013). In this study, inanimate surfaces – sinks, floor, and bench-tops were colonized by microorganism (*Pseudomonas* species). The prevalence of *Pseudomonas aeruginosa* was significantly higher (p=0.0118) in sinks compared to other inanimate surfaces. This finding agrees with the reports of Ezeador *et al*. (2020) in Onitsha, Nigeria, Rodriguez *et al*. (2020) in Brazil and Asinobi *et al.* (2021) in Enugu, Nigeria. Sinks are one of the frequently handled hospital surfaces from which ESKAPEE (*Enterococcus feacalis,/faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa , Enterobacter species, and Escherichia coli*) pathogens that are associated with hospital acquired infections colonize (Leitner *et al.,* 2015; Suleiman *et al.*, 2018). This may explain why *Pseudomonas aeruginosa* and other species of *Pseudomonas* were recorded more from sink.

Among the clinical specimens analyzed in this study, wound swabs yielded the most isolates. This is not in agreement with previous reports (Tobin *et al*., 2021; Aika and Enato, 2022). The difference could be due to fact that in this study, *Pseudomonas* selective media was used to the recover *Pseudomonas*; while in the study of Tobi *et al* (2021) and Aika and Enato (2022), the clinical specimens were processed to recover other isolates. The prevalence of *Pseudomonas aeruginosa* (p<0.0001) and other *Pseudomonas* species (p=0.0002) was significantly higher in wound swabs compared to other clinical specimens. This is in agreement with previous reports (Adejobi *et al*., 2021; Ailka and Enato 2022), but it is not consistent with the finding of Gad *et al* (2007), and Olayinka *et al* (2014). The study of Olayinka *et al* (2014), included patients from urology all of whom were on catheter (catheter specimen urine) for a long time, which they opined as being responsible for high rate of *Pseudomonas aeruginosa* recovered from urine samples compared to wound. In this study mid-stream urine were collected and processed, this may explain the difference in the finding of this study and those of Olayinka *et al* (2014).

Generally, the prevalence of qac genes in clinical isolates of *Pseudomonas aeruginosa* is 81.82 %, while in clinical isolates of other *Pseudomonas* species was 44.23 %. Clinical isolates of *Pseudomonas aeruginosa* are significantly more likely to harbor *qacE* genes than other *Pseudomonas* species. (OR=5.674, 95 % CI=2,471 13.029; p=0.0001). The finding that qacE genes were higher among clinical isolates in this study does not agree with previous reports (Kazama *et al*., 1998; Kucken *et al*., 2000; Helal and Khan, 2015). The difference may be due to geographical location. The study of Kazama *et al* (1998) was undertaken in Japan, Kucken *et al* (2000) study was done in Germany and that of Helal and Khan (2015) was carried out in Egypt, while this study was carried out Nigeria. Also, disinfection practices may differ from country to country. Thus, the prevalence of different qac genes may also differ; this may explain the variance in this study.

Among environmental isolates, the prevalence of qac genes was significantly higher in other *Pseudomonas* species than in *Pseudomonas aeruginosa* (p=0.0106). There was no *non-aeruginonsa* clinical isolates in the study of Kazama *et al* (1998). Therefore comparison could not be made with this study. *qacEΔ1* genes were more prevalent than *qacE* genes in environmental *Pseudomonas* isolate. *qacEΔ1* genes have been reported in environmental isolates of *Pseudomonas aeruginosa* and other *Pseudomonas* species (Kazama *et al.*, 1998). No publication in Nigeria reporting the prevalence of qac genes was found, thus this finding may be the first in Nigeria. The prevalence of *qacE, qacEΔ1*and *qacE+qacEΔ1* did not differ significantly between clinical and environmental isolates of *Pseudomonas aeruginosa* and other *Pseudomonas* species. This may indicate that qac genes may be widely distributed in *Pseudomonas* species in this study.

Among environmental and clinical specimens *Pseudomonas* species were recovered mostly from sinks and wounds respectively. Clinical isolates of *P. aeruginosa* was approximately 6 times more likely to habour qac genes than their non-aeruginosa counterpart, while among environmental isolates the prevalence of qac genes was higher among non-aeruginosa isolates compared with *P. aeruginosa*. The presence of these genes may be responsible for the ineffectiveness of disinfectants.

There are conflicting reports on disinfectant use and antibiotic resistance. Some authors have reported that biocide resistance could lead to antibiotic resistance (Jia *et al.*, 2022), while others have opined that there are no conclusive evidence that links biocide resistance to antibiotic resistance (Boyce, 2023). Speculatively, the presence of qac genes in this study may lead to antibiotic resistance. However , further studies would be needed to confirm this

Conclusion

This study helps to demonstrate the prevalence of *qacE* and *qacEΔ1* resistance genes in varying proportions among *Pseudomonas* species isolates in hospitals in Benin City, Nigeria. Our findings demonstrate that quaternary ammonium compound (QAC) genes were most abundant in *P. aeruginosa* than other *Pseudomonas* species from clinical source. The reverse was the case among environmental isolates, where the prevalence of qac genes was more among other *Pseudomonas* species. Future studies should be carried out to determine the correlation of qac genes with microbial tolerance to disinfectants in this setting. It is advocated that proper use of disinfectants be implemented in healthcare institutions to stem the tide of high antimicrobial resistance.

Conflict of Interest

None declared.

Reference

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