



SJMLS - 5 (2) - 003

**Antibacterial susceptibility profile of *Pseudomonas* and related bacterial species associated with wound infections in Calabar, Nigeria**Ahaotu, C.E.<sup>1</sup>, Ibeneme, E.O.<sup>2,3\*</sup>, Utsalo, S.J.<sup>2</sup>Department of Medical Microbiology/Parasitology, Federal Neuropsychiatric Hospital, Calabar-Nigeria<sup>1</sup>, Department of Medical Laboratory Science, University of Calabar, Calabar-Nigeria<sup>2</sup>, Dr. Lawrence Henshaw Memorial Hospital Reference Laboratory, Calabar, Nigeria<sup>3</sup>.

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**Abstract**

The infection of wounds by *Pseudomonas* species has till date posed a great deal of medical concern, owing largely to the potential severity of their infections and high transmissibility of the pathogens, both in hospital settings and in the community. This study sought to identify the species of *Pseudomonas* and other related bacteria implicated in wound infections as well as provide therapeutic guidelines for the species so identified. Fifty presumptive *Pseudomonas* spp. were identified using simple microbiological identification procedures. Further identification using the API 20NE identification system was carried out. Antibiotic susceptibility pattern was determined by the modified Kirby-Bauer disk diffusion method against a panel of antimicrobials of standard strength. IBM SPSS version 20.0 was utilized for statistical analysis of data obtained and statistical significance was drawn at  $p \leq 0.05$ . Results show profound misidentification of these species by the use of simple manual microbiological identification methods alone, while revealing the high occurrence of *Burkholderia* in wound infections. As a result of the more advanced API 20NE<sup>®</sup> identification method employed, it was noted that there was a high prevalence of *Burkholderia cepacia* across the age categories, and the infection of wounds peaked around age groups >1-10 years and 11-20 years, who are known to have more exposure to outdoor activities. Results showed that Imipenem produced the highest potency against all isolates whereas Ticarcillin/Clavulanic acid had no antibiotic activity on all isolated pathogens. This study emphasizes the need for the incorporation

of more advanced bacterial identification methods in our routine laboratory practice in order to forestall misdiagnosis. The study also highlights the higher occurrence of *Burkholderia cepacia* in wounds than *P. aeruginosa*.

**Keywords** *Burkholderia cepacia*, *Pseudomonas aeruginosa*, wound infections, Calabar, Nigeria

**Introduction**

Wounds occur when the intact skin covering the body is eroded at any particular area. Exposure of the underlying subcutaneous tissue, which provides a warm nutritious environment for microbes, encourages the colonization and proliferation of potential microbial pathogens, thereby resulting in infections (Bowler *et al.*, 2001). Colonization of wounds by bacteria and other micro-organisms cause a delay in wound healing, deterioration of the wound, prolonged hospital stays, high cost of treatment and susceptibility to opportunistic and nosocomial co-infections. *P. aeruginosa* accounts for a significant proportion of nosocomial infections (Hassan *et al.*, 2015).

The genus *Pseudomonas*, being an ecologically important group of bacteria are widespread in nature and possess elevated metabolic versatility owing to their complex enzymatic system (Lessie and Phibbs Jr., 1984). They are found in natural habitats like soil, fresh water and marine environment and so on, but have also been isolated from clinical instruments, hitherto aseptic solutions, cosmetics and medical products (Klausen *et al.*, 2003). The presence of *P. aeruginosa* in hospital environments is due to

its great physiological capacity to utilize a great array of organic substrates such as carbon and nitrogen sources, hence perpetrating its survival for long periods of time (Jiménez *et al.*, 2010).

The diversity of antimicrobial resistant pathogens, which are major causes of nosocomial infections, imposes a substantial burden on healthcare systems (Sirijan and Nitaya, 2016). Infections caused by *P. aeruginosa* are peculiarly problematic because the pathogen is known to utilize high levels of intrinsic and acquired resistance mechanisms to counter most antibiotics (Pang *et al.*, 2019). This advanced ability to resist antibiotics is due to its adaptive antibiotic resistance mechanisms which include biofilm-mediated resistance and formation of multidrug-tolerant persister cells, responsible for intractability and relapse of infections (Pang *et al.*, 2019). These resistance mechanisms arise as a result of mutations and the acquisition of new genes that promote the development of multidrug resistant (MDR), extremely-drug resistant (XDR) and Pan-drug resistant (PDR) pathogens, which are characteristics that induce high mortality rates due to the limited choice of antibiotics available for treatment (Frattaria *et al.*, 2019; Cohen, 2013; Lambert *et al.*, 2011; Combes *et al.*, 2006). Several studies have assessed the infection of wounds by *P. aeruginosa* and classified the bacteria as the most common microorganism isolated from chronic wounds (Davis and Brown, 2016). Likewise, a study in Nigeria conducted in Abia State University Teaching Hospital gave the prevalence rate of *P. aeruginosa* in surgical wounds as 32.9% (Nwachukwu *et al.*, 2009). Similarly, *P. aeruginosa* was the most frequent bacterial isolate from freshly-admitted and long-term admitted burn-wound patients at University of Benin Teaching Hospital where the prevalence stood at 62.7% and 37.3% respectively (Yah *et al.*, 2006).

Typing of bacterial strains is a necessary step towards infection control, prevention of community and nosocomial cross-infection and monitoring of antimicrobial therapy efficacy. The aim of this study was to assess the prevalence of *Pseudomonas* species and related

organisms implicated in wound infections in Calabar and provide informed antimicrobial chemotherapeutic guidelines for infections arising from these organisms.

## Materials and methods

### *Ethical considerations*

This study was conducted in accordance with the Helsinki Declaration of 1975 (as revised in 2000) and approved by the Medical Research Ethics Committee of the Cross-River State Government of Nigeria (Reference Number: CRS/MH/CGS/E-H/016/Vol.I/89). Written informed consent was obtained from study participants before any they were recruited to the study.

### **Study participants and sample collection**

Wound swabs were obtained from randomly-selected 242 male and female participants, who attended the University of Calabar Teaching Hospital and General Hospital, Calabar. Inclusion criteria were patients (either in- or out-patients) with wound infections who voluntarily agreed to participate in the study; patients who were not willing to partake in the study were excluded. Questionnaires were also administered to the study participants, or their parents/guardian, where applicable, to ascertain their demographic characteristics and clinical history.

### **Bacteriological examination of samples**

The obtained samples were taken to the laboratory where they were inoculated onto 5% blood, chocolate agar and Cysteine lactose electrolyte-deficient (CLED) agar. The cultured plates were thereafter incubated at 37°C for 24 hours. The blood and chocolate agar were incubated in 5% CO<sub>2</sub> incubator. Using the swabs, smears were made on clean, grease-free slides for direct Gram procedure. Preliminary identification of isolated pathogens was done using Gram staining technique, colonial appearance, haemolysis on Blood agar, pigment production, lactose-fermentation, distinctive grape-like odor of aminoacetophenone, cytochrome oxidase production, catalase production and growth at 42°C. Positive cultures were stored on nutrient agar slopes overlaid with liquid paraffin. From the stock cultures, species identification of the positive isolates was made

by use of the API 20NE identification system (Biomerieux, Craaponne, France). The results were read from the reading table provided by the manufacturers, while the identification was completed using the API Web identification software (Biomerieux, Craaponne, France).

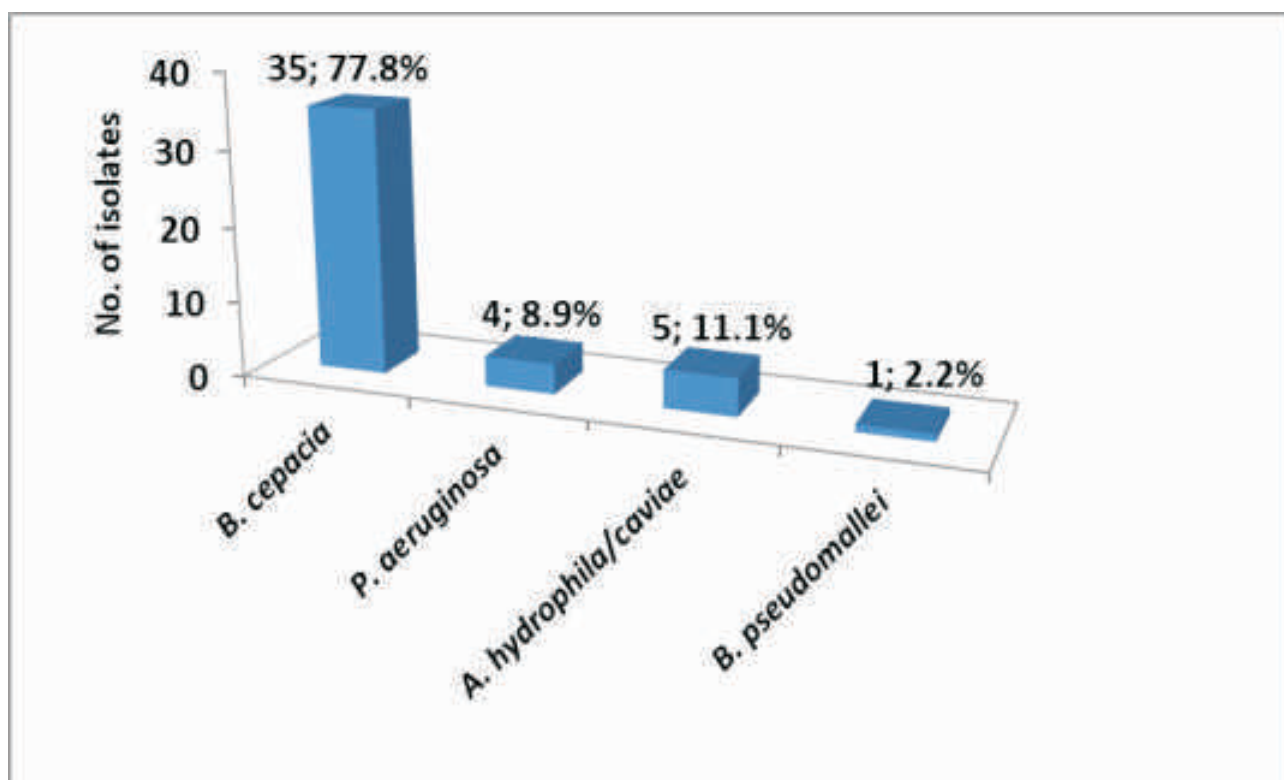
### Antibiotic Sensitivity testing

Antibiotic susceptibility testing was carried out using the modified Kirby-Bauer disk diffusion method and the inocula prepared using the direct colony suspension method as recommended by CLSI (2012). The isolates were first sub-cultured on nutrient agar from the stock cultures and incubated for 24 hours at 37°C in order to obtain discreet colonies. Two or three colonies were picked with sterile wire loop into 4ml of 0.9% NaCl solution in a sterile sample bottle and properly shaken to homogenize. The suspensions were then adjusted to a turbidity equivalent of 0.5 McFarland Standard. Within 15 minutes of the adjustment sterile cotton swabs

were dipped into each suspension and used to streak the Mueller Hinton agar plates evenly. The plates were allowed for about 3-5 minutes to absorb the culture, before application of the standard antibiotic discs with the aid of sterile forceps. The plates were thereafter incubated at 37°C for not less than 18 hours, after which the diameters of the resulting zones of inhibition were measured to the nearest whole millimeter with a ruler.

### Results

Figure 1 shows the frequency of the presumptive *Pseudomonas* spp. recovered from wound samples and further characterized using the API 20NE system. Five isolates were termed unacceptable, while of the remaining 45 isolates, only 4 (8.9%) were classified as *Pseudomonas aeruginosa*. Thirty-five (77.8%) were identified as *Burkholderia cepacia*, 5 (11.1%) as *Aeromonas hydrophila/caviae* and 1 (2.2%) as *Burkholderia pseudomallei*.



**Figure 1: Frequency of isolates characterized by API 20NE**

The isolates were obtained from 26 males and 19 females, as shown in Table 1. *Burkholderia cepacia* strains were isolated from 21 (60%) males and 14 (40%) females, whereas the *Pseudomonas aeruginosa* isolates were from 2 (50%) males and 2 (50%) females.

**Table 1: Gender-related frequency of infections by the isolates from wounds**

Gender	No. of participants	No of infections due to:			
		<i>B. cepacia</i> (n = 35)	<i>B. pseudomallei</i> (n = 1)	<i>A. hydrophila/caviae</i> (n = 5)	<i>P. aeruginosa</i> (n = 4)
Male	26	21 (80.8)	1 (3.8)	2 (7.7)	2 (7.7)
Female	19	14 (73.7)	0 (0.0)	3 (15.8)	2 (10.5)

**Table 2: Age-specific prevalence of infections by isolates from wounds**

Age (years)	No. of subjects (n = 45)	No (%) of subjects infected by:			
		<i>B. cepacia</i> (n = 35)	<i>B. pseudomallei</i> (n = 1)	<i>A. hydrophila/caviae</i> (n = 5)	<i>P. aeruginosa</i> (n = 4)
< 1 – 10	9	9 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
11 – 20	9	5 (56.0)	1 (11.1)	1 (11.1)	2 (22.2)
21 – 30	7	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
31 – 40	8	6 (75.0)	0 (0.0)	1 (12.5)	1 (12.5)
41 – 50	7	6 (85.7)	0 (0.0)	1 (14.3)	0 (0.0)
≥ 51	5	2 (40.0)	0 (0.0)	2 (40.0)	1 (20.0)

**Table 3: Comparative percentage susceptibility profiles of *Pseudomonas aeruginosa* and other bacteria isolated from wounds**

Antibiotics	Disc content	No. (%) sensitivity profiles isolates			
		<i>B. cepacia</i> (n = 35)	<i>P. aeruginosa</i> (n = 1)	<i>A. hydrophila/caviae</i> (n = 5)	<i>B. pseudomallei</i> (n = 4)
IPM	10	31 (88.6)	4 (100.0)	4 (80.0)	1 (100.0)
AK	30	24 (68.6)	3 (75.0)	3 (60.0)	1 (100.0)
PB	300iu	16 (45.7)	3 (75.0)	1 (20.0)	0 (0.0)
CAZ	30	13 (37.1)	3 (75.0)	2 (40.0)	0 (0.0)
CN	30	10 (28.6)	3 (75.0)	1 (20.0)	1 (100.0)
ATM	30	9 (25.7)	2 (50.0)	2 (40.0)	0 (0.0)
C	30	2 (5.7)	0 (0.0)	1 (20.0)	0 (0.0)
SXT	25	1 (2.9)	0 (0.0)	1 (20.0)	0 (0.0)
TIM	85	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)



**Key:** CAZ-Ceftazidime, C-Chloramphenicol, ATM-Aztreonam, PB-Polymyxin B, SXT-Sulfamethoxazole/Trimethoprim, IPM-Imipenem, AK-Amikacin, TIM-Ticarcillin/clavulanic acid, CN-Gentamicin

Of the 35 *Burkholderia cepacia* isolates tested, 31 (88.6%) were sensitive to Imipenem, 24 (68.6%) to Amikacin, 16 (45.7%) to Polymyxin B and none to Ticarcillin/clavulanic acid. All four of the *Pseudomonas aeruginosa* isolates were sensitive to Imipenem. Ceftazidime, Gentamicin, Amikacin, and polymyxin B, each produced antibacterial activity against 3 (75%) of the *P. aeruginosa* isolates, while none of the isolates was sensitive to chloramphenicol, Sulfamethoxazole/Trimethoprim and Ticarcillin/Clavulanic acid. Four (80%) of the five *A. hydrophila/caviae* isolates tested were sensitive to Imipenem, whereas 3 (60.0%), 2 (40.0%) and 2 (40.0%) showed sensitivity to Amikacin, Ceftazidime and Aztreonam respectively. None of the isolates was sensitive to Ticarcillin/Clavulanic acid. The *B. pseudomallei* isolate identified in this study showed sensitivity to Gentamicin, Imipenem and Amikacin only.

## Discussion

A survey sponsored by the World Health Organization demonstrated an average prevalence of nosocomial infections at 8.7%, with wound infections accounting for 0.5-15% of the total (World Health Organization, 2002). *Pseudomonas aeruginosa*, *Staphylococcus aureus* and beta-haemolytic Streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds (Bowler *et al.*, 2001) *Pseudomonas aeruginosa* being a common inhabitant of soil, water and vegetation (Karpecki and Shechtman, 2012) is a common nosocomial pathogen. It is constantly reintroduced into the hospital environment, and consequently spread to patients on the hands of hospital personnel as well as direct contact with contaminated reservoirs.

*Burkholderia cepacia*, formerly grouped under the genus *Pseudomonas*, has been identified as a diverse group of bacteria of evolving importance in individuals with compromised immunity, especially in patients with cystic fibrosis (Jones *et al.*, 2004) and Chronic Granulomatous

Disease (Shuvra *et al.*, 2018). However, an extensive research into its role in wound infections, to the best of my knowledge, has not been done, hence the essence of this study. Acquisition of this organism is either from the environment or through patient-to-patient transmission (Hopkins *et al.*, 2009; Ramsay *et al.*, 2013), hence the adoption of segregative policies (Leitao *et al.*, 2017). The possibility of patient-to-patient spread therefore necessitates prompt and accurate identification. The occurrence of *Burkholderia cepacia* in this study was 8.75 times more than that of *Pseudomonas aeruginosa*. This emphasizes the possible frequent misidentification of those isolates when only simple microbiological identification methods are utilized. It is also important to differentiate *B. cepacia* from *Pseudomonas aeruginosa* as *B. cepacia* complex has inherently contrasting susceptibility pattern to that of *P. aeruginosa* (Omar *et al.*, 2015). *B. cepacia* complex is ranked as the fourth most common pathogenic non-fermenting Gram-negative bacilli (NFGNB) after *P. aeruginosa*, *Acinetobacter calcoeceticus-baumannii* complex and *Stenotrophomonas maltophilia* (Gautam *et al.*, 2011). Since the effectiveness of therapies for *B. cepacia* infections is frustrated by their innate resistance to most antimicrobial agents, management efforts are therefore aimed at prevention of infection (Saiman *et al.*, 2014). The efficiency of the infection control measures however is determined by the accuracy with which *B. cepacia* is diagnosed (Rastogia *et al.*, 2019; McMenamin *et al.*, 2000).

*Aeromonas hydrophila/caviae*, another Gram-negative rod-shaped, facultative anaerobic, non-spore-producing bacterium has two divisions from soil and aquatic environments (Igbinsola *et al.*, 2012) believed to be of potential human health significance. Infections due to *Aeromonas* are typically acquired through ingestion of contaminated food or water, or through bacterial contact with broken skin (Hoel *et al.*, 2019). Wound infections with *Aeromonas* spp. were associated with contaminated soil and water-

related activities such as swimming, fishing etc, according to Miyagi *et al.* (2016). In a study by Lamy *et al.* (2009) in France, where cutaneous and pulmonary exposure to a potential *Aeromonas*-contaminated environment (water and soil) was recorded from the patients' medical histories, the infection was considered nosocomial in six (7%) cases, whereas wound infections consisted of 25 (74%) cases of purulent wound infections and 9 (26%) cases of soft tissue abscesses. The difference in prevalence from that gotten from this study would probably result from the brackish water found in Calabar, since *Aeromonas* spp. is found mainly in fresh water.

From the present study, the number of isolates obtained from in-patients is four times more (80%) than that obtained from out-patients (20%). *B. cepacia* were recovered from 29 (82.9%) and 6 (17.1%) in-patients and out-patients respectively. The recovery *P. aeruginosa* had a ratio of 3:1 (75%:25%) for in- and out-patients respectively. Several factors which may have contributed to the dominance of these organisms in the hospitalized participants of this study include: survival in an environment (hospital) with high contamination from spillages, as well as the use and reuse of antimicrobials or disinfectants which encourages development of resistant strains. Additionally, regular interactions with staff and patients as well as fomite transmissions enhance the spread of these infectious strains, especially considering their ubiquitous nature which enables them to access tissues with compromised skin integrity (Steer *et al.*, 1996).

### Conclusion

This study brought to the fore the prominent role played by *Burkholderia* spp. in wound infections, which hitherto had been reported as being most implicated in cystic fibrosis and chronic granulomatous disease. The findings of this study in Calabar emphasize the need for the use of more sensitive and specific diagnostic methods in the assessment of wound infections. This is necessary because the incidence of other Gram-negative non-fermenting bacteria in wound infections, other than *Pseudomonas* spp. seem to be underestimated in this locality. There

could therefore be resultant mismanagement of cases leading to treatment failures and the likes. The study also revealed a higher prevalence of *B. cepacia* in wounds than *P. aeruginosa* in this locality. Extra-intestinal (in this case wound) infection by *Aeromonas* spp. was also identified and requires proper attention, given that Calabar is a riverine area where these organisms abound. All the isolates tested had multidrug resistant strains but Imipenem was the drug of choice for all the isolates.

### Conflict of interest

The authors declare no conflict of interest.

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**Citation:** Ahaotu, C.E., Ibeneme, E.O., Utsalo, S.J. Antibacterial susceptibility profile of *Pseudomonas* and related bacterial species associated with wound infections in Calabar, Nigeria. *Sokoto Journal of Medical Laboratory Science*; 5(2): 19 - 26.

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