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Assessment of Antibiotics Resistance Pattern of *Pseudomonas aeruginosa* isolated from Patients Admitted in selected Hospitals in Kebbi State, Nigeria

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Abstract

Pseudomonas aeruginosa is one of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*) that induce a dignified healthcare aggravation among hospital admitted patients. This results in prolonged hospital stays, which increase costs for healthcare providers and patients' families. Therefore, this study aimed to isolate *Pseudomonas aeruginosa* and evaluate its resistance patterns from patients admitted to selected hospitals in Kebbi State. A total of 185 clinical samples, including nasal intubation, urine catheters, and wound swabs, were obtained. The bacteria were isolated and identified following standard microbiological methods. Modified Kirby Bauer techniques was used to determine the susceptibility status of the isolates. Out of 185 clinical samples collected, 43 (23.2%) bacterial isolates yield positive and 13 (30.2%) of which were *P. aeruginosa* from the studied hospitals. Prevalence of *P. aeruginosa* was found to be higher among females 08 (61.5%). The age groups 6-11 years had the highest prevalence *P. aeruginosa* 07 (53.8%). *P. aeruginosa* was isolated most from wound swab samples, 07 (24.1%). The *Pseudomonas aeruginosa* isolates exhibit high level resistance (100%) to Amoxicillin/Clavulanic acid, Cefpodoxime, Cefepime, Cepotaxime and Meropenem and showed least resistant to Imepenem 05 (38.4%). The increasing resistance of *Pseudomonas aeruginosa* isolates to multiple antimicrobial agents that are currently considered as first- line agents for the treatment of *Pseudomonas aeruginosa* infections, this highlights the need for careful use of these agents and also suggests the need for careful and up-to-date monitoring of multidrug- resistant strains diffusion in the various health care facilities of the country. Treatment options should be guided by medical laboratory scientist via microscopy culture and sensitivity testing, as well as local epidemiological surveillance data.

Keywords: *Pseudomonas aeruginosa*, Antibiotics Resistance, Hospitals, Kebbi

INTRODUCTION

Pseudomonas comprises more than 140 species, most of which are saprophytic. More than 25 species are known to be associated with humans (Kerr *et al.*, 2009). Most *Pseudomonas* known to cause diseases in humans are associated with opportunistic infections. These include *P. aeruginosa, P. fluorescens, P. putida, P. cepacia, P. stutzeri, P. maltophilia,* and *P. putrefaciens* (Kerr *et al.*, 2009). The gram-negative, aerobic rod *Pseudomonas aeruginosa* does not form spores and can cause a range of illnesses in hosts that are immunocompetent or immunocompromised (Kerr *et al.*, 2009). *Pseudomonas aeruginosa* grow well at 35-37°C on culture media especially MacConkey agar (Sinha *et al.*, 2022). On blood agar, it forms colorless, non-hemolytic, shiny colonies, smooth in context with a diameter of 1-2 mm after 24 hours of incubation at 37°C. It produces blue-green colonies on MacConkey agar which are shiny and tomb-shaped, indicating its non-lactose fermenting ability (Lodise, 2016).

Additionally, this organism is capable of producing other water-soluble pigments like pyomelanin or pyorubrin, which give colonies their distinctive red or brown colors. However, no other species of gram-negative non-fermenters produces pyocyanin, which is helpful in identifying the organism (Moore, 2011). In hospitals, *Pseudomonas aeruginosa* can live on beds, curtains, walls, roofs, medical devices, equipment, medical staff, running water sinks, phones, door handles, hand sanitizer dispensers, carts, trash cans, and computers (Lodise, 2016)

Pseudomonas aeruginosa is a threatening and emerging public health problem worldwide particularly in sub Saharan nations, where Nigeria is found. It remains the main cause of mortality and morbidity in humans (Hirsch, 2010). It is included in the critical category of the World Health Organization's (WHO) priority list of bacterial pathogens for which research and development of new antibiotics are urgently needed (Tacconelli *et al.*, 2018). It is ranked 4th among the nosocomial pathogens and is difficult to treat owing to its resistance to most commercially available antibiotics (Khan *et al.*, 2015). *Pseudomonas aeruginosa* is a major cause of nosocomial infections, including urinary tract infections, respiratory tract infections, wound and burn infections, and bacteremia (Khalid *et al.*, 2017). These infections become severe when the patients is immune compromised (Wozniak *et al.*, 2003; Pagani *et al.*, 2004).

According to epidemiological research, bacterial infections caused by multidrug-resistant bacteria affect up to 700,000 people annually (Mancuso, *et al.*, 2012). The overall resistance of *Pseudomonas aeruginosa* isolated from European populations is 12.9% (Botelho *et al.*, 2019). It has been demonstrated that *Pseudomonas aeruginosa* is resistant to multiple antibiotics. Findings from (Chah *et al.*, 2003) showed that isolates of *Pseudomonas aeruginosa* were 80% resistance to cephalexin, lower than report by (Jombo *et al.*, 2008) which showed 98.9% resistivity, while that of (Ahmad, 2003) was slightly higher than that of (Chah *et al.*, 2003) which showed 88.5% resistance to cephalexin by *Pseudomonas aeruginosa*. A study carried out in Ibadan showed that co-trimoxazole were 90% resistance for *Pseudomonas aeruginosa* isolates which was higher than a report from (Chah *et al.*, 2003) which was 73% in a study carried out in Nsukka. Thus, gentamicin resistance for *Pseudomonas aeruginosa* was higher with 90% in a report by (Oni *et al.*, 2002) compare to the study carried out by (Chah *et al.*, 2003 and Jombo *et al.*, 2008) which showed 70% and 63.7% resistance. The resistivity of cefuroxime to *Pseudomonas aeruginosa* isolate were much higher with 90% in a report by Oni *et al.*, 2002 than a report by (Ahmad, 2003) which showed 61.7% resistivity to the isolates.

Pseudomonas species isolated in Ethiopia were shown to be susceptible to gentamicin (88%), kanamycin (72%), and Amoxicillin-clavulanic acid (84%) but resistant to all regularly used antibiotics (Ferede *et al.*, 2001; Jombo *et al.*, 2008). Additionally, owing bacterial rapid mutations and adaptation to develop antibiotic resistance, *Pseudomonas aeruginosa* infections are exceedingly difficult to treat (Blomquist, 2021). Hospital infections caused by *Pseudomonas aeruginosa* continue to lead to antibiotic resistance, which is a major healthcare problem (Haque *et al.*, 2018). Therefore, this study was carried out to investigate the prevalence and antibiotic susceptibility profile of *P. aeruginosa* isolated from patients admitted in selected hospitals in Kebbi State, Nigeria.

MATERIALS AND METHODS

Research area

The study was conducted in Kebbi State, which is situated in Nigeria's northwest geopolitical zone between latitudes 10°N and 30°N and longitudes 3°E and 6°E. There are 21 local government areas in the State with the postal code 860001. According to the 2018 projected population, the metropolis has a total population of 3,238,628 (based on the 2006 population census) distributed across 21 Local Government Areas, the majority of which were civil servants, traders, farmers, teachers and students. The study was conducted at two secondary hospitals within the state; Sir Yahaya Memorial Hospital Birnin Kebbi (SYMHBK) and Kebbi Medical Center Kalgo (KMCK). Every hospital in Kebbi State was positioned with a view to serving both rural and urban populations.

Sample size

The minimum sample size for the study was calculated using a standard calculation and using the prevalence from earlier studies.

$$N = \frac{Z^2 p(1-p)}{d^2}$$

In which

N is the number of samples (sample size).

Z = Standard normal deviation with a 95% confidence interval of 1.96

P = Prevalence from prior studies = 14.0% (Bashir *et al.*, 2019).

d = 0.05 as the maximum accepted error

Consequently, the sample size will be at least 200

$$N = \frac{(1.96)^2 x 14.0\%(1-14.0\%)}{(0.05)^2}$$

$$N = \frac{3.8416x0.14\,(1-0.14)}{0.0025}$$

$$N = \frac{03.8416x0.14x0.86}{0.0025}$$

N = 185

Inclusion Criteria

All patients with the age of 1-14 in the Intensive Care Unit (ICU), pediatric and surgical wards admitted, whose relatives consented to the study were included. Patients who underwent surgery, and were intubated, mechanically ventilated, and catheterized were also included.

Exclusion Criteria

Non-consenting patients or patients' relatives, patients spending less than 24 hours in the ICU, pediatric, surgical wards, and patients older than 14 years were excluded.

Ethical approval

The Kebbi State Ministry of Health, the Sir Yahaya Memorial Hospital Research Ethics Review Committee, and the Kebbi Medical Center Kalgo Research Ethics Review Committee all granted their respective approvals for this study with the following codes: MOH/KSREC/VOL.1/57, SMHBK/SUB/011/VOL, and KMCK/EC/07.

Sample collection and transportation

A total of one hundred and eighty-five (185) samples were collected according to the method described by Ibrahim *et al.*, (2018), wound swabs, urine collected via catheter, and nasal intubation from admitted patients. In accordance with the process outlined by Odoki *et al.* (2019), all samples were transported cold chain to the Post Graduate Microbiology Laboratory at Kebbi State University of Technology, Aliero.

***Pseudomonas aeruginosa* Isolation and identification**

Freshly prepared Citrimide agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India, M173) was inoculated with all samples (urine and swabs), and the plates were then incubated for 24 hours at 37°C. After incubation, non-lactose-fermenting isolates (shining blue-green colonies) on Citrimide agar were plated on nutrient agar media and incubated for another 24 hours at 37°C. Gram stain and common Biochemical tests like indole, catalase, coagulase, methyl red, oxidase, citrate utilization, urease, Vogesproskauer, motility, and the triple sugar iron test were used to further identify the pure isolates on the nutrient agar plate (Cheesbrough, 2010).

Antibiotics susceptibility testing

The disk diffusion using a modified Kirby-Bauer method was used to assess the isolates' susceptibility to six antibiotics namely; Amoxicillin/clavolanic acid, (30 g), Cefpodoxime, (10 g), Cefepime, (30 g), Cepotaxime, (30 g), Meropenem, (10 g), and Imepenem, (10 g) were placed on freshly prepared Mueller Hinton agar (Oxoid, UK) plates with a pure isolate suspension of 0.5 McFarland standard. Plates were left to stand at room temperature for 10 minutes, the plates where incubated for 24 hours at 37°C. The zones of inhibition were then measured in mm using measuring ruler and interpreted as described by CLSI (2022).

RESULTS

Occurrence of *P. aeruginosa* and other bacterial pathogens associated with health care associated infection (HCAIs) according to clinical samples.

Out of 43/185 (23.2%) isolates, *E. coli* was the most frequently identified organism 22 (51.1%) followed by *P. aeroginosa* and *K pneumonia* with the prevalence of 13 (30.2%) and 08 (18.6%) respectively (Table 1). The bacterial isolates were most commonly isolated from the wound swab samples, 29 (67.4%), followed by urine, 08 (18.6%) and the least was the nasal intubation, only 06 (13.9%) bacteria were isolated. For *P. aeruginosa* specifically, it was isolated most from the wound swab samples, 07 (24.1%) and was least isolated from the catheter, 02 (25.0%) as presented in Table 1.

Table 1: Distribution of *P. aeruginosa* and other bacterial pathogens associated with health care associated infection (HCAIs) according to clinical samples.

Organisms	Wound swab n(%)	Urine collected via catheter n (%)	Nasal intubation n (%)	Total n (%)
P. aeruginosa	7 (24.1)	2 (25.0)	4 (66.6)	13 (30.2)
E. coli	14 (48.2)	6 (75.0)	2 (33.3)	22 (51.1)
K. pneumonia	8 (27.5)	0 (0.0)	0 (0.0)	8 (18.6)
Total	29 (67.4)	8 (18.6)	6 (13.9)	43 (100.0)

Distribution of *P. aeruginosa* based on some demographic characteristics

Out of 185 samples collected, 43/185 (23.2%) bacterial isolates were obtained in which 13/43 (30.2%) was *Pseudomonas aeruginosa* using a phenotypic approach. According to the results of the prevalence of *Pseudomonas aeruginosa* in the study hospital, the prevalence of *P. aeruginosa* in SYMHBK was higher 7/13 (53.8%) than in KMCK with the prevalence of 6/13 (46.1%). Prevalence of *Pseudomonas aeruginosa* based on age of the research participants revealed that, 6-11 recorded the highest prevalence 7/13 (53.8%). Prevalence of *P. aeruginosa* based on gender revealed that, female had the highest prevalence 8/13 (61.5%) (Table 2).

Table 2: Distribution of *P. aeruginosa* based on some demographic characteristics

	SYMHBK	KMCK	
Ages (Years)	*P. aeruginosa n(%)*	*P. aeruginosa n (%)*	Total n (%)
1-5	0 (0.0)	1 (16.6)	1 (7.6)
6-11	4 (57.1)	3 (50.0)	7 (53.8)
12-14	3 (42.8)	2 (33.3)	5 (38.4)
Total	7 (53.8)	6 (46.1)	13 (100.0)
Sex			
Male	3 (42.8)	2 (33.3)	5 (38.4)
Female	4 (57.1)	4 (66.6)	8 (61.5)
Total	7 (53.8)	6 (46.1)	13 (100.0

Key: **SYMHBK**: Sir Yahaya Memorial Hospital Birnin Kebbi, **KMCK**: Kebbi Medical Center Kalgo.

Prevalence of *P. aeruginosa* according to samples sites based on the two studied hospitals

Out of 13 *P. aeruginosa* isolates, 9 (69.2%) and 4 (30.7%) were from Sir Yahaya Memorial Hospital Birnin Kebbi (SYMHBK) and Kebbi Medical Center Kalgo (KMCK), respectively. The prevalence of *P. aeruginosa* according to the sample site showed that, wound swab had the highest prevalence 55.5% from SYMHBK, while KMCK had the least 50.0%. The prevalence of *P. aeruginosa* according to Catheter urine showed that, SYMHBK had the highest prevalence 22.2% when compared to KMCK with 0.0%. The prevalence of *P. aeruginosa* according to Nasal intubation showed that, KMCK has the highest prevalence of 50.0% while SYMHBK had the least of 22.2% (Table 3).

Table 3: Prevalence of *P. aeruginosa* according to samples sites based on the two studied hospitals

	Hospitals		
Samples	SYMHBK n (%)	KMCK n (%)	Total n (%)
Wound swab	5 (55.5)	2 (50.0)	7 (53.8)
Catheter urine	2 (22.2)	0 (0.0)	2 (15.3)
Nasal intubation	2 (22.2)	2 (50.0)	4 (30.7)
Total	9 (69.2)	4 (30.7)	13 (100.0)

Key: SYMHBK: Sir Yahaya Memorial Hospital Birnin Kebbi, KMCK: Kebbi Medical Center Kalgo

Ward-wise distribution of *P. aeruginosa* base on the two studied Hospitals

Out of 13 *P. aeruginosa* isolates, 3 (23.0%) were isolated from intensive care unit (ICU) followed by pediatric medical ward (PMW) and pediatric surgical ward (PSW) with the prevalence of 7 (53.8%) and 3 (23.0%), respectively. Sir Yahaya Memorial Hospital Birnin Kebbi (SMHBK) recorded high number of *P. aeruginosa* isolated from different ward as compared to Kebbi Medical Center Kalgo (KMCK) with the prevalence rate of 10 (76.9%) and 3 (23.0%), respectively (Table 4).

Table 4: Ward wise distribution of *P. aeruginosa* base on the two studied Hospitals

Hospital ward	SYMHBK n (%)	KMCK n (%)	Total n (%)
ICU	2 (20.0)	1 (33.3)	3 (23.0)
PMW	5 (50.0)	2 (66.6)	7 (53.8)
PSW	3 (30.0)	0 (0.0)	3 (23.0)
Total	10 (76.9)	3 (23.0)	13 (100.0

Key: SYMHBK: Sir Yahaya Memorial Hospital Birnin Kebbi, KMCK: Kebbi Medical Center Kalgo, ICU: Intensive care unit, PMW: Pediatric medical ward, PSW: pediatric surgical ward.

Antibiotic susceptibility profile of *P. aeruginosa* to the tested antibiotics

Six different antibiotics from three different classes were used to investigate the antibiotic susceptibility profile of *P. aeruginosa* isolates. *P. aeruginosa* were (100.0%) resistant to Amoxicillin/Clavolanic acid, Cefpodoxime, Cefepime, Cepotaxime and Meropenem while it showed 38.4% resistant against Imepenem (Table 5).

Table 5: Antibiotics susceptibility pattern of *P. aeruginosa* using antibiotics belonging to different class

Antibiotics (µg)	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Amoxicillin/Clavolanic acid (30)	00 (0.0)	00 (0.0)	13 (100.0)
Cepodoxime (10)	00 (0.0)	00 (0.0)	13 (100.0)
Cefepime (30)	00 (0.0)	00 (0.0)	13 (100.0)
Cepotaxime (30)	00 (0.0)	00 (0.0)	13 (100.0)
Meropenem (10)	00 (0.0)	00 (0.0)	13 (100.0)
Imepenem (10)	06 (46.1)	02 (15.3)	05 (38.4)

DISCUSSION

Pseudomonas aeruginosa has emerged as one of the leading cause of hospital acquired infection (HAI) worldwide (Samad *et al.*, 2017). The prevalence of *P. aeruginosa* (30.2%) obtained in our study correspond with the findings of Bukholm *et al.*, (2002) who reported prevalence of *P. aeruginosa* infection (33.5%) from Intensive Care Unit department. However, our findings are higher than the prevalence reported in studies carried out in China by Wang *et al.*, (2005) who reported 9.0%, Similarly; Park *et al.*, (2011) reported 4.6% *P. aeruginosa* infection in Chosun University Hospital, Gwangju South Korea and (9.0%) was reported in Pakistan by Khan *et al.*, (2014).

Our finding is also higher compared to the findings of Hussain *et al.*, (2017) who reported 20.9% for *P. aeruginosa*. The observed prevalence was also higher than 14.5% obtained by Samad *et al.*, (2017), 13.9% by Qureshi *et al.*, (2018), and 9.0% by Khan *et al.*, (2014). Similarly, Ijaz *et al.*, (2019) reported a lower prevalence of 8.4% of *P. aeruginosa* from the ICU of a tertiary hospital in Pakistan. Also, Abootaleb *et al.*, (2011) reported a lower prevalence of 0.9% in Iran. However, Procop *et al.*, (2017) reported 38% prevalence of *P. aeruginosa* among casualties from the battlefields of Afghanistan and Iraq which is higher when compared with the prevalence of this study.

Prevalence of *P. aeruginosa* based on the age of the participants revealed that, the age of 6-11had the highest prevalence (53.8%). The immunity issues could be the reason for higher prevalence, long time hospitalization and use of invasive procedures which also raise the risk of infections by *P. aeruginosa*. The immunity issues was justified based on the Schober *et al.*, (2007) report, who published in their research that age group of 41-70 are more prompt to *P. aeruginosa*, this showed that, infection due to *P. aeruginosa* may occur in any age group.

Prevalence of *P. aeruginosa* based on the participants gender revealed that, female had the highest prevalence 66.6% and 57.1% at KMCK and SYMHBK respectively. This was aligned with finding of Samad *et al.*, (2017), who reported prevalence of (57.4%) among female participants. This finding is higher when compared with the study carried out in Alex Ekwue Federal University Teaching Hospital by Ikonomidis *et al.*, (2006), who reported the Prevalence of 39.3% in females. Furthermore, this finding is lower when compared with the study carried out in Federal Medical Center Abeokuta by Egwuenu *et al.*, (2018), who reported the prevalence of 73.2% in females.

The distribution of *P. aeruginosa* based on the clinical samples revealed, higher prevalence of 53.8% from wound swab samples which was in line with Hussain *et al.*, (2017), who reported the prevalence of 53.6% *P. aeruginosa* from wound swab.

Hence, this finding was contrary to the report by Qureshi *et al.*, (2018), who revealed that *P. aeruginosa* isolates were from ventilator machine 54.6% and intubated tube 23.5%. Recent study showed that, ward distribution of *P. aeruginosa* was higher from pediatric medical ward with the prevalence of 53.8%, the immunity issues could be the reason for higher prevalence in pediatric medical ward.

Most of the isolates in this research were resistant to widely used antibiotics such cefpodoxime, cefepime, cepotaxime, and meropenem. Sensitivity was only found to be present in Imepenem. In this study, *P. aeruginosa* showed 100% resistant to Amoxicillin/cluvanic acid, Cefpodoxime, Cefepime Cepotaxime and Meropenem, and 38.4% resistant to Imepenem. Carbapenems have been the drug of choice for the treatment of *Pseudomonas* infections, but unfortunately, carbapenem-resistant *Pseudomonas aeruginosa* is becoming the largest global health threat (Bukholm *et al.*, 2002). The present study observed Imipenem resistance rate of (38.4%) which is lower than 85.7% as reported by Ijaz *et al.*, (2019) and higher than 32% and 36% reported by (Wang *et al.*, (2010); Park *et al.*, 2010) respectively.

CONCLUSION

The increasing resistance of *Pseudomonas aeruginosa* isolates to multiple antimicrobial agents that are currently considered as first- line agents for the treatment of *Pseudomonas aeruginosa* infections, this highlights the need for careful use of these agents and also suggests the need for careful and up-to-date monitoring of multidrug- resistant strains diffusion in the various health care facilities of the country. Treatment options should be guided by medical laboratory scientist via microscopy culture and sensitivity testing, as well as local epidemiological surveillance data.

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