



Antibiotic susceptibility pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from some drinking wells in Ondo town southwest Nigeria

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ABSTRACT: Antibiotic-resistant bacteria (ARB) strains have become a global health threat. This study aimed to determine the antibiotic susceptibility pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from wells in Ondo town, Southwest Nigeria. Twenty-eight well water samples were analyzed for the presence of *K. pneumoniae* and *P. aeruginosa* by standard pour plate technique. The bacterial isolates were tested against eight commonly used antibiotics using Kirby Bauer disc diffusion method. The percentage occurrence of *K. pneumoniae* and *P. aeruginosa* in the well water samples were 17.86% and 21.43%, respectively. Two multi-drug resistant strains of *K. pneumoniae* were isolated, which were resistant to at least three classes of antibiotics. Fifty percent of the *P. aeruginosa* isolates were resistant to ceftazidime, cefuroxime, nitrofurantoin, and ampicillin. None of the isolates was fully susceptible to cefuroxime, but have all showed resistance to β -lactam (ceftazidime, cefuroxime augmentin, and ampicillin) antibiotics. Cefuroxime may not be effective an effective drug in the treatment of *K. pneumoniae* and *P. aeruginosa* implicated infections in these communities in Ondo. Also, the over-use of antibiotics should be discouraged in order to curtail the menace of antibiotic resistance.

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Poor access to safe drinking water is a major challenge in many developing countries and this has resulted in waterborne related illnesses and even death in some cases, especially among infants (Novelo *et al.*, 2018; WHO/UNICEF, 2010). In Nigeria, stream water is the main source of water supply in the rural areas, while hand-dug wells supply many communities in the urban centers. Individual owners of residential building in Nigeria construct their septic wells around the building, which are not always far away from the hand-dug wells that supply water to the house. Some of these hand-dug wells are prone to contaminations from septic wells and municipal waste effluents, and these are major repositories for pathogenic microorganisms. Coliforms are members of the family *Enterobacteriaceae*. Their presence in water could be used as indicator for the presence of some pathogenic microorganisms. The coliform group includes *Escherichia coli*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*. Although over the years, more attention has been given to antibiotic susceptibility pattern of *Escherichia coli* in well water, in recent studies, there has been an increase in the incidence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in drinking water sources in Nigeria (Ajayi and Agangan, 2011; Aboh *et al.*, 2015; Laniyan *et al.*,

2016; Bello *et al.*, 2017; Onuoha, 2017). Some strains of *Klebsiella pneumoniae* isolated from some surface water in Schleswig-Holstein, Germany have been described as equal virulence as clinical strains (Podschun *et al.*, 2001; Kumar and Shrutikirti, 2013). More than eighty percent of *Klebsiella pneumoniae* isolated from surface water of Tigris River within Baghdad Province were carrying CTX-M genes and were resistant to some antibiotics (Abd Al-kareem *et al.*, 2015). Barati *et al.* (2016) and Kumar and Shrutikirti (2013) reported the isolation of *Klebsiella pneumoniae* from environmental samples which could have some public health concerns, such as recurrent urinary tract infection, diarrhea, respiratory tract infection, primary liver abscess, wounds, and soft tissue infections. *Pseudomonas aeruginosa* is very ubiquitous in the environment and can cause serious infections such as pneumonia, liver abscess, urinary tract infection, and wound infection (Aljanaby, 2008). It has also been implicated in fatal cases of mastitis in humans and animals (Szita *et al.*, 2007). *Pseudomonas aeruginosa* forms biofilms in clinical and water facilities which are often resistant to many antibiotics (Aljanaby, 2008). Their resistance to antibiotics can be associated with their loss of membrane permeability and ability of efflux pumping of antimicrobial agents

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(Alnour, 2017). Virulent strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have been isolated from the aquatic environments in Malaysia and Iraq, respectively (Aljanaby, 2008; Barati *et al.*, 2016). Antibiotics are usually prescribed in cases of infections and contaminated water has been identified as one of the sources of growth and spread of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* which may colonize humans (Podschun *et al.*, 2001; Szita *et al.*, 2007). Antibiotic resistance is a global health concern and a serious threat in the treatment of infections (Zhang *et al.*, 2006; Roca *et al.*, 2015; Coleman, 2018; WHO, 2020). Many studies have been carried out to determine the presence and antibiotic susceptibility pattern of *E. coli* in wells in Nigeria, but there are only a few reports on *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In our previous study within communities in Ondo town, we reported high total heterotrophic bacteria and coliform counts. More than 60% of the wells sampled were positive for *E. coli*, of which 52.78% were resistant to at least one antibiotic (Aromolaran *et al.*, 2016). Therefore, this study was carried out, as a pilot study to determine the antibiotics susceptibility pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from wells in Ondo town, Southwest Nigeria.

MATERIALS AND METHODS

Study site and sample collection: A total of twenty-eight (28) water samples were collected from private hand-dug wells in various communities within Ondo town, Southwest Nigeria. The wells selected for this study were used primarily for domestic purposes, such as drinking, cooking, and bathing. The water samples were collected in sterile glass bottles, transported immediately to the laboratory, and analyzed within 6 h of collection.

Isolation and identification of bacterial isolates from well water: Isolation of bacteria from the water samples was carried out using the pour plate technique. The media used include nutrient agar, triple-sugar-iron agar, phenol-red-carbohydrate broth, and cysteine-lactose-electrolyte deficient (CLED) agar. After 24 h of incubation, the broth samples were sub-cultured on nutrient agar plates and incubated at 37°C for 24 h. Thereafter, discrete colonies were streak on sterile nutrient agar plates to obtain pure culture and biochemical tests were carried out on the isolates. The isolates were identified based on the results of the biochemical tests carried out. Further studies were carried out on isolates identified as *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Antibiotic susceptibility test: The bacteria isolates (*K. pneumoniae*, and *P. aeruginosa*) were subjected to

commonly used antibiotics in Nigeria using Kirby Bauer agar disc diffusion technique as described by Aromolaran and Badejo (2014) and Akinyemi *et al.* (2005). Pure colonies of bacterial isolates were streaked on sterile Muller Hinton agar (MHA) plates and incubated at 37°C for 24 h. The bacterial cells were harvested into sterile normal saline solution and standardized using 0.5 McFarland standards. The cultured cells were introduced on the surface of sterile MHA using sterile swab sticks and multi-disc antibiotics were placed on the culture media aseptically and incubated at 37°C for 24 h. The antibiotic discs used were ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), nitrofurantoin (300µg), ampicillin (10µg), and augmentin (30µg). The diameter of the zones of inhibition around each disc was measured after the incubation period and recorded.

RESULTS AND DISCUSSION

Based on the biochemical tests carried out, six *P. aeruginosa*, and five *K. pneumoniae* bacterial strains were isolated and identified from the well water samples within the study area. The percentage occurrence of *K. pneumoniae* and *P. aeruginosa* in the well samples was 17.86%, and 21.43%, respectively. This is consistent with the report of Bello *et al.* (2017) who observed an occurrence of *K. pneumoniae* (9.52%) and *P. aeruginosa* (23.81) in sachets and bottled water brands sold in Ondo town. Similarly, Venkatesan *et al.* (2014) also reported a high frequency of occurrence of *K. pneumoniae* (11.9%) and *P. aeruginosa* (28.6) in packaged drinking water sold in Chennai. Table 1 presents the biochemical characteristics of the isolates. *P. aeruginosa* were able to ferment glucose with the production of acid, while the *Klebsiella* species were able to utilize all the sugars tested. The result of this study implied that the bacterial isolates were resistant to some of the antibiotics (Table 2). None of the isolates was sensitive to cefuroxime, while four *K. pneumoniae* and three *P. aeruginosa* showed intermediate resistance. Table 3 showed the antibiotic-resistant pattern of the isolates to some commonly used antibiotics. All the isolates showed resistance to at least β -lactam class of antibiotics except one of the *P. aeruginosa*. Figure 1 represents the percentage resistance of *K. pneumoniae* and *P. aeruginosa* to the tested antibiotics. None of the *P. aeruginosa* isolated from the well water samples showed full resistance to ofloxacin and augmentin. At least one *Klebsiella* species was resistant to one of the antibiotics. None of the isolates was fully susceptible to cefuroxime, but have all showed resistance to β -lactam (ceftazidime, cefuroxime augmentin, and ampicillin) antibiotics (Table 4). Poonia *et al.* (2014)

reported that 57.5% of the bacteria isolated from natural water in rural communities of East Sikkim were resistant to ampicillin, which is similar to this study. About 60% of the *Klebsiella* species isolated in this study were resistant to gentamicin and ampicillin. *Klebsiella pneumoniae* is commonly implicated in re-occurring urinary tract infection (Kumar and

Shrutikirti, 2013; Barati *et al.*, 2016). Twenty percent of the *K. pneumoniae* isolated from the well water were resistant to ciprofloxacin, which is the most common antibiotic used in the treatment of UTI in Ondo town. The resistance could be due to the uncontrolled use of antibiotics, aiding the development of resistance by the bacteria.

Table 1: Biochemical characteristics of *P. aeruginosa* and *K. pneumoniae* isolated from well water samples

S/N	Biochemical tests	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
1	Grams reaction	-	-
2	Catalase	+	+
3	H ₂ S production	-	-
4	Urease	-	+
5	Oxidase	+	-
6	Sucrose	-	AG
7	Lactose	-	AG
8	Glucose	AG	AG
9	Mannitol	-	AG
10	Sorbitol	-	AG
11	Mannose	-	AG
12	Inositol	-	AG
13	Maltose	-	AG

Keys: + positive; - negative; A - Production of acid; G - Formation of gas

Table 2: Antibiotic susceptibility of *P. aeruginosa* and *K. pneumoniae* isolated from well water samples

Location code	Isolates	Zone of inhibition in millimeters							
		CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
OD2	<i>Klebsiella pneumoniae</i>	13(R)	15(I)	13(I)	21(S)	28(S)	11(R)	19(S)	17(S)
OD4	<i>Pseudomonas aeruginosa</i>	15(I)	0(R)	12(R)	21(S)	30(S)	28(S)	25(S)	0(R)
OD5	<i>Klebsiella pneumoniae</i>	17(I)	18(I)	21(S)	23(S)	12(R)	14(I)	18(S)	15(I)
OD7	<i>Klebsiella pneumoniae</i>	18(S)	14 (R)	0(R)	21(S)	15(I)	14(I)	14(R)	0(R)
OD8	<i>Pseudomonas aeruginosa</i>	14(R)	0(R)	19(S)	18(I)	15(I)	21(S)	0(R)	17(S)
OD12	<i>Klebsiella pneumoniae</i>	19(S)	15(I)	0(R)	17(I)	19(S)	21(S)	19(S)	0(R)
OD13	<i>Pseudomonas aeruginosa</i>	17(I)	12(R)	16(S)	15(R)	21(S)	17(I)	15(I)	0(R)
OD15	<i>Pseudomonas aeruginosa</i>	17(I)	18(I)	15(S)	14(R)	19(S)	16(I)	0(R)	15(I)
OD17	<i>Pseudomonas aeruginosa</i>	14(R)	17(I)	15(S)	19(I)	13(I)	15(I)	0(R)	0(R)
OD23	<i>Klebsiella pneumoniae</i>	17(I)	18(I)	0(R)	0(R)	15(I)	17(I)	19(S)	0(R)
OD28	<i>Pseudomonas aeruginosa</i>	14(R)	19(I)	21(S)	18(I)	15(I)	19(S)	18(S)	17(S)

Keys: CAZ - Cefazidime, CRX - Cefuroxime, GEN - Gentamicin, CPR - Ciprofloxacin, OFL - Ofloxacin, AUG; - Augmentin, NIT - Nitrofurantoin, AMP - Ampicillin; R - Resistant; S - Susceptible

Table 3: Antibiotic resistant pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from well water samples

Bacteria isolates	Resistant pattern (class of antibiotics)
<i>K. pneumoniae</i>	OFL (β-lactam)
	GEN, AMP (aminoglycosides, β-lactam)
	CAZ, AUG (β-lactam)
	GEN, CPR, AMP (aminoglycosides, fluoroquinolones, β-lactam)
	CRX, GEN, NIT, AMP (β-lactam, aminoglycosides, nitrofurantoin)
<i>P. aeruginosa</i>	CAZ (β-lactam)
	CPR, NIT (fluoroquinolones, nitrofurantoin)
	CAZ, NIT, AMP (β-lactam nitrofurantoin)
	CRX, CPR, AMP (β-lactam, fluoroquinolones)
	CRX, GEN, AMP (β-lactam, aminoglycosides)
	CAZ, CRX, NIT (β-lactam, nitrofurantoin)

Keys: CAZ - Cefazidime, CRX - Cefuroxime, GEN - Gentamicin, CPR - Ciprofloxacin, OFL - Ofloxacin, AUG; - Augmentin, NIT - Nitrofurantoin, AMP - Ampicillin

Fifty percent of the *Pseudomonas* species were resistant to ceftazidime, cefuroxime, nitrofurantoin, and ampicillin. Ampicillin and penicillin have been used in the treatment of wound infection for a very long time. The development of resistance to ampicillin by *P. aeruginosa* could also be attributed to extensive use (Lambert, 2002).

The overall antibiotic susceptibility and resistance profile of the bacteria isolates (*K. pneumoniae*, and *P. aeruginosa*) is presented in Table 4. None of the isolates was fully susceptible to cefuroxime. However, the isolates showed 54.5% sensitivity to nitrofurantoin, but 54.5% resistance to gentamicin and ofloxacin.

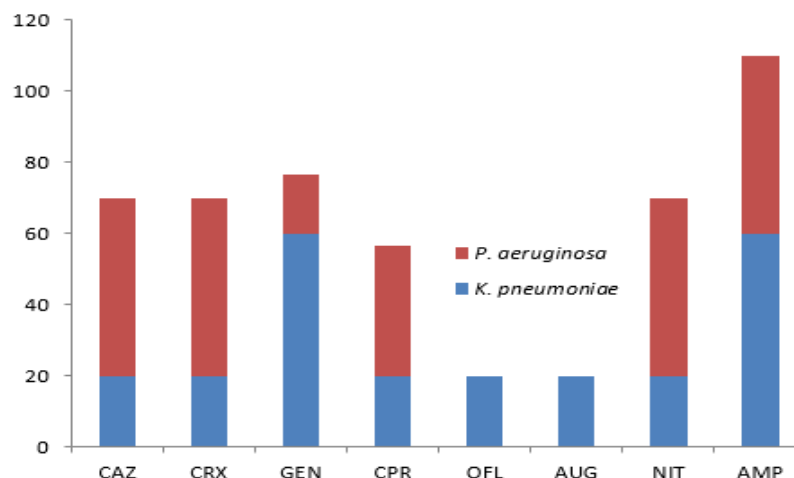


Fig 1: Percentage resistance of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to antibiotics

Table 4: Antibiotic susceptibility and resistant profile of *K. pneumoniae* and *P. aeruginosa* isolated from well water samples (N=11)

Antibiotics	Resistant isolates	Susceptible isolates
Nitrofurantoin	5 (45.5%)	6 (54.5%)
Ampicillin	8 (72.7%)	3 (27.3%)
Ceftazidime	9 (81.8%)	2 (19.2%)
Cefuroxime	11 (100%)	0 (0%)
Gentamicin	6 (54.5%)	5 (45.5%)
Ciprofloxacin	7 (63.6%)	4 (36.4%)
Ofloxacin	6 (54.5%)	5 (45.5%)
Augmentin	7 (63.6%)	4 (36.4%)

*Isolates showing intermediate-resistance were indicated as resistant isolate

The percentage occurrence of *K. pneumoniae*, *P. aeruginosa* in the well water samples was 17.86%, and 21.43%, respectively. None of the isolate was fully susceptible to cefuroxime, but have all showed resistance to β -lactam (ceftazidime, cefuroxime augmentin, and ampicillin) antibiotics. It can be inferred from this study that cefuroxime may not be effective in the treatment of *K. pneumoniae* and *P. aeruginosa* implicated infections in the study area.

Also, the release of antibiotic wastewater into the environment and the use of over-the-counter service without the appropriate prescription should be discouraged in our urban centers, so as to reduce the menace of drug resistance.

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