# Characterization and Identification of Antibiotic-resistant *Acinetobacter baumannii* from Hospital Wastewaters

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**Abstract** : The present study was designed to characterize antibiotic-resistant *Acinetobacter baumannii* from some selected hospital environments in Benin City, Nigeria. A total of sixty (60) wastewater samples (15 samples from each hospital) were collected from hospital drains in University of Benin Teaching Hospital (UBTH); Health Centre (University of Benin); Central Hospital Benin and Faith Mediplex, Benin City between January and March 2014. Standard culture-based methods and biochemical approach were used for identification of the bacterial strains. Antimicrobial susceptibility profiles of the isolates were carried out using disc diffusion method. The mean cell density of heterotrophic bacteria ranged from $2.41x10^6 \pm 0.03$ to $3.35x10^7 \pm 0.02$ cfu/ml while the mean *Acinetobacter* species count ranged from $2.05x10^3 \pm 0.01$ to $7.55x10^4 \pm 0.07$ cfu/ml. Antibiotic-resistant profiles of the isolates revealed that 67/72 (93.06%) were resistant to amoxicillin (Penicillins), 62/72 (86.11%) were resistant to gentamycin (Aminoglycosides), 69/72 (95.83%) were resistant to ampiclox ([3-lactam/p-lactamase inhibitors); while 5/72 (6.94%) were sensitive to cefuroxime (Cephalosporin II) and chloramphenicol (Phenicols). Multidrug-resistant profile revealed that 33/72 (45%) of the isolates were resistant to $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$, $CHL^R$, $PEF^R$, $CXM^R$, $TET^R$ with multiple antibiotic-resistant index of 0.9. The findings from this study revealed *A. baumannii* in the hospital effluent were resistant to clinically relevant antibiotics. The multiple antibiotic-resistant index suggest a potential risk to public health and the surrounding communities.

*Keywords: Acinetobacter baumannii,* hospital, health risk, multidrug-resistant, antibiotic.

## Introduction

The *Acinetobacter baumannii* is a Gram-negative, non-fermenting, aerobic, oxidase-negative, and non-motile coccobacilli nosocomial causing infections globally that are widespread in the environment (Bedenic *et al.*, 2015; Atrouni *et al.*, 2016). *Acinetobacter* spp. have been isolated from different environmental sources, including vegetables, soil, wastewater, water, humans and animals (Dahiru and Enabulele, 2015; Ece *et al.*, 2015; Atrouni *et al.*, 2016; Goic-Barisic *et al.*, 2016; Hrenovic *et al.*, 2016). A significant consideration was channelled to this genus since some of the members, particularly *A. baumannii* evolved as worldwide opportunistic pathogens, initiating some hospital outbreaks ranging from pneumonia to wound infections and bacteremia (Wei *et al.*, 2015; Goic-Barisic *et al.*, 2016; Maravic *et al.*, 2016).

Infections originating from antibiotic-resistant *A. baumannii* have an important impact on the morbidity and mortality of patients (Goic-Barisic *et al.*, 2016; Hrenovic *et al.*, 2016; Krahn *et al.*, 2016). In addition, *A. baumannii* tend to reveal notable ability to develop resistance to lots of frequently used classes of antibiotics, such as fluoroquinolones and aminoglycosides, culminating to a phenotype of multidrug-resistant strain and restricted alternatives for antibiotic therapy (Ece *et al.*, 2015; Jia *et al.*, 2015).

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Therapeutic options emanating from infections due to *Acinetobacter* species is a task due to relapses. Some resistance genes are described which can demonstrate genetic variance. Hence, the strain can acquire resistance to different antibiotics (Ece *et al.*, 2015; Peng *et al.*, 2015).

*Acinetobacter baumannii* has during the past three decades transpired to an infectious agent in hospitals and its environs worldwide (Jia *et al.*, 2015; Wei *et al.*, 2015). The encounter with *A. baumannii* is the capability to attain antimicrobial-resistance genes tremendously swiftly, culminating to multidrug resistance (Hrenovic *et al.*, 2016). As such, the occurrence of *Acinetobacter* has resulted in a public health task not only in a clinical environment but, also in a population with poor socioeconomic status quo (Dahiru and Enabulele, 2015; Kitti *et al.*, 2015). The aim of this study was to investigate the antibiotic resistance profile of *Acinetobacter baumanii* recovered from wastewater in a hospital environment in Benin City, Nigeria.

## Materials and Methods

### Collection of Sample

A total of sixty (60) wastewater samples from hospital drains were obtained from four different healthcare facilities in Benin metropolis, Nigeria. Fifteen (15) wastewater samples were obtained from each health care facility between January and March 2014. The healthcare facilities include University of Benin Teaching Hospital (UBTH); Health Centre

(University of Benin); Central Hospital, Benin City; and Faith Mediplex, Benin City, Nigeria.

*Isolation and Enumeration of Microorganisms from* Samples

One (1 ml) millilitre of wastewater samples was measured into a sterile test tube containing 9 ml of sterilized distilled water and serially diluted to the order of $10^{-1}$ to $10^{-10}$ dilution. Aliquot of 100 µl from each dilution was inoculated on nutrient agar (Lab M, United Kingdom) for the enumeration of heterotrophic bacteria and CHROMagar *Acinetobacter* (Oxoid, United Kingdom) for *Acinetobacter* species, both incubated at 37 °C for 24-48 h. The colony count on each plate of a given dilution was used to estimate the total count for the samples in colony forming units per millilitre (cfu/ml). Two to four distinct reddish colonies per plate on CHROMagar *Acinetobacter* were selected and purified on nutrient agar. The purified *Acinetobacter* species were stored on slants at 4 °C until ready for further characterization.

Characterization and Identification of *Acinetobacter* Isolates from Hospital Wastewater

Bacterial colonies were identified by standard microbiological culture-based tests which included Gram-staining, catalase testing (using 3% hydrogen peroxide), indole, oxidase, coagulase, citrate, urease, Voges-Proskauer, DNase test, sugar fermentation and the oxidation and fermentation of mannitol salt agar (Cheesbrough, 2000). All tests were performed according to standard guidelines. *Acinetobacter baumannii* ATCC1605 was used as a positive control for each test protocol.

**Antimicrobial Susceptibility Test** on *Acinetobacter baumannii* from Hospital Wastewater

All the identified *Acinetobacter baumannii* isolates were tested for resistance or sensitivity to different antibiotics using the standard disc diffusion method (Kirby Bauer test). For the disc diffusion assay, bacteria were grown for 18 and 24 h on Mueller-Hinton agar, harvested and then suspended in 0.85% sterile physiological saline solution adjusted to a 0.5 McFarland turbidity standard, corresponding to $10^6$ cfu/ml. The inoculum was streaked onto plates of Mueller-Hinton agar using a sterile cotton swab and impregnated with commercially available antibiotics discs. All *A. baumannii* isolates were tested against cephalosporin II (cefuroxime (30µg)), penicillins (penicillin (10 units); ampicilin (10µg); amoxicillin (10µg)), quinolones (nalidixic acid (30µg); perfloxacin (30µg)), tetracyclines (tetracycline (30µg)], phenicols (chloramphenicol (10µg)), β-Lactam/β-lactamase inhibitor combinations (ampiclox (10µg)), and aminoglycosides (gentamicin (10 µg)). Resistance patterns of the isolates against 10 different antibiotics (1 dose/disc), grouped into 7 different classes of antimicrobials were interpreted as resistant (R), intermediate resistant (I) or sensitive (S) in accordance with the recommended standard established by the Clinical Laboratory Standards Institute (CLSI, 2017).

*Statistical Analysis*

Data obtained in this study were analysed using the statistical software SPSS version 21.0 and Microsoft Excel 2013. One Way Analysis of Variance (ANOVA) and Duncan multiple range tests was used in analysing the data. The p-values that were less than 0.05 were considered statistically significant.

## Results

Isolation and identification of the *Acinetobacter baumannii* from hospital wastewater. Out of the 165 *Acinetobacter* species isolates that were purified and stored, 72/165 (43.6%) were identified as *Acinetobacter baumannii* based on the biochemical characteristics. The biochemical characterization of the isolates revealed that 72/165 (43.6%) isolates were non-motile, catalase positive and oxidase negative. Their capacity to ferment different sugars showed that the isolates fermented glucose, galactose, lactose, maltose and raffinose, while none of the isolates fermented sucrose and mannitol. In addition, the isolates were able to utilize citrate as sole carbon source, not able to reduce nitrate or react with methyl red as well as negative to Voges-Proskauer test.

**Population density of the heterotrophic bacteria and *Acinetobacter* species**

The population density of the heterotrophic and *Acinetobacter* species from hospital wastewater in this study is presented in Table 1. The aerobic bacterial counts from Central Hospital Benin ranged from $6.25\times10^5 \pm 0.00$ to $8.23\times10^9 \pm 0.01$ cfu/ml with a mean density of $3.15\times10^7 \pm 0.12$ cfu/ml; Faith Mediplex, Benin ranged from $5.00\times10^4 \pm 0.27$ to $6.35\times10^8 \pm 0.34$ cfu/ml with a mean density of $2.41\times10^6 \pm 0.03$ cfu/ml; Health Centre (UNIBEN) ranged from $5.22\times10^4 \pm 0.34$ to $1.21\times10^8 \pm 0.01$ cfu/ml with a mean density of $4.01\times10^6 \pm 0.42$ cfu/ml; UBTH ranged from $7.13\times10^6 \pm 0.00$ to $7.21\times10^9 \pm 0.04$ cfu/ml with a mean density of $3.35\times10^7 \pm 0.02$ cfu/ml.

The *Acinetobacter* species cell densities from Central Hospital Benin ranged from $1.98\times10^2 \pm 0.04$ to $9.60\times10^6 \pm 0.14$ cfu/ml with a mean density of $5.79\times10^4 \pm 0.04$ cfu/ml; Faith Mediplex, Benin ranged from $1.04\times10^1 \pm 0.12$ to $3.05\times10^5 \pm 0.06$ cfu/ml with a mean density of $2.05\times10^3 \pm 0.01$ cfu/ml; Health Centre (UNIBEN) ranged from 0 to $8.35\times10^5 \pm 0.07$ cfu/ml with a mean density of $4.78\times10^3 \pm 0.01$ cfu/ml; UBTH ranged from $5.45\times10^2 \pm 0.07$ to $9.55\times10^6 \pm 0.03$ cfu/ml with a mean density of $7.55\times10^4 \pm 0.07$ cfu/ml.

**Distribution of antimicrobial susceptibility profile of the *Acinetobacter baumannii* isolates**

The antimicrobial susceptibility profile of the bacterial isolates revealed that 17/22 (77%) of the isolates from UBTH were resistant to cefuroxime, amoxicillin, nalidixic acid, and gentamycin. More so,

isolates from UBTH were also 21/22 (96%) resistant to penicillin, 18/22 (82%) resistant to ampicillin, 14/22 (64%) resistant to perfloxacin, 11/22 (50%) resistant to tetracycline and chloramphenicol, and 19/22 (86%) resistant to ampiclox. The isolates from Faith Mediplex revealed 12/17 (71%) resistance to tetracycline and chloramphenicol; 11/17 (65%) resistant to nalidixic acid and perfloxacin, 17/17 (100%) resistant to amoxicillin, gentamycin and ampiclox. Of the isolates from Central Hospital were 22/22 (100%) resistant to cefuroxime, penicillin, amoxicillin, perfloxacin, tetracycline, and ampiclox. Also isolates from Health Centre were 22/22 (100%) resistant to cefuroxime, penicillin, amoxicillin, perfloxacin, tetracycline and ampiclox. The isolates from Health centre were 11/11 (100%) resistant to penicillin, amoxicillin, gentamycin and ampiclox (Table 2). Similarly, isolates from UBTH were 2/22 (9%) sensitive to cefuroxime and chloramphenicol. Isolates from Faith Mediplex were 3/17 (17%) sensitive to cefuroxime; Central Hospital were 2/22 (9%) sensitive to chloramphenicol and Health Centre were 1/11 (9%) sensitive to chloramphenicol.

Total percentage antibiotic profile of *Acinetobacter baumannii* from the hospital wastewater

The overall resistance profile of the *Acinetobacter baumannii* from the hospital wastewater is shown in Figure 1. It was observed that 57/72 (79.17%) were resistant to cefuroxime, 68/72 (94.44%) were resistant to penicillin, 49/72 (68.06%) were resistant to ampicillin, 67/72 (93.06%) were resistant to amoxicillin, 48/72 (66.67%) were resistant to nalidixic acid, 57/72 (79.17%) were resistant to perfloxacin, 50/72 (69.44%) were resistant to tetracycline, 46/72 (63.88%) were resistant to chloramphenicol, 62/72 (86.11%) were resistant to gentamycin, 69/72 (95.83%) were resistant to ampiclox. Hence, the antimicrobial susceptibility profile of the *A. baumannii* revealed that all the isolates were 46-100 % resistant to cephalosporin II (Cefuroxime), penicillins (Penicillin and Amoxicillin), quinolones (Nalidixic acid, perfloxacin), tetracyclines (Tetracycline), aminoglycosides (Gentamycin), and β-lactam/β-lactamase inhibitors (Ampiclox).

The overall sensitive profile revealed that 0/72 (0%) was sensitive to penicillin and amoxicillin (Penicillins), tetracycline (Tetracycline), gentamycin (Aminoglycosides), and ampiclox (β-lactam/β-lactamase inhibitors). In addition, 1/72 (1.38%) were sensitive to nalidixic acid and perfloxacin (Quinolones). More so, 2/72 (2.77%) were sensitive to ampicillin (Penicillins), while 5/72 (6.94%) were sensitive to cefuroxime (Cephalosporin II) and chloramphenicol (Phenicols).

Multidrug-resistant profile of the *Acinetobacter baumannii* isolated from hospital wastewater

Multidrug-resistant profile of the isolates revealed that 60/72 (83%) of the *Acinetobacter baumannii* isolates which comprised UBTH [16/22 (73%)], Faith Mediplex [17/17 (100%)], Central Hospital [16/22 (73%)], and Health Centre [11/11 (100%)] were resistant to $AMX^R$, $GEN^R$, $APX^R$ with a multiple antibiotic-resistant index of 0.3. Similarly, 33/72 (45%) of the *A. baumannii* isolates which comprised UBTH [9/22 (41%)], Faith Mediplex [9/17 (53%)], Central Hospital [10/22 (46%)], and Health Centre [5/11 (46%)] were resistant to $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$, $CHL^R$, $PEF^R$, $CXM^R$, $TET^R$ with a multiple antibiotic resistant index of 0.9 (Table 4).

**Table 1.** Population density of the *Acinetobacter* species and heterotrophic counts

| Population profile | Sample location | Minimum (cfu/ml) | Maximum (cfu/ml) | Mean (cfu/ml) |
|---|---|---|---|---|
| *Acinetobacter* species | Central Hospital Benin | $1.98\times10^{2}\pm0.04$ | $9.60\times10^{6}\pm0.14$ | $5.79\times10^{4}\pm0.04^{b}$ |
| | Faith Mediplex, Benin | $1.04\times10^{1}\pm0.12$ | $3.05\times10^{5}\pm0.06$ | $2.05\times10^{3}\pm0.01^{a}$ |
| | Health Centre [UNIBEN] | 0 | $8.35\times10^{5}\pm0.07$ | $4.78\times10^{3}\pm0.01^{a}$ |
| | UBTH | $5.45\times10^{2}\pm0.07$ | $9.55\times10^{6}\pm0.03$ | $7.55\times10^{4}\pm0.07^{b}$ |
| | *p*-value | | | 0.003 |
| Heterotrophic bacteria | Central Hospital Benin | $6.25\times10^{5}\pm0.00$ | $8.23\times10^{9}\pm0.01$ | $3.15\times10^{7}\pm0.12^{b}$ |
| | Faith Mediplex, Benin | $5.00\times10^{4}\pm0.27$ | $6.35\times10^{8}\pm0.34$ | $2.41\times10^{6}\pm0.03^{a}$ |
| | Health Centre [UNIBEN] | $5.22\times10^{4}\pm0.34$ | $1.21\times10^{8}\pm0.01$ | $4.01\times10^{6}\pm0.42^{a}$ |
| | UBTH | $7.13\times10^{6}\pm0.00$ | $7.21\times10^{9}\pm0.04$ | $3.35\times10^{7}\pm0.02^{b}$ |
| | *p*-value | | | 0.012 |

**Legend**: UNIBEN: University of Benin; UBTH: University of Benin Teaching Hospital. Mean values across columns which carry the same lowercase superscript alphabet show no significant difference ($p<0.05$).

**Table 2:** Distribution of antimicrobial susceptibility profile of the *Acinetobacter baumannii* isolates

| Antimicrobial Class | Antibiotics | UBTH (*n*=22) | | | Faith Mediplex (*n*=17) | | | Central Hospital (*n*=22) | | | Health Centre (*n*=11) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | S | I | R | S | I | R | S | I | R | S | I |
| Cephalosporin II | Cefuroxime (30µg) | 17 (77) | 2 (9) | 3 (14) | 13 (77) | 3 (17) | 1 (6) | 22 (100) | 0 (0) | 0 (0) | 5 (46) | 0 (0) | 6 (54) |
| Penicillins | Penicillin (10units) | 21 (96) | 0 (0) | 1 (4) | 14 (82) | 0 (0) | 3 (17) | 22 (100) | 0 (0) | 0 (0) | 11 (100) | 0 (0) | 0 (0) |
| | Ampicillin (10µg) | 18 (82) | 0 (0) | 4 (18) | 14 (82) | 1 (6) | 2 (12) | 17 (77) | 1 (5) | 4 (18) | 0 (0) | 0 (0) | 11 (100) |
| | Amoxicillin (10µg) | 17 (77) | 0 (0) | 5 (23) | 17 (100) | 0 (0) | 0 (0) | 22 (100) | 0 (0) | 0 (0) | 11 (100) | 0 (0) | 0 (0) |
| Quinolones | Nalidixic acid (30µg) | 17 (77) | 1 (5) | 4 (18) | 11 (65) | 0 (0) | 6 (35) | 14 (64) | 0 (0) | 8 (36) | 6 (55) | 0 (0) | 5 (46) |
| | Perfloxacin (30µg) | 14 (64) | 0 (0) | 8 (36) | 11 (65) | 1 (6) | 5 (29) | 22 (100) | 0 (0) | 0 (0) | 10 (91) | 0 (0) | 1 (9) |
| Tetracyclines | Tetracycline (30µg) | 11 (50) | 0 (0) | 11 (50) | 12 (71) | 0 (0) | 5 (29) | 22 (100) | 0 (0) | 0 (0) | 5 (46) | 0 (0) | 6 (54) |
| Phenicols | Chloramphenicol (30µg) | 11 (50) | 2 (9) | 9 (41) | 12 (71) | 0 (0) | 5 (29) | 17 (77) | 2 (9) | 3 (14) | 6 ( 5) | 1 (9) | 4 (36) |
| Aminoglycosides | Gentamycin (10µg) | 17 (77) | 0 (0) | 5 (23) | 17 (100) | 0 (0) | 0 (0) | 17 (77) | 0 (0) | 5 (23) | 11 (100) | 0 (0) | 0 (0) |
| β-lactam/β-lactamase inhibitors | Ampiclox (10µg) | 19 (86) | 0 (0) | 3 (14) | 17 (100) | 0 (0) | 0 (0) | 22 (100) | 0 (0) | 0 (0) | 11 (100) | 0 (0) | 0 (0) |

**Legend:** R: Resistant, S: Sensitive, I: Intermediate, *n*: Number of isolates, Values in parenthesis represent percentage (%)

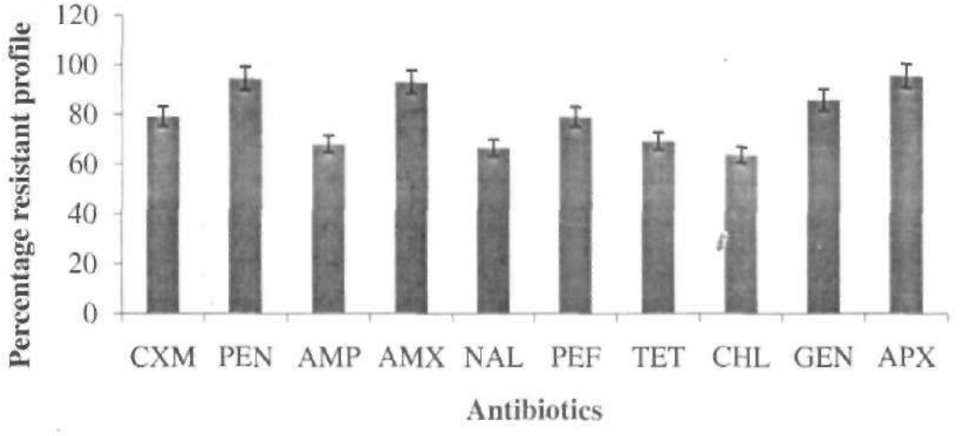


**Fig. 1:** Total percentage resistant profile of *Acinetobacter baumannii* from the hospital wastewater
**Legend:** CXM: Cefuroxime, PEN: Penicillin, AMP: Ampicillin, AMX: Amoxicillin, NAL: Nalidixic acid, PEF: Perfloxacin, TET: Tetracycline, CHL: Chloramphenicol, GEN: Gentamycin, APX: Ampiclox

**Table 3:** Multidrug resistant profile of the screened *Acinetobacter baumannii* isolates from hospital wastewater

| Antimicrobial Class | Resistant phenotype | UBTH (*n*=22) | Faith Mediplex (*n*=17) | Central Hospital (*n*=22) | Health Centre (*n*=11) | Total (*n*=72) | MAR index |
|---|---|---|---|---|---|---|---|
| 3 | $AMX^R$, $GEN^R$, $APX^R$ | 16 (73) | 17 (100) | 16 (73) | 11 (100) | 60 (83) | 0.3 |
| 3 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$ | 17 (77) | 13 (77) | 17 (77) | 11 (100) | 58 (80) | 0.4 |
| 4 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$ | 16 (73) | 10 (59) | 12 (55) | 5 (46) | 43 (59) | 0.5 |
| 4 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $PEF^R$ | 12 (55) | 9 (53) | 15 (68) | 9 (82) | 45 (62) | 0.5 |
| 5 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$, $CHL^R$ | 10 (46) | 10 (59) | 13 (59) | 5 (46) | 38 (52) | 0.6 |
| 5 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$, $CHL^R$, $PEF^R$ | 9 (41) | 10 (59) | 12 (55) | 5 (46) | 36 (50) | 0.7 |
| 6 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$, $CHL^R$, $PEF^R$, $CXM^R$ | 9 (41) | 8 (47) | 12 (55) | 5 (46) | 34 (47) | 0.8 |
| 7 | $PEN^R$, $GEN^R$, $APX^R$, $AMX^R$, $NAL^R$, $CHL^R$, $PEF^R$, $CXM^R$, $TET^R$ | 9 (41) | 9 (53) | 10 (46) | 5 (46) | 33 (45) | 0.9 |

**Legend:** CXM: Cefuroxime, PEN: Penicillin, AMX: Amoxicillin, NAL: Nalidixic acid, PEF: Perfloxacin, TET: Tetracycline, CHL: Chloramphenicol, GEN: Gentamycin, APX: Ampiclox, MAR: Multiple antibiotic resistant index. Values in parenthesis represent percentage.

**Discussion**

The development and selection of antibiotic-resistant bacteria are a potential concern as it correlates to the use of antibiotics in healthcare, food, animal and environmental sources (Kümmerer, 2003). These bacteria are able to spread their elements into the environment as a potential risk to public health. This present study has assessed the occurrence of multiple antibiotic-resistant *Acinetobacter baumannii* in hospital wastewater from Benin City, Nigeria. Factors that may affect the dissemination of microorganisms from one environment to another and cross-contamination rates are sources and destination surfaces, type of microorganisms, size of inoculum and humidity levels (Russotto *et al.*, 2015). However other factors that may play a role in the contamination and cross-contamination rate in hospital environments may include hygiene compliance, number of colonized or infected individuals in the hospital and adoption of antibiotic stewardship programs (Pittet *et al.*, 2006). The issue of environmental contamination may pose an even more serious threat where individuals are critically ill with several risk factors for nosocomial infection (Shih *et al.*, 2008). The heterotrophic cell densities and *Acinetobacter* population counts have been described previously by Zhang *et al.* (2009b) which was slightly lower than the cell densities in our study and could be attributed to geographical differences and environmental hygiene status. The infectious dose is the amount of pathogen (measured in a number of microorganisms) required to cause an infection in the host. Usually, it varies in accordance with the pathogenic agent, the patient age and overall well-being. Although there is currently no precise infectious dose for *A. baumannii*, cell densities as high as $10^4$-$10^6$ cfu/ml as reported in this study is suggestive of initiating or causing any possible infections.

*Acinetobacter genus* is found in many environments sources, including soil, water, food and sewage (Bedenic *et al.*, 2015; Dahiru and Enabulele, 2015; Ece *et al.*, 2015; Hrenovic *et al.*, 2016). At least 0.001% of the total culturable, heterotrophic cell densities in soil and water is estimated to be *Acinetobacter* spp. (Berlau *et al.*, 1999). *Acinetobacter* spp. have an unprecedented capacity to cultivate resistance to antimicrobial agents, and a phenomenal ability to develop new resistance gene determinant thereby portraying this genus principally suitable for monitoring antibiotic resistance in the environment (Guardabassi *et al.*, 1998; Zhang *et al.*, 2009b).

Hospital wastewater contains a significant amount of chemicals released from the hospital setting. The residues of pharmaceuticals can be detected in hospital wastewater, due to their inefficient preventive/control/disposal strategies in the conventional systems. Such residues comprise iodinated X-ray contrast media which are used for X-ray imaging of soft tissues, as well as non-prescription drugs mainly used in hospitals (Carballa *et al.* 2004). Besides recalcitrant and potent chemicals, hospitals wastewater harbours lots of undesired potentially pathogenic microbiota in the likes of antibiotic-resistant bacteria. Situations may arise where a total exclusion of emission from the hospital is required, for instance in the case of multiple antibiotic-resistant strains.

The findings from this study revealed that antibiotic resistance *A. baumannii* was high from hospital wastewater environments investigated. Although a high occurrence of antibiotic resistance we observed in a significant percentage of the isolates in this study; the isolates from this study were generally more resistant to antibiotics than levels reported for other environmental sources other than clinical environments (Dahiru and Enabulele, 2015; Maravić *et al.*, 2016), but consistent with other previous reports on clinical environments (Perilli *et al.*, 2014; Bedenic *et al.*, 2015). Since *A. baumannii* has a marked capacity to develop antibiotic resistance, along with the fact that antibiotics were comprehensively used in clinical settings, it was not unexpected that clinical isolates revealed a higher level of antibiotic resistance. Marked resistance by *A. baumannii* to the antibiotics used could be as a result of hydrolysis, altered target, efflux, phosphorylation, *nucleotidylation*, monooxygenation and acetylation (Davies and Davies, 2010).

Bacterial resistance to antibiotics reduces therapeutic regimens thus, increases morbidity and mortality, as well as an increase in the risk of antibiotic-associated adverse effects. Resistance is prevalent where antibiotics are severely used which was justified in this study with a MAR index (0.3-0.9). In addition, antibiotic-resistant bacteria are resident in surface water, wastewater, groundwater, soils and sediments, and significantly in aquatic environs (Baquero *et al.*, 2008; Zhang *et al.*, 2009a). Antibiotic applications select for novel resistance mutations and for prevailing resistance mechanisms. Resistance can also be developed via horizontal gene transfer through uptake of resistance genes as a result of transduction, transformation and conjugation (Thomas and Nielsen, 2005; Zhang *et al.*, 2009b). Only a little fraction of antibiotic compounds are partially biodegraded in aquatic ecosystems where most of them are persistent. The genotoxicity of compounds such as metronidazole or quinolones has been studied. Quinolones, for instance, adsorb intensely onto soils, sediments, and sewage sludge without easily been biodegraded (Kümmerer, 2003). The multi-drug resistant (MDR) *A. baumannii* strains cause difficulty in treatment. Therefore, molecular studies on clonal relationship and data on antimicrobial resistance are important in preventing epidemics and initiating effective therapy.

**Conclusion**

The findings from this study revealed that *Acinetobacter baumannii* present in the wastewater environment from hospital settings in Benin City, Nigeria, were resistant to significant numbers of

conventional antibiotics. High multiple antibiotic-resistant profiles observed in the isolates suggest that their presence in such environmental sources is a potential threat to public health. Thus, could serve as possible reservoirs for resistance genes transfer to other bacteria in the environment.

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