Antibiotics Resistance and Plasmid Profile of Clinical Bacterial Isolates Obtained from Brait-Whyte Memorial Specialist Hospital, Port Harcourt, Rivers State, Nigeria

Y. S. Wali¹, E. Effiong²* and N. N. Ndukwe³

¹Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.  
²Faculty of Sciences, University of Port Harcourt, Rivers State, Nigeria.  
³Faculty of Sciences, Federal University of Kashere, Gombe State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Author YSW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EE managed the design and analyses of the study. Author NNN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2019/v2i430078

Editor(s):
(1) Dr. Khadiga Ahmed Ismail Eltris, Professor, Ain Shams Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Reviewers:
(1) Dr. Syed Umer Jan, University of Balochistan, Pakistan.  
(2) Asit Kumar Chakraborty, Vidyasagar University, India.  
(3) Hjideharu Shintani, Chuo University, Japan.

Complete Peer review History: http://www.sdiarticle3.com/review-history/48920

Received 27 February 2019  
Accepted 13 May 2019  
Published 03 June 2019

Original Research Article

ABSTRACT

Antibiotics resistance pattern of bacterial isolates has caused both economic and societal loses to mankind. In this study, ethical approval and samples were sought and obtained from the Brait-Whyte Memorial specialist hospital in Rivers State. Two hundred and seven (207) samples were obtained from both female (71%) and male (21%) sourced samples covering endocervical, throat, ear, wound, urethral, skin and high vaginal swabs. Biochemical and Molecular approaches were employed in the identification of multidrug-resistant isolates from the isolates obtained from the study. Kirby-Bauer method was employed in the determination of antibiotics susceptibility profile. Female patients with the age 25-35 years and 35-44 years were observed to be most frequent for High vaginal and wound swab bacterial isolate colonization cases. Twenty-six isolates were observed to be resistant to Augmentin, Cefazidime, Gentamicin, Ofloxacin, Cefuroxime. Over 90%
of the bacterial isolates were resistant to Cloxacillin, 76% for Ceftriaxone. Enterobacter ludwigi was identified to be both multidrug resistant and plasmid-mediated form of resistance with multiple plasmids with Molecular weight of 13065, 9139, 2350 and 854bps whereas Klebsiella aerogenes was observed to have three distinct plasmid-bands with 10173, 2525 and 2118 bp. This study further supports the role of plasmids in the resistance profile and drug idiosyncrasies of nosocomial and pathogenic bacterial flora, reported in Port Harcourt metropolis. There is a necessary to intensify public campaigns and awareness against unsafe drug administration practices and self-medication trends.

Keywords: Plasmids; antibiotics susceptibility profile; antibiotics resistance pattern; Kirby-Bauer method; multidrug-resistant indices; drug idiosyncrasies.

1. INTRODUCTION

The increasing costs of health care delivery increased population and the increase in resistance of bacterial pathogens to conventional antibiotics have necessitated the need to underscore the battle between man and microbes in the administration of antimicrobial therapies. These have become imperative to ascertain the role of plasmids in the trends of microbial resistance. Antibiotics are microbial-derived substances, can either be produced by microbial activities or they can be synthesized naturally or even both. They can be used to eliminate systemic or topical infections. Mostly they can inhibit the growth of pathogens or even kill them. Antimicrobial therapies can be categorized into two major groups, technically, the coverage or effectiveness of such antibiotics to both gram-negative and gram-negative is said to have a broad (wide) spectrum, but when it is effective against either the gram negatives or gram-positive it regarded to have a narrow spectrum [1]. Bacterial resistance is a survival route in which bacterial groups either react to a strange toxicant, biological substances or even to a novel ecosystem [2].

Bacterial resistance to chemical substances has been linked to the mutational changes and uptake of novel plasmids by some form of horizontal gene transfer. These resistance genes are often encoded on plasmids or on gene locus of the genome. Plasmids are extra-chromosomal genetic material, can be either linear or circular, with a self-replicating ability. They carry genes most essential for the initiation and control of replication while some others carry genes that ensure stable uptake of genes encoded in the locus of the genomes. These ones are often referred to as transposons also known as jumping genes (Carattoli et al. 2001). This present study examined Occurrence, antibiotic susceptibility and plasmid profile of clinical isolates in Braith Waite Memorial Specialist Hospital, Port Harcourt, Rivers State, Nigeria.

2. METHODS

2.1 Location of Study

This study was carried out in Brait-Whyte Memorial Specialist Hospital, Port Harcourt, Rivers State, Nigeria. Hence, For the purpose of advancement with this study in accordance with the ethical guidelines for Biomedical research involving human subjects, ethical approval was sought and obtained from the Rivers State Health Research Ethics, Rivers state Hospital Management Board, Port Harcourt, Rivers State.

2.2 Sample Size

This study was investigating the proportion of swab samples in the total hospital attendees with clinical infection. Therefore, the sample size was determined using a qualitative variable (Charan and Biswas, 2013 employing the following equation:

\[
{\text{Sample size}} = \frac{Z_{\alpha/2}^2 \cdot p(1-p)}{d^2}
\]

\[
= \frac{1.96^2 \times 0.16(1-0.16)}{0.05^2} = 206.544
\]

Sample size = 207

Where: \(Z_{\alpha/2}\) is the standard normal variate (at 5% type 1 error \((\alpha<0.05)\) it is 1.96 and at 1% type error \((\alpha<0.01)\) it is considered significant below 0.05 hence 1.96 is used in the formula.

\(p=\) expected variation in population based on previous studies or pilot studies.

\(d=\) absolute error or precision (has to be decided by the researcher)

Hence, the proportion of patients hypothetically with possible infections collected from clinical swabs specimens in the hospital with bacterial origin among all age group according to laboratory statistics are estimated to be 16%. Using an absolute error of 5% therefore,
2.3 Population Studies

During this study, bacterial isolates was obtained from swab specimens of different patients. The populations of patients comprise males and females across all ages that were clinically diagnosed for possible infection that attended the hospital within the period of sample collection. Therefore, the specimen population is as follows (Table 1).

2.4 Isolation of Pathogen from Samples

The swabs were obtained from the patients by a physician, information on the form was obtained and document, with information on the age, sex, nature of sample and site of sample collection were reported. The swab stick was smeared on the solidified nutrient agar plates.

2.5 Biochemical Reaction

The method of Cheesbrough [3] was employed in the identification of the clinical pathogens. Gram reaction, spore staining, catalase, oxidase, sugar fermentation, Tripple sugar ion, Methyl red Voges-Proskauer and colonial morphology were used in the characterization of the isolates using dichotomous key response.

2.6 Standardization of Inoculums

Approximately 85 ml of 1% sulfuric acid (H2SO4) was added to a 100 ml volumetric flask. Using a volumetric pipette, 0.5 ml of 1.175% anhydrous barium chloride (BaCl2) was added drop-wise to the 1% sulfuric acid (H2SO4) while constantly swirling the flask. Then the solution was brought to a volume to 100 ml with 1% H2SO4. Stir for 5 minutes while examining visually, until the solution appears homogeneous and free of clumps. The optical density (OD) of the McFarland standard was checked at a wavelength of 625 nm.

2.7 Determination of Antimicrobial Susceptibility

According to the Clinical and Laboratory Standards Institute [4] recommendations, the modified Kirby-Bauer disc diffusion method was used in determining the susceptibility pattern of the clinical isolates to antibiotics. Hence, a freshly prepared eighteen-hour culture media of the bacterial isolates was inoculated into sterile distilled water, and compared with the equivalent of 0.5 Macfarland standard. About 0.1 ml of the sample was spread on Mueller-Hinton agar and incubated at 37°C for 2 hrs. Hence, a multi-disc (the Gram-negative and Gram-positive disc, containing 100 discs manufactured by Abtek Biological Ltd., UK) were used to determine the drug sensitivity and resistance pattern of the isolates) was aseptically placed on the Mueller-Hinton agar then incubated at 37°C, after 24 h the zones of inhibition were measured and compared with the zones of inhibition (breakpoints) as recommended by the Clinical and Laboratory Standards Institute [4].

2.8 Calculation of Multi-drug Antibiotics Resistance

The MAR (Multi-drug Antibiotics Resistance) index for Gram Positive and Gram Negative isolates was calculated using the method as described by Blasco et al. [5] and Odjadjare et al. [6] as follows:

\[ \text{MAR} = \frac{a}{b} \]

where:

- \( a \) = No. of antibiotics to which the isolate was resistant;
- \( b \) = Total No. of antibiotics against which individual isolate was tested.

| Specimens-type | Total |
|----------------|-------|
| TS             | 5     |
| SS             | 1     |
| HVS            | 88    |
| WS             | 80    |
| ECS            | 5     |
| US             | 12    |
| ES             | 16    |
| Total          | 207   |

Key: Wound Swabs-WS, High vaginal Swabs-HVS, Urethral Swabs-US, Endocervical Swabs-ECS, Ear Swabs-ES, Throat Swabs-TS, Skin Swabs-SS, M-Male, F-Female

Abtek disc (manufactured by abtek biochemicals ltd) were used for sensitivity:

| Antibiotics   | Symbol | Concentration (µg) |
|---------------|--------|--------------------|
| Cloxacillin   | CXC    | 30 µg              |
| Ceftriaxone   | CTR    | 30 µg              |
| Cefuroxime    | CRX    | 30 µg              |
| Cefixime      | CXM    | 5 µg               |
| Augmentin     | AUG    | 30 µg              |
| Erythromycin  | ERY    | 5 µg               |
| Gentamicin    | GEN    | 10 µg              |
| Ciprofloxacin | CPR    | 5 µg               |
| Ofloxacin     | OFL    | 5 µg               |
| Nitrofurantoin| NIT    | 300 µg             |
3. RESULTS

Table 2 describes the summarized antibiogram of the clinical isolates to conventional isolates, eight (8) isolates did not reveal antibiotics resistance pattern, hence had 0-MAR indices. Table 3 revealed that a total of 26 isolates were resistant to Augmentin, Ceftazidime, Gentamicin, Ofloxacin and Cefuroxime. Twenty (20) isolates were observed to be resistant to augmentin, alone, whereas 18 had no resistance (Tables 4, 5). Fig. 9 reveals the plasmid profiling of multidrug-resistant clinical isolates gel bands for the bacterial isolates. Fig. 10 (Table 6) describes the molecular weight determination protocol. Table 7 describes the correlation of plasmid gene-typing of the experimented samples in both male and females.

### Table 2. Number of antibiotics showing resistance pattern of clinical isolates

| Number of antibiotics resistant to | Number of isolates showing pattern | MAR index |
|-----------------------------------|-----------------------------------|-----------|
| None                              | 8                                 | 0         |
| One                               | 5                                 | 0.1       |
| Two                               | 9                                 | 0.3       |
| Three                              | 14                                | 0.4       |
| Four                               | 24                                | 0.5       |
| Five                               | 14                                | 0.6       |
| Six                                | 13                                | 0.8       |
| Seven                              | 7                                 | 0.9       |
| Eight                              | 8                                 | 1.0       |

Key: Multi-Drug Resistant index (MAR index)

### Table 3. Antimicrobial profiles of isolates resistant to broad-spectrum antibiotics

| Antimicrobial resistance profile | Number of isolates showing profile |
|---------------------------------|-----------------------------------|
| No Resistance                   | 18                                |
| Augmentin                       | 20                                |
| Ceftazidime                     | 14                                |
| Gentamicin                      | 12                                |
| Ofloxacin                       | 3                                 |
| Cefuroxime                      | 1                                 |
| Augmentin, Ceftazidime          | 9                                 |
| Augmentin, Ofloxacin            | 2                                 |
| Augmentin, Cefuroxime           | 17                                |
| Ceftazidime, Gentamicin         | 8                                 |
| Ceftazidime, Ofloxacin          | 8                                 |
| Ceftazidime, Cefuroxime         | 6                                 |
| Gentamicin, Ofloxacin           | 2                                 |
| Gentamicin, Cefuroxime          | 1                                 |
| Augmentin, Ceftazidime, Gentamicin | 1                            |
| Augmentin, Ceftazidime, Ofloxacin | 6                           |
| Augmentin, Ceftazidime, Cefuroxime | 5                           |
| Augmentin, Gentamicin, Ofloxacin | 1                              |
| Augmentin, Ofloxacin, Cefuroxime | 7                              |
| Ceftazidime, Gentamicin, Ofloxacin | 3                             |
| Ceftazidime, Gentamicin, Cefuroxime | 2                             |
| Ceftazidime, Ofloxacin, Cefuroxime | 1                             |
| Gentamicin, Ofloxacin, Cefuroxime | 1                             |
| Augmentin, Ceftazidime, Gentamicin, Ofloxacin | 9 |
| Augmentin, Ceftazidime, Gentamicin, Cefuroxime | 12 |
| Augmentin, Ceftazidime, Cefuroxime, Cefuroxime | 4 |
| Augmentin, Gentamicin, Cefuroxime, Ofloxacin | 3 |
| Ceftazidime, Gentamicin, Ofloxacin, Cefuroxime | 5 |
| Augmentin, Ceftazidime, Gentamicin, Ofloxacin, Cefuroxime | 26 |

Zone of inhibition: *Ab*≤0*Antibiotics*
Table 4. Percentage of isolates showing resistance pattern to broad-spectrum antibiotics

| Number of antibiotics resistant to | Number of isolates showing pattern |
|-----------------------------------|-----------------------------------|
| Zero                              | 18 (8.7%)                         |
| One                               | 50 (24.2%)                        |
| Two                               | 53 (25.6%)                        |
| Three                             | 27 (13.0%)                        |
| Four                              | 33 (15.9%)                        |
| Five                              | 26 (12.6%)                        |

Table 5. Percentage occurrence of broad-spectrum antibiotics resistant isolates

|             | Total number of isolates-n | Total number of isolates resistant to broad-spectrum antibiotics-n' | Percentage when \( \sum n=26 \) | Percentage when \( \sum n=207 \) |
|-------------|-----------------------------|---------------------------------------------------------------------|----------------------------------|----------------------------------|
| HVS         | 88                          | 7                                                                   | 27                               | 3.4                              |
| WS          | 80                          | 13                                                                  | 50                               | 6.3                              |
| ES          | 16                          | 3                                                                   | 12                               | 1.4                              |
| US          | 12                          | 2                                                                   | 8                                | 1.0                              |
| TS          | 5                           | 1                                                                   | 4                                | 0.5                              |
| Total       | 207                         | 26                                                                  | 12.6                             |                                  |

*Key:* Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES) and Wound Swab (WS)

Table 6. Plasmid profile standard curve

| Distance of ladda | Base pairs |
|-------------------|------------|
| 44.1              | 25130      |
| 50.1              | 9416       |
| 56                | 6557       |
| 63                | 4361       |
| 79                | 2322       |
| 84                | 2027       |
| 135.3             | 564        |

Fig. 1. Population study by gender in percentages

Female was 71% and males 29%
Fig. 2. A clustered column chart showing population study by age distribution  
Key: Wound Swabs-WS, High vaginal Swabs-HVS, Urethral Swabs-US, Endocervical Swabs-ECS, Ear Swabs-ES, Throat Swabs-TS, Skin Swabs-SS, AD-patients with unknown age, NIL-patients without age record

Fig. 3. A column chart showing a summary of the population study in the percentage of age distribution  
Key: AD-patients with unknown age, NIL-patients without age record

Fig. 4. A clustered column chart showing antibiotics susceptibility profile  
Key: CXC-Cloxacillin; CAZ-Ceftazidine, CTR-Ceftriaxone, CRX-Cefuroxime; CXM-Cefixim; AUG-Augmentin; ERY-Erythromycin; GEN-Gentamicin, CPR-Ciprofloxacin, OFL-Oflaxacin; NIT-Nitrofurantoin; S-Susceptible; I-Intermediate and R-Resistant
Fig. 5. A clustered column chart showing sample-type and percentage distribution for gram-positive isolates by gender from Brait Wait Memorial Hospital, Rivers State.
Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES), Wound Swab (WS)

Fig. 6. Percentage occurrence of gram-positive pathogens in clinical isolates from Brait Wait Memorial Hospital, Rivers State.
Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES), Wound Swab (WS)

Fig. 7. A column chart showing percentages of gram-negative isolates according to gender.
Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES), Wound Swab (WS)
Fig. 8. A pie chart showing the percentage occurrence of gram-negative pathogens in clinical isolates from brait waite memorial hospital, Rivers State

Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES) and Wound Swab (WS)

Table 7. Correlation of plasmid gene-typing

| PMC | Isolate identity   | Isolate code | The molecular weight of plasmid (bp) | Age | Sex |
|-----|--------------------|--------------|-------------------------------------|-----|-----|
| E1  | Enterobacter ludwigi | E3           | 13065, 9139, 2350, 864               | 68  | F   |
| E2  | Bacillus sp         | H6           | 0                                   | 2Mt | M   |
| E3  | Pseudomonas fluorescens | W5     | 0                                   | 2Mt | F   |
| H1  | Staphylococcus epidermis | W8     | 0                                   | 27  | F   |
| H2  | Pseudomonas sp.     | W11          | 0                                   | 28  | F   |
| H4  | Staphylococcus epidermis | H2     | 0                                   | 34  | F   |
| H5  | Staphylococcus epidermis | W9     | 48                                  | 34  | F   |
| H6  | Pseudomonas marginalis | W10    | 0                                   | 37  | F   |
| H7  | Burkholderia cenocepsia | H7    | 10173                               | 40  | F   |
| H8  | Enterobacter sp.    | W2           | 10173                               | 41  | F   |
| T1  | Enterobacter cloaeae | E1           | 0                                   | 14  | F   |
| U1  | Brevibacillus sp.   | W6           | 0                                   | 61  | M   |
| U2  | Streptococcus pyogenes. | H8     | 0                                   | 65  | M   |
| W1  | Streptococcus sp.   | W4           | 0                                   | 12  | M   |
| W10 | Providencia vermicola | T1    | 10173                               | 77  | F   |
| W11 | Pseudomonas aeruginosa strain | W3 | 0                                   | 72  | F   |
| W12 | Bacillus subtilis   | U1           | 0                                   | 75  | M   |
| W13 | Bacillus subtilis   | E2           | 0                                   | 71  | M   |
| W2  | Providencia vermicola | W13  | 10173                               | 25  | F   |
| W3  | Proteus mirabilis   | H5           | 0                                   | 29  | M   |
| W4  | Citrobacter sp.     | H4           | 0                                   | 35  | F   |
| W5  | Klebsiella sp.      | W12          | 10173                               | 53  | M   |
| W6  | Staphylococcus aureus | W7    | 0                                   | 55  | F   |
| W7  | Klebsiella aerogenes | U2    | 10173, 2525, 2118                   | 62  | F   |
| W9  | Enterobacter sp.    | H1           | 10173                               | 70  | F   |

Key: Throat Swab (T), High Vaginal Swab (H), Urethral Swab (U), Ear Swab (E), Wound Swab (W), Male (M), Female (F), Month (Mt), PMC-Plasmid and Molecular study Code, Gram-positive (+), Gram-negative (-)
4. DISCUSSION

Epidemiological survey of clinical isolates for both resistance and multidrug resistance is critical for a robust curative and preventive measure for limiting the spread of these microorganisms [7]. The emergence of infections caused by antibiotic-resistant pathogens is a growing problem and has now become a major health issue [8]. Pathogenicity of clinical isolates has the ability to increase in resistance profile [9]. The study conducted by Umolu et al. [7]
observed in a total of eighty-six clinical samples collected high vaginal swab (HVS) for samples reported for enteric pathogen nted for 4 while wound samples were 14 for similar cases. In this study over 207 samples were collected wound swab (80), 88 (HVS), Ear Swab (16), Urethra Swab (12). The occurrence of the enteric pathogen in females was significantly higher in the samples collected for females than for males as seen in urethra swab samples. This line of argument negates or disagrees with the position of Jombo et al. [10] Although the loss in medical history, presentation of disease cases might have been identified as crucial.

The impact of age of patients presented with obvious microbial surface colonization. When the age ranges of 25-34 and 65± displayed the highest cases resistance. Hassan et al. [11] in a separate but related study reported similar findings but had a far different report where. The HVS had the highest cases still within the ages of 25-34 while wound swab also had a high occurrence. The female patients had the highest cases of this multidrug resistance compared to the male. Surprisingly, the children within the ages 0-4 were also observed to have these multidrug resistances as well.

The most resisted were the beta-lactam antibiotic, in this case, was Cloxacillin (86%) these findings agree with the report of Aibuin et al. [12], Stelling et al. (2005) and who reported a 100% amoxicillin. Also resisted Ceftrixoxin (76%), Augmentin (60%), Cefixime (59%) while the most susceptible antibiotics were Erythromycin (71%), Nitrofurantoin (70%), Ofloxacin (58%), Gentamicin (52%) and Cefuroxime (50%) whereas. The use of Nitrofurantoin is currently discouraged due to its toxicity and side effects, but in extreme cases of urinary tract infections it is limited for use for children. Jumbo et al. 2012 reported that children have uncontrolled ability to produce the enzyme beta-lactamases which neutralizes most penicillin group, further suggesting the use of Ofloxacin with a remarkable sensitivity of 58%. This agrees with the findings of this current study cephalosporin like Erythromycin and Ciprofloxacin was slightly resistant.

Since the widespread use of antibiotics in animal husbandry and agricultural activities such propelled by economic motives, the especially higher yield from the veterinary and agricultural world, and from food producers and pharmaceutical companies, to combat the spread of multi-drug resistant bacteria effectively [13]. Strong adherence of appropriate chemotherapy to disease treatment and control has its foundations surveillance, control of resistance, administration of the proper antibiotics and adjustment of doses. Health facilities must be encouraged to adhere to these recommendations [14,15]. Also, the high rate of resistance (100%) of ESBL producing strains of E. coli against cephalosporins in Turkey [16]; the high rate of resistance of Enterobacteriaceae against quinolones in Sweden [17]; and the high resistance of Enterobacter against ceftazidime in Brazil [18] clearly shows the global variations in antimicrobial susceptibility patterns. Laboratory physicians and scientists should always develop local antimicrobial susceptibility profiles (antibiograms) of local bacterial isolates, and the patterns regularly updated for ready consultations [19,20].

The number of samples reported for Gram-negative were 105 samples while Gram-positive had 102 two samples. Among these samples, enteric pathogens were most frequent [11]. This agrees with this study result when they reported that the Enterobacteriaceae groups suggesting that Escherichia coli as the dominant isolate especially in High vaginal swab than in wound swab. The susceptibility isolates like the Enterobacter ludwigi, Pseudomonas fluorescens, Pseudomonas sp., Pseudomonas marginalis, Enterobacter cloacae, Providencia vermicola, and Enterobacter sp. and Burkholderia Cenocepacia. The isolates obtained in this study agrees with the findings of Hassan et al. [11] whom in his study was able to identify Enterobacter cloacae, Citrobacter freundii, Sadly the preponderance of Pseudomonas sp. were hardly seen in previous studies Proteus mirabilis.

5. CONCLUSION

This study reveals the role of plasmids in the resistance profile and drug idiosyncrasies of nosocomial and pathogenic bacterial flora, in Port Harcourt metropolis, Nigeria. It is a necessary to intensify public campaigns and awareness programmes against unsafe drug administration practices and self-medication trends.

ETHICAL APPROVAL

The clearance for this research was sought for and approved by the hospital management board.
COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. Zhang Y. Mechanisms of antibiotic resistance in the microbial world. Baltimore, USA; 2007.
2. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews. 2010;74(3):417–433.
3. Cheesbrough M. Microbiological tests. District Laboratory Practice in Tropical Countries. 2006;II:35-234.
4. Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing, document M100-S16. Wayne, PA: Clinical and Laboratory Standards Institute; 2006
5. Blasco MD, Esteve C, Alcarde E. Multiresistant waterborne pathogens isolated from water reservoirs and cooling systems. Journal of Applied Microbiology. 2008;105:469–475.
6. Odjadjare EE, Igbinoso EO, Mordi R, Igere B, Igeleke CL, Okoh AI. Prevalence of multiple antibiotics resistant (MAR) Pseudomonas species in the final effluents of three municipal wastewater treatment facilities in South Africa. International Journal of Environmental Research and Public Health. 2012;9:2092–2107.
7. Umolu PI, Ominig O, Taffeng Y, Omorogbe FI, Asabokhale F, Ugbdagah OP. Antimicrobial Susceptibility and Plasmid Profiles of Escherichia coli Isolates Obtained from Different Human Clinical Specimens in Lagos – Nigeria. Journal of American Sciences. 2006;2(4):70-76.
8. Yoshikawa TT. Antimicrobial resistance and aging: Beginning of the end of the antibiotic era? The American Geriatrics Society. SUPPLEMENT. 2002;50(7).
9. Karlowsky JA, Jones ME, Draghi DC, Thornsbery C, Sahm DF, Volturo GA. Prevalence of antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Annals of Clinical Microbiology and Antimicrobials. 2004;3:7.
10. Jombo GTA, Emanehe UE, Amefule EN, Damen JG. Urinary tract infections at a Nigerian University Hospital: Causes, patterns and antimicrobial susceptibility profile. Journal of Microbiology and Antimicrobials. 2011;3(6):153-159.
11. Hassan AO, Hassan RO, Muhibi MA, Adeibimpe WO. A survey of Enterobacteriaceae in hospital and community acquired infections among adults in a tertiary health institution in Southwestern Nigeria. African Journal of Microbiology Research. 2012;6(24):5162-5167.
12. Aibinu I, Adenikeun E, Oduegbemi. The emergence of quinolone resistance amongst Escherichia coli strains isolated from clinical infections in some Lagos state hospitals, in Nigeria. Nigerian Journal of Health and Biomedical Sciences. 2004;3(2):73–78.
13. Lutter SA, Currie ML, Milz LB, Greenbaum LA. Antibiotic resistance patterns in children hospitalized for urinary tract infections. Archives of Paediatrics and Adolescent Medicine. 2005;159(10):924-928.
14. Cheng CH, Tsai MH, Huang YC, Su LH, Tsau YK, Lin CJ, Chiu CH, Lin TY. Antibiotic resistance patterns of community-acquired urinary tract infections in children with vesicourethral reflux receiving prophylactic antibiotic therapy. Paediatric. 2009;122(6):1212-1217.
15. Chakupurakal R, Ahmed M, Sobithadevi DN, Chinnappan S, Reynolds T. Urinary tract pathogens and resistant patterns. Journal of Clinical Pathology. 2010;63(7):652-654.
16. Akyr I. Antibiotic resistance rates of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella spp. strains isolated from urinary tract infections in a private hospital. Mikrobiyology Bulletin. 2008;42(4):713-715.
17. Osthholm-Balkhed A, Tarnberg M, Nilsson M, Johnsson AV, Hanberger H, Monstein HJ, Nilsson LE. Prevalence of extended spectrum beta-lactamase-producing Enterobacteriaceae and trends in antibiotic consumption in a county of Sweden. Scandinavian. Journal of Infection of Diseases. 2010;42(11-12):831-835.
18. Sader HS, Farrell DJ, Jones RN. Tigecycline activity tested against multidrug-resistant Enterobacteriaceae and Acinetobacter species isolated in US medical centres (2005-2009). Diagnostic Microbiology Infection and Diseases. 2011;69(2):223-227.
19. Morosini MI, Castillo MG, Coque TM, Valverde A, Novais A, Loza E, Baquero F, Canton R. Antibiotic resistance in extended spectrum beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline. Antimicrobial Agents and Chemotherapy. 2006;50(9):2695-2699.

20. Bouchillon SK, Hoban DJ, Johnson BM, Stevens TM, Dowzicky MJ, Wu DH, Bradford PA. In vitro evaluation of tigecycline and comparative agents in 3,049 clinical isolates: 2001-2002. Diagnostic and Microbiology of Infections and Diseases. 2005;51:201-205.

© 2019 Wali et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.