ABSTRACT

The antibiotic resistance and susceptibility profiles of some bacterial isolates including Listeria monocytogenes, Erwinia stewartii, Legionella pneumophila, Carnobacterium gallinarum, Staphylococcus caseolyticus, Enterobacter dissolves, Pseudomonas mallei, Klebsiella pneumonia, Aeromonas media and Lactobacillus sp. were determined using some broad and narrow spectrum antibiotics by the disk diffusion technique. Based on the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria, some isolates were found to be resistant to some of the tested antibiotics but susceptible to others. Among the Gram-positive bacterial isolates, Lactobacillus specie had the highest susceptibility profile with the zone of clearance ranging from 28 - 30 ± 8 mm in diameter. However, among the Gram-negative bacterial isolates, Pseudomonas mallei, Klebsiella pneumoniae and Aeromonas media were susceptible to all tested antibiotics, with 30mm ± 0 mm zones of clearance. CLSI standards were used to interpret results; while Lactobacillus sp. was the most susceptible isolate, while Erwinia stewartii was resistant to all the test antibiotics except ceporex.

Keywords: Gram-positive, Gram-negative, Antibiotics, Resistance, Susceptibility, Zone of clearance

INTRODUCTION

Antibiotics, also known as antibacterials are chemotherapeutic agents that majorly control the growth of bacteria, by bactericidal or bacteriostatic means. They are classified majorly based on their structure, spectrum of activity, route of administration, mode and mechanism of action. Understanding how these antibiotics induce their action is centered on the essential cellular function inhibited when they interact with bacterial cell. These specific antibacterial-cellular function interactions are termed drug-target interactions. Antibiotic susceptibility of bacteria is to a large extent dependent on the spectrum of activity, the mode and mechanism of action of the antibiotics. According to Neonakis . (2003), resistance to aminoglycoside antibiotics such as amikacin by members of the Enterobacteriaceae family is usually due to an aminoglycoside-modifying enzymes- aminoglycoside 6’-N-acetyltransferases which modifies amikacin, tobramycin, kanamycin and netilmicin but not gentamicin. Bacterial spectrum is the number of bacteria a broad-spectrum or narrow-spectrum antibiotic is effective against. Broad-spectrum antibiotics are effective against a broad range of microorganisms (Gram-negative and Gram-negative bacteria), whereas narrow-spectrum antibiotics treat few infections caused especially either by Gram-negative or Gram-negative bacteria. Antibiotics are either bactericidal or bacteriostatic in terms of their mode (how they induce) action. Generally, bactericidal antibiotics completely destroy bacterial cell
walls or other cell organelles. Examples may include penicillins, fluoroquinolones, daptomycin, metronidazole, nitrofurantoin and co-trimoxazole.

Bacteriostatic antibiotics stop bacterial proliferation and multiplication by interfering with bacterial protein synthesis, DNA replication or any other aspect of bacterial cell metabolism. Examples may include tetracyclines, macrolides, lincosamides, sulphonamides, trimethoprim, streptomycin and chloramphenicol.

Antibiotic activities based on mechanisms of action are group into inhibitors of cell wall synthesis. This group is further divided into inhibitors of peptidoglycan synthesis including bacitracin and cycloserine, and inhibitors of peptidoglycan cross-linking like vancomycin and b-lactams as penicillins and cephalosporins (Joanne., 2013).

RESULTS AND DISCUSSION

The antibiotic susceptibility/resistance for the Gram-positive bacteria was determined using streptomycin, ciproflox, gentamicin, amoxil, ampiclox, chloramphenicol, erythromycin, levofloxacin, norfloxacin, rifampicin while that of the Gram-negative bacteria was determined using streptomycin, gentamicin, ciproflox, augmentin, ceporex, nalidixic acid, tarivid, reflacine, ampicillin and septrin. Results were calculated according to the zones of clearing observed in mm ± standard deviation within the antibiotics for the individual bacterial isolates. Tables 1 and 2 below show raw results ± standard deviation.

MATERIALS AND METHOD

Antibiotic susceptibility and resistance study for Gram-positive and Gram-negative bacterial isolates obtained from Malabor tap water was carried out by the discs diffusion method using Mueller Hinton agar (MHA). The isolates were uniformly streaked on aseptically prepared and solidified MHA on duplicated petri dishes for each identified isolate. The choice of antibiotics was based on the Gram reaction of the isolates and the mechanisms of action of the antibiotics. The antibiotic discs were placed on the duplicate MHA plates for each of the isolates and incubated at 37°C for 24 hours.

By disk diffusion technique, the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk were measured. The Petri plate was held a few inches above a black, nonreflecting background illuminated with reflected light. The zone margins were considered: area showing no obvious, visible growth as detected with the unaided eye. However, faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth were ignored (CLSI, 2016).
### 4.2.1 Antibiotic susceptibility profile for Gram-negative bacterial isolates

**Table 1: Antibiotic Susceptibility of Gram-negative Bacterial Isolates**

| S/N | Suspected bacteria                      | AMX (20µg) | APX (20µg) | CH (30µg) | CPX (10µg) | CN (10µg) | E (30µg) | LEV (20µg) | NB (10µg) | RD (20µg) | S (30µg) |
|-----|----------------------------------------|------------|------------|-----------|------------|-----------|-----------|------------|-----------|-----------|---------|
| 1   | *Listeria monocytogenes*               | 20±12mm    | 16±12mm    | 30±12mm   | 30±12mm    | 0±12mm    | 30±12mm   | 12±12mm    | 28±12mm   | 0±12mm    |         |
| 2   | *Carnobacterium gallinarum*            | 14±8mm     | 0±8mm      | 16±8mm    | 14±8mm     | 28±8mm    | 16±8mm    | 16±8mm     | 16±8mm    | 14±8mm    |         |
| 3   | *Staphylococcus caseolyticus*          | 18±8mm     | 18±8mm     | 12±8mm    | 12±8mm     | 28±8mm    | 16±8mm    | 24±8mm     | 20±8mm    | 18±8mm    | 0±8mm   |
| 4   | *Lactobacillus spp*                    | 10±8mm     | 16±8mm     | 28±8mm    | 30±8mm     | 30±8mm    | 30±8mm    | 28±8mm     | 28±8mm    | 12±8mm    |         |

**Key:** AMX: Amoxil, APX: Ampiclox, CH: Chloramphenicol, CPX: Ciproflox, CN: Gentamicin, E: Erythromycin, LEV: Levofloxacin, NB: Norfloxacin, RD: Rifampicin, S: Streptomycin

### 4.2.2 Antibiotic susceptibility profile for Gram-negative bacterial isolates

**Table 2: Antibiotic Susceptibility of Gram-negative Bacterial Isolates**

| S/N | Suspected bacteria               | AU (30µg) | CEP (10µg) | CN (10µg) | CPX (10µg) | NA (30µg) | OFX (10µg) | PEF (10µg) | PN (30µg) | S (30µg) | SXT (30µg) |
|-----|---------------------------------|-----------|------------|-----------|------------|-----------|------------|------------|-----------|---------|-----------|
| 1   | *Erwinia stewartii*             | 10±10mm   | 30±10mm    | 0±10mm    | 0±10mm     | 12±10mm   | 0±10mm     | 0±10mm     | 10±10mm   | 0±10mm  |           |
| 2   | *Legionella pneumophilia*       | 14±8mm    | 14±8mm     | 0±8mm     | 24±8mm     | 10±8mm    | 10±8mm     | 14±8mm     | 10±8mm    | 16±8mm  | 28±8mm    |
|   | **Enterobacter dissolves** | 30±5mm | 30±5mm | 28±5mm | 30±5mm | 14±5mm | 30±5mm | 30±5mm | 30±5mm | 30±5mm |
|---|-----------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 4 | **Pseudomonas mallei**      | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm |
| 5 | **Klebsiella pneumonia**    | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm |
| 6 | **Aeromonas media**        | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm | 30±0mm |

**Key:** AU: Augmentin, CEP: Ceporex, CN: Gentamicin, CPX: Ciproflox, NA: Nalidixic Acid, OFX: Tarivid, PEF: Reflacine, PN: Ampicillin, S: Stretomycin, SXT: Septrin.
Gram-positive Antibiotic Susceptibility and Resistance

In terms of Gram-positive bacterial isolates, there was significant difference in the mean antibiotic susceptibility within Gram-positive bacterial isolates, at 95% confidence interval, hence $F_{cal.} (9.7)$ is greater than $F_{crit.} (3.6)$. Similarly, there was a significant difference in the mean antibiotic sensitivity of test antibiotics at 95% confidence interval with $F_{cal.} (4.7)$ being greater than $F_{crit.} (2.6)$. Interpreting, using the Clinical and Laboratory Standards Institute (CLSI, 2016), all the Gram-positive bacterial isolates were susceptible to levofloxacin, a narrow-spectrum Gram-positive antibiotic while majority of the isolates were resistant to streptomycin- a broad-spectrum antibiotic. Results also show that *Lactobacillus* specie isolated from Hall9 tap water in the Malabor hostel was susceptible to almost all the tested broad-spectrum and Gram-positive antibiotics.

Figures 1 – 4 show the individual susceptibility/resistance results of Gram-positive bacterial isolates.

**Staphylococcus caseolyticus**

**Listeria monocytogenes**

**Carnobacterium gallinarum**
Gram-negative Antibiotic Susceptibility and Resistance

There was significant difference in the mean antibiotic susceptibility within Gram-negative bacterial isolates, at 95% confidence interval, hence Fcal. (24.3) is greater than Fcrit. (2.7). Meanwhile, there is no significant difference in the mean antibiotic sensitivity of test antibiotics hence Fcal. (1.3) is less than Fcrit. (2.2) at 95% confidence interval. Interpreted using CLSI results revealed that all Gram-negative isolates except *Erwinia stewartii* are susceptible to the test broad-spectrum antibiotics: gentamicin and streptomycin. Results also revealed that all the Gram-negative bacterial isolates were resistant to ampicillin. Also, *Erwinia stewartii* shows resistance to all the test broad-spectrum and Gram-negative antibiotics. Conversely, *Pseudomonas* sp., *Klebsiella* sp. and *Aeromonas* sp. were susceptible to all the tested broad-spectrum and Gram-negative antibiotics. Figures 5–10 show the antibiogram results of each Gram-negative bacterial isolate against the tested antibiotics.
According to WHO (2006), the spectrum of organisms detected by HPC testing includes organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers; and organisms that rapidly
proliferate in treated water in the absence of residual disinfectants. Some drinking-water treatment processes, such as coagulation and sedimentation, reduce the number of HPC organisms in water. However, the numbers of HPC organisms are reduced significantly by disinfection practices, such as chlorination, ozonation and UV light irradiation. In practice, none of the disinfection processes sterilizes water while under suitable conditions such as the absence of disinfectant residuals, HPC organisms can grow rapidly. In distribution systems, a high HPC number can indicate deterioration in cleanliness, possibly stagnation and the potential development of biofilms (WHO, 2006).

Moreover, new macrolide antibiotics, such as clarithromycin and azithromycin, show more effective in-vitro activity and have better intracellular and tissue penetration than erythromycin, as do the quinolones.

**Antibiotic susceptibility profile compared with CLSI standards**

Table 3 below shows CLSI standards for antibiotic susceptibility test.
Table 3: CLSI Interpretive Standards of antibiotics

| Antibiotic     | Disk Content | Spectrum of Activity | Zone Diameter Breakpoints Nearest Whole mm |
|----------------|--------------|----------------------|-------------------------------------------|
|                |              |                      | S  | I  | R  |
| Ciproflox      | 10 µg        | Broad Spectrum       | ≥21| 16 - 20 | ≤15 |
| Gentamicin     | 10 µg        | Broad Spectrum       | ≥15| 13 - 14 | ≤12 |
| Streptomycin   | 30 µg        | Broad Spectrum       | ≥15| 12 - 14 | ≤11 |
| Levofloxacin   | 20 µg        | Gram-negative bacteria | - | - | - |
| Norfloxacin    | 10 µg        | Gram-negative bacteria | ≥17| 13 - 16 | ≤12 |
| Rifampicin     | 20 µg        | Gram-negative bacteria | ≥20| 17 - 19 | ≤16 |
| Erythromycin   | 30 µg        | Gram-negative bacteria | ≥23| 14 - 22 | ≤13 |
| Chloramphenicol | 30 µg    | Gram-negative bacteria | ≥18| 13 - 17 | ≤12 |
| Ampicillin     | 30 µg        | Gram-negative bacteria | ≥17| 14 - 16 | ≤13 |

CLSI (2011). Zone Diameter of Inhibition

Key: S: Susceptible, I: Intermediate, R: Resistant

All Gram positive isolates were susceptible to levofloxacin even though it does not sustain a CLSI standard, but the zone of inhibition is quit fascinating. Lactobacillus spp was susceptible to all the test antibiotics but not streptomycin. Staphylococcus spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within three to four days after initiation of therapy. Testing of repeat isolates may be warranted (CLSI, 2011).

Majority of the gram negative bacterial isolates including Legionella, Enterobacter, Pseudomonas, Klebsiella and Aeromonas spp. were susceptible to ciproflox and streptomycin, while Erwinia sp. is resistant all the relative test antibiotics. Also, ampicillin is not a drug of of choice for the gram negative spp especially members of the Enterobacteriaceae family. According to CLSI (2011), members of the family Enterobacteriaceae are susceptible to trimethoprim, a sulphonamide and ceftolozane, a cephalosporin and b-lactamase-inhibitor combination. This is evident in the like Klebsiella, Pseudomonas sp, Enterobactersp, Erwiniasp. Usually, quinolones (like ciproflox) are synergistic with β-lactams (like ampicillin) and aminoglycosides (Catherine et al., 2002).

Similarly, urinary tract infections are often treated with different broad-spectrum antibiotics even when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms (Yakubuet al., 2010). Fluoroquinolones are preferred as initial agents for empiric therapy of UTI in areas where resistance is likely to be of concern (Biswasetal., 2006; Schaeffer, 2002) this is evident in all Gram negative isolates except for Legionella and Erwinia spp. This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance among most common uropathogens (Tankhiwaleet al., 2004).

Antibiotics have proven an effective weapon against bacterial contamination and infection. However, the presence of multiple drug resistant microorganisms will compromise our ability in treating infections caused by such pathogens. Thus, the isolation of Erwiniasp from the tap water stands as a major public health threat to people using the water as drinking water source. This corroborates the report by Bashir et al. (2014) that multiple antibiotic resistant bacteria living in various drinking-water sources suggests that contaminated...
water may be a primary source of severe infectious diseases and according to Mmuoegbulam et al. (2016), enteopathogenic bacteria when not treated, are not only enterotoxigenic but also induce some histological changes. The emergence of bacteria resistant to most of the commonly used antibiotics is of considerable medical significance, because of public health implications; hence the prevalence of drug resistant organisms poses a great challenge to clinicians and the consumption of water containing these antibiotic resistant organisms may prolong the treatment of water borne pathogens, thus bringing about the need for a new and more expensive antibiotics (Tagoe et al. (2011).
Table 4:

| S/N | Bacterial Isolates            | Antibiotic Susceptibility Interpretations for Gram-positive bacteria |
|-----|------------------------------|---------------------------------------------------------------------|
|     |                              | CPX | CN | S | LEV | NB | RD | E | CH |
| 1   | *Listeria monocytogenes*     | S   | R  | R | S   | R  | S  | S | S  |
| 2   | *Carnobacterium gallinarum* | R   | S  | I | S   | I  | I  | I | I  |
| 3   | *Staphylococcus caseolyticus* | R   | S  | R | S   | S  | I  | I | R  |
| 4   | *Lactobacillus*              | S   | S  | R | S   | S  | S  | S | S  |

Antibiotic Susceptibility Interpretations for Gram-positive bacteria

Key: S: Susceptible, I: Intermediate, R: Resistant

Table 5:

| S/N | Bacterial Isolates | Antibiotic Susceptibility Interpretations for Gram-negative bacteria |
|-----|--------------------|---------------------------------------------------------------------|
|     |                    | Ciproflo x (CPX) | Gentamcin (CN) | Streptomycin | Ampicillin (PN) |
| 1   | *Erwinia stewartii* | R                  | R              | R            | R             |
| 2   | *Legionella pneumophilia* | S          | R              | S            | R             |
| 3   | *Enterobacter dissolves* | S            | S              | S            | R             |
| 4   | *Pseudomonas mallei*  | S                  | S              | S            | R             |
| 5   | *Klebsiella pneumoniae* | S                | S              | S            | R             |
| 6   | *Aeromonas media*     | S                  | S              | S            | R             |

Key: S: Susceptible, I: Intermediate, R: Resistant

CONCLUSION

There is need to treat the Malabor tap water so as to reduce the coliform count to zero (0) as required by the water quality standards. However, such pathogens when present (even after water treatment) are susceptible to some broad and narrow spectrum antibiotics except for *Erwinia stewartii* which was susceptible to ceporex alone.

NOTE: The “resistant” category of antibiotics confirms that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms (eg, β-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

REFERENCES

Bashir, A., Mehnaz, L., Javid A., Shumaila, B., Sher M., Sudhair, A. & Said, H. (2014). Microbiology and Evaluation of Antibiotic Resistant Bacterial Profiles of Drinking Water in Peshawar, Khyber Pakhtunkhwa. *World Applied Sciences Journal*. 30 (11): 1668-1677.

Biswa, D., Gupta, P., Prasad, R., Singh, V., Arya, M. & Kumar, A. (2006). Choice of Antibiotic for Empirical Therapy of Acute Cystitis in a Setting of High Antimicrobial Resistance. *Indian Journal of Medical Science*, 60: 53-58.

Bomberger, J. M., Maceachran, D. P., Coutermarch, B. A., Ye, S., O’Toole, G. A. & Stanton, B. A. (2009). Long distance delivery of bacterial virulence factors by *Pseudomonas aeruginosa* outer membrane vesicles. *Plos Pathogenic* 5: 100 – 382.

Catherine, M, Oliphant P. D., Gary M. & Green M. D. (2002). Quinolones: A Comprehensive Review. *American Academy of Family Physicians*, 65: 455-464
Clinical and Laboratory Standards Institute-CLSI (2011). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. Approved Standard, 10: 33-98

Joanne, W., Linda, S. & Chris, W. (2013). Prescott’s Microbiology, 9th edition. McGraw-Hill, United States. Pp. 332 – 338.

Mmuoegbulam, O. A., Prof. S. P. Antai. & Dr. E. B. E. Asikong (2016). Histopathological effects of enterotoxigenic Klebsiella variicola and Enterobacter species isolated from Iko River- Nigeria. International Journal of Science and Research, 5(4): 488 – 492.

Neonakis, I., Gikas, A., Scoulica, E., Manios, A., Georgeiladakis, A. & Tselentis, Y. (2003). Evolution of aminoglycoside resistance phenotypes of four Gram-negative bacteria: an 8-year survey in a University hospital in Greece. International Journal of Antimicrobial Agents, 22: 526 – 531.

Schaeffer, A. J. (2002). The Expanding Role of Fluoroquinolones. American Journal of Medicine, 113: 45S-54S.

Yakubu B. N., Mark, O. A., Obidake E. R., Adebukola A. S., Adebola, O. & Samuel O. O. (2010) Antimicrobial susceptibility of Escherichia coli and other coliforms isolated from urine of asymptomatic students in Bayelsa State, Nigeria. African Journal of Microbiology Research, 5 (3): 184-191

WHO.(2006). Guidelines for Drinking-water Quality. World Health Organization Recommendations, 3(1): 107-142

Tagoe, D. N. A., Nyarko, H., Arthur S. A. & Birikorang E. (2011). A Study of Antibiotic Susceptibility Pattern of Bacterial Isolates in Sachet Water Sold in the Cape Coast Metropolis of Ghana. Research Journal of Microbiology, 6(2): 153 - 158

Tankhiwale, S. S, Jalgaonkar, S. V., Ahamad, S. & Hassani, U. (2004). Evaluation of Extended Spectrum Beta-lactamase in Urinary Isolates. Indian Journal of Medical Research, 120: 553-556.