



## **Antibiotic Susceptibility Profile of Bacterial Pathogens Isolated from Malabor Hostel Tap Water, Calabar- Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author OAM designed the study and gave the protocol. Authors AAU and IUB managed the analyses and tabulation of the study. Authors OAM and EEI managed the literature searches and wrote the first draft of the manuscript while author EEI performed the statistical analysis. All authors read and approved the final manuscript.*

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### **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 30 mm ± 0 mm zones of clearance. CLSI standards were used to interpret results; while *Lactobacillus* sp. was the most susceptible isolate, *Erwinia stewartii* was resistant to all the test antibiotics except ceporex.

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**Keywords:** Gram-positive; gram-negative; antibiotics; resistance; susceptibility; zone of clearance.

## 1. INTRODUCTION

Antibiotics are chemotherapeutic agents that control the growth of bacteria. They are also known as antibacterials and can be bactericidal or bacteriostatic in action. They are classified based on their structure, spectrum of activity, route of administration, mode and mechanism of action. Based on the essential cellular function inhibited when they interact with bacterial cell, one will be able to understand how each antibiotic induces its action. These specific antibacterial-cellular function interactions are termed drug-target interactions.

Antibiotic susceptibility of bacteria to a large extent is dependent on the spectrum of activity, the mode and mechanism of action of the antibiotics. According to [1], 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.

Antibiotic can be broad-spectrum or narrow-spectrum based on its bacterial spectrum or the number of bacteria an antibiotic is effective against. Broad-spectrum antibiotics are effective against a broad range of microorganisms (Gram-negative and Gram-negative bacteria), while the narrow-spectrum antibiotics treat few infections caused especially either by Gram-negative or Gram-negative bacteria.

The bactericidal or bacteriostatic nature of antibiotics depends solely on their mode or how they induce their antibacterial actions. Generally, bactericidal antibiotics completely destroy bacterial cell walls or other cell organelles resulting to an outright killing of the bacteria. Among the members of this group are the penicillins, fluoroquinolones, daptomycin, metronidazole, nitrofurantoin and co-trimoxazole. Bacteriostatic antibiotics simply inhibit bacterial proliferation and multiplication by interfering with bacterial protein synthesis, DNA replication or any other aspect of bacterial cell metabolism without complete destruction or killing of the bacteria. These include tetracyclines, macrolides, lincosamides, sulphonamides, trimethoprim, streptomycin and chloramphenicol.

Antibiotic have been grouped based on mechanisms of action. One of such groups is the 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  $\beta$ -lactams such as penicillins and cephalosporins [2].

Antibiotics can also disrupt cell membrane structure and according to [3] antibiotics can cause disruption through membrane lysis death pathway, vesicle-vesicle contact pathway and/or by hydroxyl radical pathway. This mechanism according to [4] is common with the polymyxins including polymyxin B and E.

## 2. MATERIALS AND METHODS

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 [5].

## 3. 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 show raw results ± standard deviation.

### 3.1 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 [5], 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. Figs. 1 – 4 show the individual susceptibility/resistance results of Gram-positive bacterial isolates.

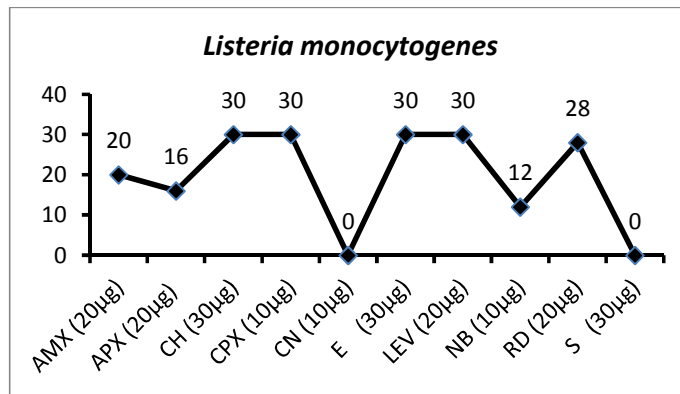


Fig. 1. Antibiotic susceptibility of *L. monocytogenes*

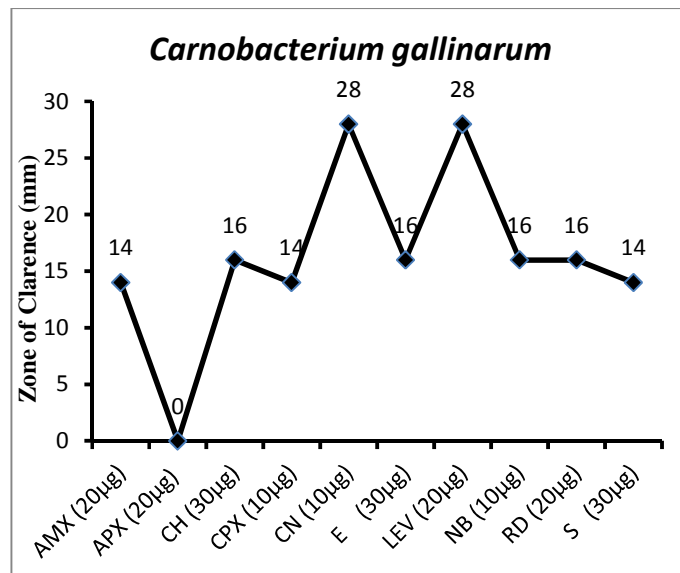


Fig. 2. Antibiotic susceptibility of *C. gallinarum*

**Table 1. Antibiotic susceptibility of gram-positive bacterial isolates**

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

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

**Table 2. Antibiotic susceptibility of gram-negative bacterial isolates**

| S/N | Suspected bacteria            | AU<br>(30 µg) | CEP<br>(10 µg) | CN<br>(10 µg) | CPX<br>(10 µg) | NA<br>(30 µg) | OFX<br>(10 µg) | PEF<br>(10 µg) | PN<br>(30 µg) | S<br>(30 µg) | SXT<br>(30 µg) |
|-----|-------------------------------|---------------|----------------|---------------|----------------|---------------|----------------|----------------|---------------|--------------|----------------|
| 1   | <i>Erwinia stewartii</i>      | 10±10 mm      | 30±10 mm       | 0±10 mm       | 0±10 mm        | 0±10 mm       | 12±10 mm       | 0±10 mm        | 0±10 mm       | 10±10 mm     | 0±10 mm        |
| 2   | <i>Legionella pneumophila</i> | 14±8 mm       | 14±8 mm        | 0±8 mm        | 24±8 mm        | 10±8 mm       | 10±8 mm        | 14±8 mm        | 10±8 mm       | 16±8 mm      | 28±8 mm        |
| 3   | <i>Enterobacter dissolves</i> | 30±5 mm       | 30±5 mm        | 28±5 mm       | 30±5 mm        | 14±5 mm       | 30±5 mm        | 30±5 mm        | 30±5 mm       | 30±5 mm      | 30±5 mm        |
| 4   | <i>Pseudomonas mallei</i>     | 30±0 mm       | 30±0 mm        | 30±0 mm       | 30±0 mm        | 30±0 mm       | 30±0 mm        | 30±0 mm        | 30±0 mm       | 30±0 mm      | 30±0 mm        |
| 5   | <i>Klebsiella pneumonia</i>   | 30±0 mm       | 30±0 mm        | 30±0 mm       | 30±0 mm        | 30±0 mm       | 30±0 mm        | 30±0 mm        | 30±0 mm       | 30±0 mm      | 30±0 mm        |
| 6   | <i>Aeromonas media</i>        | 30±0 mm       | 30±0 mm        | 30±0 mm       | 30±0 mm        | 30±0 mm       | 30±0 mm        | 30±0 mm        | 30±0 mm       | 30±0 mm      | 30±0 mm        |

Key: AU: Augmentin, CEP: Ceporex, CN: Gentamicin, CPX: Ciprofloxx, NA: Nalidixic Acid, OFX: Tarivid, PEF: Reflaxine, PN: Ampicillin, S: Stretomycin, SXT: Septrin

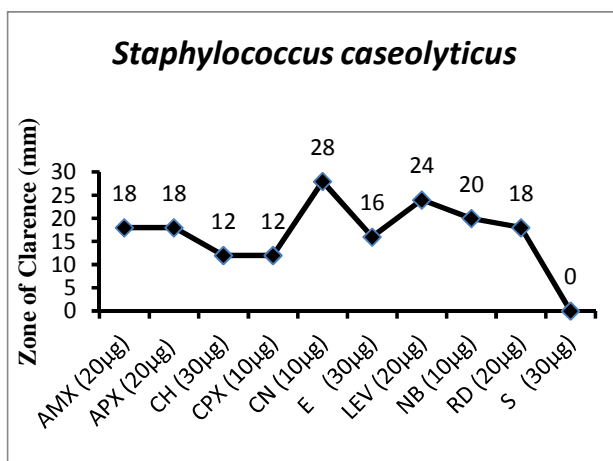


Fig. 3. Antibiotic susceptibility of *S. caseolyticus*

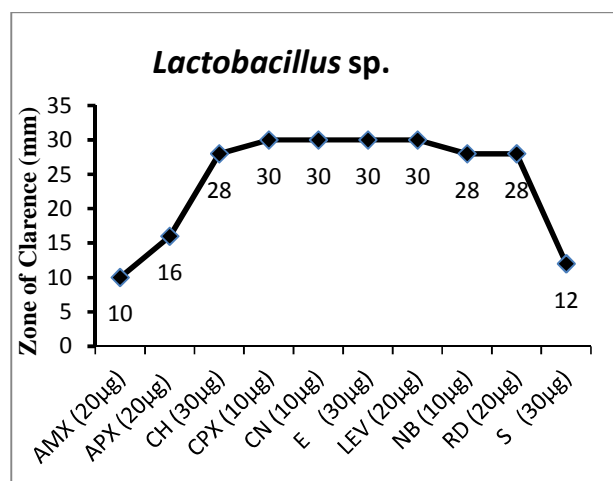


Fig. 4. Antibiotic susceptibility of *Lactobacillus sp.*

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

### 3.2 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  $F_{cal.}$  (24.3) is greater than  $F_{crit.}$  (2.7). Meanwhile, there is no significant difference in the mean antibiotic sensitivity of test antibiotics hence  $F_{cal.}$  (1.3) is less than  $F_{crit.}$  (2.2) at 95% confidence interval. Interpreted using CLSI standard, results revealed that all Gram-negative isolates except *Erwinia stewartii* were susceptible to the tested broad-spectrum antibiotics: gentamycin and streptomycin. The results also revealed that all the Gram-negative bacterial isolates (with the exception of two) were resistant to ampicillin while *Erwinia stewartii* showed

resistance to all the tested broad-spectrum and Gram-negative antibiotics except ceporex. Conversely, *Pseudomonas mallei*, *Klebsiella pneumonia* and *Aeromonas media* were susceptible to all the tested broad-spectrum and Gram-negative antibiotics by giving zones of inhibition of  $30 \pm 0$  mm in all the tested antimicrobials. This therefore means that infections from any of these three pathogens can effectively be taken care of using any of the antibiotics they were susceptible to. Figs. 5–10 show the antibiogram results of each Gram-negative bacterial isolate against the tested antibiotics.

According to [6], 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 [6].

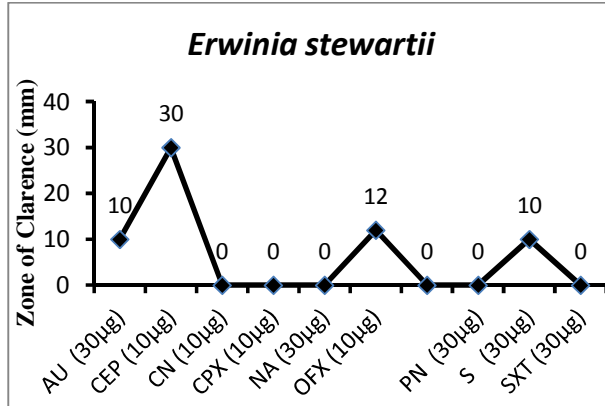


Fig. 5. Antibiotic susceptibility of *E. stewartii*

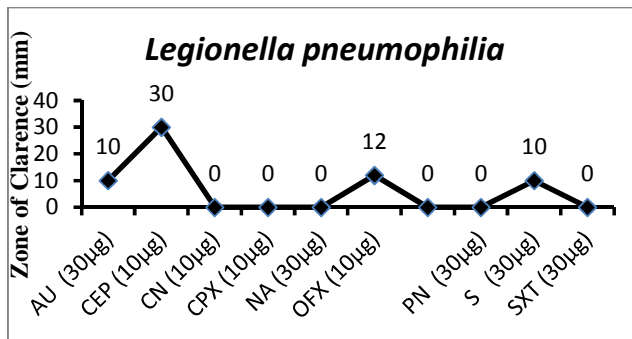


Fig. 6. Antibiotic susceptibility of *L. pneumophila*

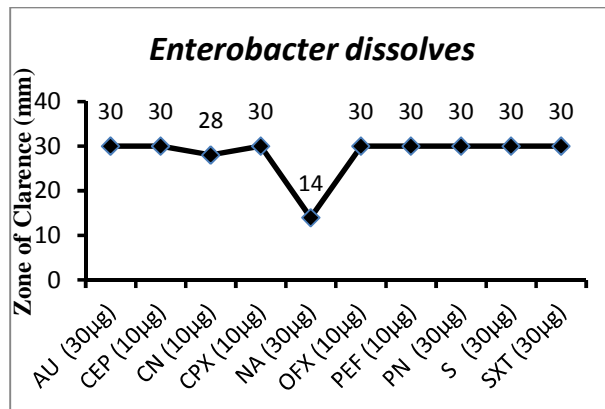


Fig. 7. Antibiotic susceptibility of *E. dissolves*

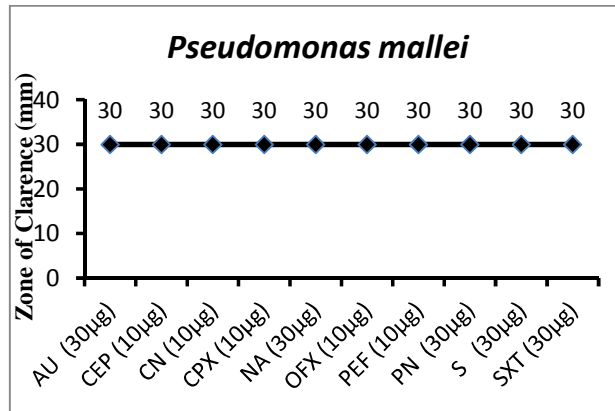


Fig. 8. Antibiotic susceptibility of *P. mallei*

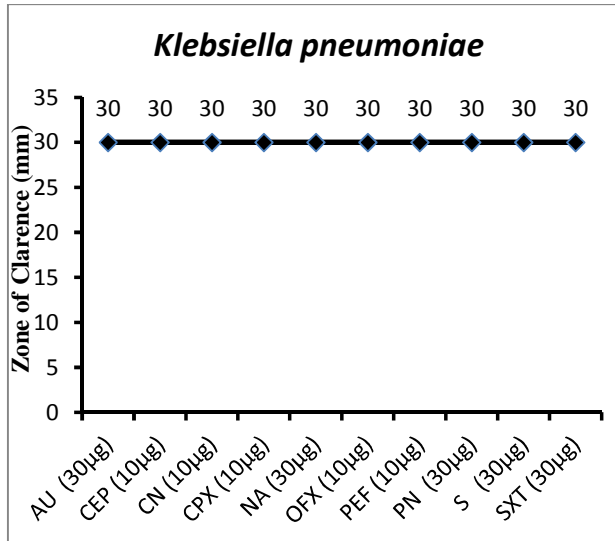


Fig. 9. Antibiotic susceptibility of *K. pneumoniae*

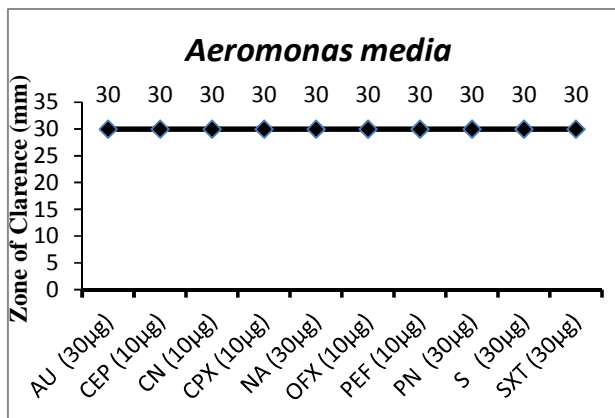


Fig. 10. Antibiotic susceptibility of *A. media*

Key: AU: Augmentin, CEP: Ceporex, CN: Gentamicin, CPX: Ciproflox, NA: Nalidixic Acid, OFX: Tarivid, PEF: Reflacine, PN: Ampicillin, S: Streptomycin, SXT: Seprin

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.

### 3.3 Antibiotic Susceptibility Profile Compared with CLSI Standards

Table 3 shows CLSI standards for antibiotic susceptibility test.

All Gram positive isolates were susceptible to levofloxacin even though it does not sustain a CSLI standard, but the zone of inhibition is quit fascinating. *Lactobacillus* sp 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 [5].

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 a drug of choice for the gram negative spp especially members of the Enterobacteriaceae family except for *E. stewartii* and *L. pneumophilla* which were resistant to ampicillin. According to [5], 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 likes of *Klebsiella* sp, *Pseudomonas* sp, *Enterobacter* sp,

*Erwinia* sp. According to [7], quinolones (like ciproflox) are synergistic with β-lactams (like ampicillin) and aminoglycosides. Usually, 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 [8]. Fluoroquinolones are preferred as initial agents for empiric therapy of UTI in areas where resistance is likely to be of concern [9,10]. This is evident in all Gram negative isolates except for *Legionella* and *Erwinia* spp. This is because of their high bacteriological and clinical cure rates, as well as low rates of resistance among most common uropathogens [11].

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 *Erwinia* sp 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 [12] 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 [13] and [14], enteropathogenic bacteria when not treated, are not only enterotoxigenic (as shown by members of the Enterobacteriaceae and the enterotoxigenic *Staphylococcus aureus*) but also induce some histological changes. Thus, the emergence of bacteria resistance to most of the commonly used antibiotics is of considerable medical significance because of public health

**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 |

[5]: Zone diameter of inhibition

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



**Table 4. Antibiotic susceptibility interpretations for gram-positive bacteria**

| S/N | Bacterial isolates                 | Antibiotic susceptibility interpretations for gram-positive bacteria |    |   |     |    |    |   |    |
|-----|------------------------------------|--|----|---|-----|----|----|---|----|
|     |                                    | CPX  | CN | S | LEV | NB | RD | E | CH |
| 1   | <i>Listeria monocytogenes</i>      | S  | R  | R | S   | R  | S  | S | S  |
| 2   | <i>Carnobacterium gallinarum</i>   | R  | S  | I | S   | I  | I  | I | I  |
| 3   | <i>Staphylococcus caseolyticus</i> | R  | S  | R | S   | S  | I  | I | R  |
| 4   | <i>Lactobacillus</i> sp.           | S  | S  | R | S   | S  | S  | S | S  |

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

**Table 5. Antibiotic susceptibility interpretations for gram-negative bacteria**

| S/N | Bacterial isolates             | Antibiotic susceptibility interpretations for gram-negative bacteria |                 |                  |                 |
|-----|--------------------------------|--|-----------------|------------------|-----------------|
|     |                                | Ciproflox (CPX)  | Gentamicin (CN) | Streptomycin (S) | Ampicillin (PN) |
| 1   | <i>Erwinia stewartii</i>       | R  | R               | R                | R               |
| 2   | <i>Legionella pneumophilia</i> | S  | R               | S                | R               |
| 3   | <i>Enterobacter dissolves</i>  | S  | S               | S                | R               |
| 4   | <i>Pseudomonas mallei</i>      | S  | S               | S                | R               |
| 5   | <i>Klebsiella pneumoniae</i>   | S  | S               | S                | R               |
| 6   | <i>Aeromonas media</i>         | S  | S               | S                | R               |

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

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, thereby bringing about the need for a new and more expensive antibiotics [15].

**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 demonstrated MICs or zone diameters fall in the range where specific microbial resistance mechanisms (eg,  $\beta$ -lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

#### 4. CONCLUSION

From the results obtained, it shows that broad-spectrum antibiotics such as gentamicin, ciproflox and streptomycin together with the Gram-negative antibiotics (except ceporex) are not good drugs of choice for *Erwinia stewartii* but are good for treating infections caused by *Enterobacter dissolves*, *Pseudomonas mallei*, *Klebsiella pneumonia* and *Aeromonas media*.

Although the broad-spectrum antibiotics were observed to be good for members of the Enterobacteriaceae family, *E. stewartii* infections cannot be treated with any of the tested broad-spectrum antibiotics. On the other hand, *Legionella pneumophilia* infections are not to be treated with gentamicin (a broad-spectrum antibiotic and an aminoglycoside) since the organism showed resistance to the antibiotic.

Thus, the presence of *E. stewartii* in the Malabor hostel tap water is a public health problem because of its resistance to all the tested antibiotics (multiple antibiotic resistance) and that will compromise the ability of health care professionals (clinicians) in treating infections caused by this pathogen.

Furthermore, infections resulting from ingestion of *Listeria monocytogenes* (a foodborne pathogen) can be treated with ciproflox, levofloxacin, rifampisin, erythromycin and chloramphenicol (based on its susceptibility result) but not with gentamicin, streptomycin or norfloxacin to which the organism showed resistance.

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.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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