

# Antibiogram of Bacteria Isolated from Tuberculosis Patients Attending Hospital within Izzi-Abakaliki in Ebonyi State

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## Abstract

The Antibiogram of the bacterial isolates from Tuberculosis (TB) patients attending hospitals within Izzi-Abakaliki was evaluated. The bacterial isolates were isolated and identified from the sputum samples according to microbiological principles while the antibiotics susceptibility test was done by disc diffusion method using Ofloxacin, Pefloxacin, Ciprofloxacin, amoxicillin/clavulanate, Gentamycin, Streptomycin, Cephalosporin, Cotrimoxazole, Nalidixic acid and Ampicillin. Bacteria isolated include 5 *E. coli* (25%), 3 *Streptococcus pyogenes* (15%), 2 *Streptococcus pneumoniae* (10%), 1 *Klebsiella* spp. (5%), 3 *Haemophilus influenza* (15%), 2 *Pseudomonas* (10%), 3 *Proteus* spp. (15%), 1 *Staphylococcus aureus* (10%). The result of Antibiogram shows that *E. coli* was 100% resistant to Amoxicillin/clavulanate and cotrimoxazole, followed by Streptomycin (80%) and 100% susceptible to Pefloxacin with inhibition zone diameter of 18 mm and 18 mm for Ofloxacin (60%). *S. pneumoniae* and *Klebsiella* spp. were highly resistant to Amoxicillin/clavulanate (100%), Gentamycin (100%) and Ampicillin (100%) and 100% susceptible to Pefloxacin with inhibition zone 18 mm, Ciprofloxacin (17 mm). *S. pyogenes* was resistant to streptomycin and Ceporex, with 100% sensitivity to Ofloxacin, Ciprofloxacin and Pefloxacin. *Pseudomonas* spp. and *S. aureus* were both 100% resistant

to all antibiotics except Ofloxacin, Ciprofloxacin, and Pefloxacin respectively. *Proteus* spp. was susceptible to Pefloxacin (100%), Ofloxacin (66.7%) and Ciprofloxacin (66.7%) but highly resistant to Streptomycin (100%) and Ampicillin (100%). *Haemophilus influenzae* were susceptible to Ofloxacin (100%) and Pefloxacin (100%), with high resistance to Amoxicillin/clavulanate (100%) and Ceporex (100%). From the study, Ofloxacin and Pefloxacin are susceptible to all bacteria isolated and are recommended for treatment of the bacterial infection with TB patient.

## Keywords

Tuberculosis, *pneumoniae*, Infection, Sputum, Antibiotics

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## 1. Introduction

Tuberculosis is among the Lower Respiratory Tract Infections (RTIs) that are frequently reported human infections, Lower Respiratory Tract Infections (LRTIs) generally account for almost 90% [1]. Unlike Upper Respiratory Tract Infections that are prevalently caused by viruses rather than bacteria. Lower Respiratory Tract Infections including tuberculosis are most commonly caused by bacterial pathogens [2]. It is also the most prevalent disease in humans globally. These diseases directly have annual mortality rate of about 7 million deaths yearly in persons of all ages. According to WHO, tuberculosis and acute lower respiratory tract infections constitute the two among the 6 leading causes of mortality in the world [3].

The most important complication among tuberculosis patients is caused by bacterial infection [4]. The extensive use of antibiotics and steroids has recently caused a widespread prevalence of bacterial pulmonary infection in patients [5]. Tuberculosis patients become susceptible to secondary bacterial infection because of many reasons. The major reason is the inhibition of human immunological defense system during the cause of active tuberculosis [6]. Several studies carried out worldwide report that the potent pathogens of the respiratory tracts are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., *Streptococcus pyogenes* and some other enteric Gram-negative bacteria [7] [8]. These bacteria are known to be the normal flora of the human respiratory tract. So, it is clear that most of the time, the infection is initiated by normal flora and secondary infection from other invading bacteria [9]. The probability of developing TB disease is much higher among individuals with medical conditions that weaken the immune system such as HIV/AIDS, diabetes, cancer, organ transplantation, renal disease, alcohol abuse, malnutrition, severe fungal infections, tobacco use, air pollution, malignancies, an aging population and many others [10]. According to WHO guidelines, there are two major groups of anti-TB drugs, Group-I comprising of four first-line drugs and Groups-2 comprising of second-line anti-TB drugs. The four first-line anti-TB agents that form the threshold of treatment regimen

for drug-susceptible TB in new patients (with active TB disease but who have not undergone prior TB treatment) include isoniazid, rifampicin, pyrazinamide, ethambutol [11]. Among the first-line drugs, rifampicin, isoniazid and pyrazinamide are bactericidal whereas ethambutol is bacteriostatic. More than 90% of patients with drug-sensitive TB remain curable in 6 months using oral administration of combinations of first-line drugs [12]. When the bacterial strain becomes resistant to one or more of these drugs, second-line drugs are used. These include streptomycin, kanamycin, fluoroquinolones, ethionamide, and p-aminosalicylic acid. Generally, second-line drugs are less effective and more toxic compared to the first-line drugs [13]. Drug-resistant TB strains emerging in both hospitals and communities exhibit different levels of drug resistance such as Rifampicin Resistance (RR), Multidrug Resistance (MDR) and Extensive Drug Resistance (XDR) [14] [15]. RR-TB is resistant only towards rifampicin and not to other first-or second-line drugs. MDR-TB is defined as resistance to at least two most powerful anti-TB drugs, isoniazid and rifampicin [16]. It was found that 558,000 TB cases reported worldwide in 2017 were rifampicin resistant (RR-TB) and of these, 82% were Multidrug-resistant TB (MDR-TB). XDR-TB is a form of TB which is defined as MDR-TB as well as resistance to at least one drug from each of the two important classes of second-line agents (fluoroquinolones and injectable) used in MDR treatment regimen. This implies that it involves resistance to isoniazid and rifampicin, in addition to resistance towards any of the fluoroquinolones (such as levofloxacin or moxifloxacin) [17]. Many works have been reported on isolation of different bacteria from sputum sample of tuberculosis patients alongside its antibiogram and drug resistance mechanism. Hence, this present study was designed to evaluate the antibiogram of bacteria isolated from tuberculosis patients attending hospital within Izzi-Abakaliki, Nigeria.

## 2. Materials and Method

### *Study Area*

This study was conducted within the Izzi-Abakaliki in Ebonyi State. The study was undertaken and analyzed at Alex Ekwueme Federal University Ndufu-Alike Biotechnology Laboratory.

### *Sample Collection*

An early morning expectorated sputum sample was collected in a clean, sterile, leak proof containers from all patients included in the study. Patients presented before a physician with signs and symptoms such as cough lasting more than two weeks after commencing antibiotics, fever, night sweats, chest pain and other symptoms suggestive of lower respiratory tract infections and were thus sent for AFB testing. Samples were collected in the morning. Patients were asked not to eat 1 hour before expectoration, to rinse their mouth with sterile water and then to cough deeply to expectorate into a provided sterile container. The quality of sputum samples was assessed macroscopically. Watery and non-purulent sputa were considered suitable for further processing. All unsuitable specimens were

discarded and new specimens collected.

#### *Isolation and Identification of Bacteria Isolates*

##### *Culturing*

Sputum specimens were inoculated onto chocolate agar. The inoculated plates were incubated at 37°C for 24 hours aerobically. The plates were sub-cultured to get a pure culture.

##### *Gram Staining*

Little portion of the bacterial pure culture was collected using a sterile wire loop and it was smeared on a clean microscopic slide which was used for Gram staining applying all Gram staining principles.

### 3. Results

The various colonies formed by the isolates on chocolate agar, gram staining microscopy, and various biochemical test carried out, give a result that 5 *E. coli*, 3 *S. pyogenes*, 3 *Haemophilus influenzae*, 3 *proteus* spp., 2 *S. pneumoniae*, 2 *Pseudomonas* spp., 1 *Klebsiella* spp. and 1 *S. aureus* were isolated from the sputum. The number of gram-negative bacteria mostly rods 14 (*E. coli*, *Haemophilus influenzae*, *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp.) and gram-positive bacteria were 6 with cocci in chains (*Streptococcus pyogenes* and *Streptococcus pneumoniae*) and cocci in clusters (*Staphylococcus aureus*). Oxidase test shows that *Haemophilus influenzae* is positive while the rest are negative. All isolates were positive to glucose and lactose as a result of change in the indicator (phenol red) used during the test with some producing gas (bubbles) as seen in **Table 1** below.

**Table 2** below shows *Escherichia coli* has the highest percentage of bacteria present, followed by *S. pyogenes* (15%), *Haemophilus influenzae* (15%), *Proteus* spp. (15%), *S. pneumoniae* (10%), *Pseudomonas* spp. (10%), *Klebsiella* spp. (5%) and *S. aureus* (5%). There is no significant difference in the percentage of occurrence of isolates among the group ( $P < 0.05$ ).

**Table 1.** Colony appearance, gram staining and biochemical test.

| S/N | Macroscopy                         | Microscopy          | Gram Staining | Oxidase | Catalase | Indole | Voges Proskauer | Glucose | Lactose | Suspected Organism            |
|-----|------------------------------------|---------------------|---------------|---------|----------|--------|-----------------|---------|---------|-------------------------------|
| 1   | White, Creamy, Smooth              | Rod in chains       | -             | -       | +        | +      | +               | Ag      | Ag      | <i>Escherichia coli</i>       |
| 2   | White, creamy                      | Cocci in chains     | +             | -       | -        | +      | -               | Ag      | A       | <i>Streptococcus pyogenes</i> |
| 3   | White, mucoid, smooth              | Rod in chain        | -             | -       | +        | +      | -               | Ag      | A       | <i>Escherichia coli</i>       |
| 4   | White, creamy, irregular surface   | Long rods in chains | -             | -       | +        | +      | +               | Ag      | Ag      | <i>Escherichia coli</i>       |
| 5   | Light brown, creamy                | Rod in chain        | -             | -       | +        | +      | -               | Ag      | A       | <i>Escherichia coli</i>       |
| 6   | Buff, smooth, mucoid and irregular | Long rods in chains | +             | -       | +        | -      | -               | Ag      | Ag      | <i>Proteus</i> spp.           |

## Continued

|    |                                 |                               |   |   |   |   |   |    |    |                                 |
|----|---------------------------------|-------------------------------|---|---|---|---|---|----|----|---------------------------------|
| 7  | White, filamentous, wrinkled    | Cocci in short chains         | + | - | - | - | - | A  | Ag | <i>Streptococcus pyogenes</i>   |
| 8  | Buff, irregular, mucoid         | Small rods                    | - | - | + | - | - | A  | A  | <i>pseudomonas</i>              |
| 9  | Creamy and clustered            | Rod in chains                 | - | - | + | - | - | A  | A  | <i>Proteus</i> spp.             |
| 10 | Brownish, mucoid                | Small rods in clusters        | - | - | + | - | - | A  | A  | <i>Pseudomonas</i> spp.         |
| 11 | Buff, filamentous, dull surface | Cocci in chains               |   | - | - | - | - | Ag | Ag | <i>Streptococcus pneumoniae</i> |
| 12 | White, mucoid                   | Long rods                     | - | - | + | - | - | Ag | Ag | <i>Proteus</i> spp.             |
| 13 | White, creamy, smooth           | Rod                           | - | - | + | + | + | A  | Ag | <i>Escherichia coli</i>         |
| 14 | White, creamy, smooth           | Cocci in clusters             | + | - | + | - | + | Ag | Ag | <i>Staphylococcus aureus</i>    |
| 15 | White, mucoid, smooth           | Rod                           | - | - | + | + | + | Ag | A  | <i>Klebsiella</i> spp.          |
| 16 | White, mucoid, rough            | Long rod with terminal spores | - | + | + | - | - | Ag | A  | <i>Haemophilus influenzae</i>   |
| 17 | Buff, creamy                    | Cocci in pairs                | + | - | - | - | - | Ag | Ag | <i>Streptococcus pneumoniae</i> |
| 18 | Brown with distinct colony      | Rod shape in chains           | + | + | - | - | - | Ag | A  | <i>Streptococcus pyogenes</i>   |
| 19 | White creamy rough              | Rod with terminal spores      | - | + | + | + | - | Ag | Ag | <i>Haemophilus influenzae</i>   |
| 20 | White, creamy, smooth           | Rod with terminal spores      | + | + | + | + | - | A  | Ag | <i>Haemophilus influenzae</i>   |

**Table 2.** The occurrence of bacteria isolates from suspected TB patients.

| S/N          | Isolates                        | Occurrence | % of Occurrence | $\chi^2$ | P-value |
|--------------|---------------------------------|------------|-----------------|----------|---------|
| 1            | <i>Escherichia coli</i>         | 5          | 25%             | 10.746   | 0.1501  |
| 2            | <i>Streptococcus pyogenes</i>   | 3          | 15%             |          |         |
| 3            | <i>Streptococcus pneumoniae</i> | 2          | 10%             |          |         |
| 4            | <i>Klebsiella</i> spp.          | 1          | 5%              |          |         |
| 5            | <i>Haemophilus influenzae</i>   | 3          | 15%             |          |         |
| 6            | <i>Pseudomonas</i> spp.         | 2          | 10%             |          |         |
| 7            | <i>Proteus</i> spp.             | 3          | 15%             |          |         |
| 8            | <i>Staphylococcus aureus</i>    | 1          | 5%              |          |         |
| <b>Total</b> |                                 | 20         |                 |          |         |

The antibiotics susceptibility pattern of *E. coli* and *S. pyogenes* in **Table 3** below shows that *E. coli* has significant higher percentage susceptible to CN (60%), S (20%), CEP (40%), and PN (20%) than *S. pyogenes* ( $P < 0.05$ ) while *S. pyogenes* has significant higher percentage susceptible to OFX (100%), CPX (100%), A/C (33.3%), and SXT (33.3%) than *E. coli* ( $P < 0.05$ ). Conversely, *E. coli* has significant higher percentage resistant to OFX (40%), CPX (60%), A/C (100%), and SXT (100%) than *S. pyogenes* ( $P < 0.05$ ) while *S. pyogenes* has significant higher percentage resistant to CN (66.7%), S (100%), CEP (100%), and PN (100%) than *E. coli* ( $P < 0.05$ ). However, *E. coli* and *S. pyogenes* are not significantly difference in their percentage susceptible and resistant to PEF and NA ( $P > 0.05$ ).

**Table 4** below shows that the *Streptococcus pneumoniae* has significant higher percentage sensitivity to CPX (100%), S (50%), NA (50%), and SXT (50%) than *Haemophilus influenzae* ( $P < 0.05$ ) while *Haemophilus influenzae* has significant higher percentage sensitivity to OFX (100%), and CN (33.3%), than *Streptococcus pneumoniae* ( $P < 0.05$ ). Conversely, the *Streptococcus pneumoniae* has significant higher percentage resistant to OFX (50%), and CN (100%) than *Haemophilus influenzae* ( $P < 0.05$ ) while *Haemophilus influenzae* has significant higher percentage resistant to CPX (66.7%), S (66.7%), NA (66.7%), and SXT (100%) than *Streptococcus pneumoniae* ( $P < 0.05$ ). However, *Streptococcus pneumoniae* and *Haemophilus influenzae* are not significantly difference in their percentage sensitivity and resistant to PEF, AU, CEP and PN ( $P > 0.05$ ).

**Table 5** below shows that the *Proteus* spp. has significant higher percentage sensitivity to CPX (66.7%), AU (33.3%), CN (33.3%), CEP (33.3%), NA (66.7%), and SXT (33.3%) than *Pseudomonas* spp. ( $P < 0.05$ ) while *Pseudomonas* spp. has significant higher percentage sensitivity to OFX (100%) than *Proteus* spp. ( $P < 0.05$ ). Conversely, the *Proteus* spp. has significant higher percentage resistant to OFX (33.3%) than *Pseudomonas* spp. ( $P < 0.05$ ) while *Pseudomonas* spp. has significant higher percentage resistant to CPX (100%), AU (100%), CN (100%), CEP (100%), NA (50%) and SXT (100%) than *Proteus* spp. ( $P < 0.05$ ). However, *Proteus* spp. and *Pseudomonas* spp. are not significantly difference in their percentage sensitivity and resistant to PEF, S, and PN ( $P > 0.05$ ).

**Table 3.** Antibiotic sensitivity pattern for *Escherichia coli* and *Streptococcus pyogenes*.

| Antibiotics | Conc of disc ( $\mu\text{g}$ ) | <i>Escherichia coli</i> |           |           |           | <i>Streptococcus pyogenes</i> |           |           |           | $\chi^2$ | P-value |
|-------------|--------------------------------|-------------------------|-----------|-----------|-----------|-------------------------------|-----------|-----------|-----------|----------|---------|
|             |                                | Sensitive               | Sensitive | Resistant | Resistant | Sensitive                     | Sensitive | Resistant | Resistant |          |         |
| OFX         | 10                             | 3                       | 60        | 2         | 40        | 3                             | 100       | 0         | 0         | 50.00    | <0.001  |
| PEF         | 10                             | 5                       | 100       | 0         | 0         | 3                             | 100       | 0         | 0         | 0.000    | 1.000   |
| CPX         | 10                             | 2                       | 40        | 3         | 60        | 3                             | 100       | 0         | 0         | 85.71    | <0.001  |
| A/C         | 30                             | 0                       | 0         | 5         | 100       | 1                             | 33.3      | 2         | 66.7      | 39.52    | <0.001  |
| CN          | 10                             | 3                       | 60        | 2         | 40        | 1                             | 33.3      | 2         | 66.7      | 14.65    | <0.001  |
| S           | 30                             | 1                       | 20        | 4         | 80        | 0                             | 0         | 3         | 100       | 22.22    | <0.001  |
| CEP         | 10                             | 2                       | 40        | 3         | 60        | 0                             | 0         | 3         | 100       | 50.00    | <0.001  |

## Continued

|     |    |   |    |   |     |   |      |   |      |       |        |
|-----|----|---|----|---|-----|---|------|---|------|-------|--------|
| NA  | 30 | 2 | 40 | 3 | 60  | 1 | 33.3 | 2 | 66.7 | 1.06  | 0.304  |
| SXT | 30 | 0 | 0  | 5 | 100 | 1 | 33.3 | 2 | 66.7 | 39.52 | <0.001 |
| PN  | 30 | 1 | 20 | 4 | 80  | 0 | 0    | 3 | 100  | 22.22 | <0.001 |

**Table 4.** Antibiotic sensitivity pattern for *Streptococcus pneumoniae* and *Haemophilus influenzae*.

| Antibiotics | Conc of disc ( $\mu\text{g}$ ) | <i>Streptococcus pneumoniae</i> |           |           |           | <i>Haemophilus influenzae</i> |           |           |           | $\chi^2$ | P-value |
|-------------|--------------------------------|---------------------------------|-----------|-----------|-----------|-------------------------------|-----------|-----------|-----------|----------|---------|
|             |                                | Sensitive                       | Sensitive | Resistant | Resistant | Sensitive                     | Sensitive | Resistant | Resistant |          |         |
| OFX         | 10                             | 1                               | 50        | 1         | 50        | 3                             | 100       | 0         | 0         | 66.67    | <0.001  |
| PEF         | 10                             | 2                               | 100       | 0         | 0         | 3                             | 100       | 0         | 0         | 0.000    | 1.000   |
| CPX         | 10                             | 2                               | 100       | 0         | 0         | 1                             | 33.3      | 2         | 66.7      | 100.75   | <0.001  |
| AU          | 30                             | 0                               | 0         | 2         | 100       | 0                             | 0         | 3         | 100       | 0.000    | 1.000   |
| CN          | 10                             | 0                               | 0         | 2         | 100       | 1                             | 33.3      | 2         | 66.7      | 39.52    | <0.001  |
| S           | 30                             | 2                               | 50        | 2         | 50        | 1                             | 33.3      | 2         | 66.7      | 5.95     | 0.015   |
| CEP         | 10                             | 0                               | 0         | 3         | 100       | 0                             | 0         | 3         | 100       | 0.000    | 1.000   |
| NA          | 30                             | 2                               | 50        | 2         | 50        | 1                             | 33.3      | 2         | 66.7      | 5.95     | 0.015   |
| SXT         | 30                             | 2                               | 50        | 2         | 50        | 1                             | 33.3      | 2         | 66.7      | 5.95     | 0.015   |
| PN          | 30                             | 0                               | 0         | 3         | 100       | 0                             | 0         | 3         | 100       | 0.000    | 1.000   |

**Table 5.** Antibiotic susceptibility pattern for *Proteus* spp. and *Pseudomonas* spp.

| Antibiotics | Conc of disc ( $\mu\text{g}$ ) | <i>Proteus</i> spp. |           |           |           | <i>Pseudomonas</i> spp. |           |           |           | $\chi^2$ | P-value |
|-------------|--------------------------------|---------------------|-----------|-----------|-----------|-------------------------|-----------|-----------|-----------|----------|---------|
|             |                                | Sensitive           | Sensitive | Resistant | Resistant | Sensitive               | Sensitive | Resistant | Resistant |          |         |
| OFX         | 10                             | 2                   | 66.7      | 1         | 33.3      | 2                       | 100       | 0         | 0         | 39.52    | <0.001  |
| PEF         | 10                             | 3                   | 100       | 0         | 0         | 2                       | 100       | 0         | 0         | 0.000    | 1.000   |
| CPX         | 10                             | 2                   | 66.7      | 1         | 33.3      | 0                       | 0         | 2         | 100       | 100.75   | <0.001  |
| AU          | 30                             | 1                   | 33.3      | 2         | 66.7      | 0                       | 0         | 2         | 100       | 39.52    | <0.001  |
| CN          | 10                             | 1                   | 33.3      | 2         | 66.7      | 0                       | 0         | 2         | 100       | 39.52    | <0.001  |
| S           | 30                             | 0                   | 0         | 3         | 100       | 0                       | 0         | 2         | 100       | 0.000    | 1.000   |
| CEP         | 10                             | 1                   | 33.3      | 2         | 66.7      | 0                       | 0         | 2         | 100       | 39.52    | <0.001  |
| NA          | 30                             | 2                   | 66.7      | 1         | 33.3      | 1                       | 50        | 1         | 50        | 5.92     | 0.015   |
| SXT         | 30                             | 1                   | 33.3      | 2         | 66.7      | 0                       | 0         | 2         | 100       | 39.52    | <0.001  |
| PN          | 30                             | 0                   | 0         | 3         | 100       | 0                       | 0         | 2         | 100       | 0.000    | 1.000   |

The result in **Table 6** below showing *Klebsiella* spp. and *Staphylococcus aureus* shows that *Klebsiella* spp. has significant higher percentage sensitivity to CPX (100%) than *Staphylococcus aureus* ( $P < 0.05$ ) while *Staphylococcus aureus* has significant higher percentage sensitivity to CN (100%) than *Klebsiella* spp. ( $P < 0.05$ ). Conversely, the *Klebsiella* spp. has significant higher percentage resistant to CN (100%) than *Staphylococcus aureus* ( $P < 0.05$ ) while *Staphylococcus aureus*

has significant higher percentage resistant to CPX (100%) than *Klebsiella* spp. ( $P < 0.05$ ). However, *Klebsiella* spp. and *Staphylococcus aureus* are not significantly difference in their percentage sensitivity and resistant to OFX, PEF, AU, S, CEP, NA, SXT, and PN ( $P > 0.05$ ).

**Table 6.** Antibiotic susceptibility of *Klebsiella* spp. and *Staphylococcus aureus*.

| Antibiotics | Conc of disc( $\mu$ g) | <i>Klebsiella</i> spp. |           |           |           | <i>Staphylococcus aureus</i> |           |           |           | $\chi^2$ | P-value |
|-------------|------------------------|------------------------|-----------|-----------|-----------|------------------------------|-----------|-----------|-----------|----------|---------|
|             |                        | Sensitive              | Sensitive | Resistant | Resistant | Sensitive                    | Sensitive | Resistant | Resistant |          |         |
| OFX         | 10                     | 1                      | 100       | 0         | 0         | 1                            | 100       | 0         | 0         | 0.000    | 1.000   |
| PEF         | 10                     | 1                      | 100       | 0         | 0         | 1                            | 100       | 0         | 0         | 0.000    | 1.000   |
| CPX         | 10                     | 1                      | 100       | 0         | 0         | 0                            | 0         | 1         | 100       | 200.0    | <0.001  |
| AU          | 30                     | 0                      | 0         | 1         | 100       | 0                            | 0         | 1         | 100       | 0.000    | 1.000   |
| CN          | 10                     | 0                      | 0         | 1         | 100       | 1                            | 100       | 0         | 0         | 200.0    | <0.001  |
| S           | 30                     | 0                      | 0         | 1         | 100       | 0                            | 0         | 1         | 100       | 0.000    | 1.000   |
| CEP         | 10                     | 0                      | 0         | 1         | 100       | 0                            | 0         | 1         | 100       | 0.000    | 1.000   |
| NA          | 30                     | 1                      | 100       | 0         | 0         | 1                            | 100       | 0         | 0         | 0.000    | 1.000   |
| SXT         | 30                     | 0                      | 0         | 1         | 100       | 0                            | 0         | 1         | 100       | 0.000    | 1.000   |
| PN          | 30                     | 0                      | 0         | 1         | 100       | 0                            | 0         | 1         | 100       | 0.000    | 1.000   |

#### 4. Discussion

The result from this study shows that TB patients have bacterial growth in their sputum. Bacteria isolated from the sputum samples of TB patients include 5 *E. coli*, 1 *Klebsiella* spp., 3 *Proteus* spp., 3 *Haemophilus influenza*, 3 *S. pyogens*, 2 *S. pneumonia*, 2 *Pseudomonas* spp. and 1 *S. aureus* with prevalence of 25%, 5%, 15%, 15%, 15%, 10%, 10%, 5% within Izzi-Abakaliki, Ebonyi State. Susceptibility of the isolates were tested against 10 Antibiotics; Ofloxacin (10  $\mu$ g), Pefloxacin (10  $\mu$ g), Ciprofloxacin (10  $\mu$ g), Amoxicillin/clavulanate (30  $\mu$ g) Gentamycin (10  $\mu$ g), Streptomycin (30  $\mu$ g), Cephalosporin (10  $\mu$ g), Cotrimoxazole (30  $\mu$ g), Nalidixic acid (30  $\mu$ g) and Ampicillin (30  $\mu$ g).

*E. coli* was the most predominant bacteria isolate recovered in 25% of the total sample showing high resistance to Amoxicillin/clavulanate (100%) and Cotrimoxazole (100%), Streptomycin (80%), Ampicillin (80%), Ciprofloxacin (60%) with 100% sensitivity to Pefloxacin, Ofloxacin (60%) and Gentamycin (60%). From the study of Michael (2013), 44 *E. coli* (22%) were isolated from 200 sputum sample in Ibadan, which was sensitive to Amoxicillin/clavulanate (100%) and Ofloxacin (90.91%), resistant to Streptomycin (27.27%) and Ampicillin (18.18%). The isolation of *Escherichia coli* in his work was a result of high Nitrogen content in the media used. Nitrogen content supports growth of *Escherichia coli*. Also, the isolation of the *Staphylococcus aureus* is because of the mixture of some salivary content during the extraction of the sputum through the buccal cavity which may be a prominent reason why *S. aureus* (5%) was isolated in this work because most patients tend to expel saliva instead of sputum during sample collection. In

contrast it also showed that Ampicillin and Streptomycin cannot be used in the treatment of disease associated with *E. coli*. *H. influenzae* was isolated more commonly in association with *Streptococcus pneumoniae* and *Klebsiella pneumoniae* in a study conducted in South India by Shenoy *et al.* (2016) [18]. Majority of the *Haemophilus* isolates (14884.6%) were found susceptible to the antibiotics tested. Maximum resistance was observed to Ampicillin (17, 9.71%) followed by Amoxicillin-clavulanic acid (9, 5.14%) and Ceftriaxone (1, 0.57%) contrasting with the result above *H. influenzae* (15%) was seen resistant to Augmentin (100%), Cephalosporin (100%) and Ampicillin (100%), indicating that Ampicillin cannot be used for its treatment while antibiotics like Ofloxacin and Pefloxacin were 100% sensitive as indicated in **Table 4** above.

*S. pneumoniae* and *S. pyogenes* from the result above are susceptible to both Pefloxacin (100%) and Ciprofloxacin (100%). Zeinab *et al.* (2020) expressed *S. pyogenes* as a pathogen that cause serious respiratory disease such as meningitis and pneumonia which is sensitivity to Amoxicillin/clavulanate (81%) and Ampicillin (43%) while being resistant to Gentamycin in their study [19]. It was observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment. In another instant the high resistance of *Klebsiella pneumoniae* isolates to commonly used antibiotics is probably due to some factors ranging from the use of fake antibiotics, abuse and misuse of those antibiotics found commonly in circulation among the general individual and health resources centers, in this study *klebsiella* spp. was susceptible to Ofloxacin and Ciprofloxacin as shown in **Table 6** which is similar to the study carried out in Kano [20] that indicated the overall susceptibility by *K. pneumoniae* over antibiotic used.

*Pseudomonas* spp. is considered opportunistic bacterial pathogens that rarely cause disease in healthy persons. A study reveals that 12% (61/150) of the total TB patients were found co-infected with *Pseudomonas aeruginosa* [21]. *Pseudomonas* spp. in this study shows great resistance to 7 of the antibiotics used with make it a very serious and complicated among other bacteria isolates in this work as in **Table 5**.

Results of many years ago reported decreasing susceptibility of *Proteus* spp. to Ciprofloxacin x from 100 to 46% over a 6-year period in their institution; are in the same trend with our results reporting 66.7% susceptibility of *Proteus* spp. Isolates [22]. In a similar work, results showed that isolated *Proteus* spp. from 74 sputum sample were resistant to Amplicin which from the result above correlate to Ampicillin at 100% resistant as showed in **Table 5**. *S. pneumoniae* was seen as resistant to gentamycin (80%) in a result from Uzma *et al.* (2005), also adding that *S. pneumoniae* and *S. pyogenes* are seen to be on a verge of developing resistance to the cheaper antimicrobial agents. In this study, both were seen sensitive to Pefloxacin (100%) and Ciprofloxacin (100%), with *S. pneumoniae* resistant to gentamycin and Ampicillin as in **Table 4** and **Table 5**. In this study, all the bacteria isolated from the sputum were highly susceptible to Pefloxacin (100%), Ofloxacin (80%), and Ciprofloxacin (55%). Also showing high resistance to Ampicillin

(95%), Amoxicillin/clavulanate (90%), Streptomycin (85%), Cephalosporin (85%) and Cotrimoxazole (80%) as indicated in **Table 5** above.

## 5. Conclusions

In this study, it was seen that all of the TB patients whose sputum was analyzed for this work were associated with different bacteria isolates with *E. coli* having the highest frequency. Some of these patients as a result of the secondary infection caused by the bacteria pathogens isolated are prone to most of the respiratory diseases such as bronchitis, meningitis, pneumonia, etc. Ofloxacin and Pefloxacin can be administered as the results of the study shown and carefully observe the patient for further improvement.

Microorganisms are ubiquitous, proper health hygiene should be observed as those with respiratory disease should use frequent facemask in covering their nostrils because it is communicable via inhalation of the droplet expelled from an infected individual. Proper control measures should be ensured in hospitals because Lower Respiratory Infections are mostly Nosocomial infections. Also, the use of antibiotics should only be recommended by a doctor after a series of tests have been done to control antibiotic resistance to a bacterial infection.

## Ethical Approval and Informed Consent

Ethical clearance with reference number (EBSHREC/0310/HC/99) was obtained from Ebonyi State Ministry of Health. All participants were duly informed of the objectives of the study and the protocol for sample collection. All participants signed an informed consent form were signed. Participation was voluntary.

## Authors' Contribution

OEN and IDC conceptualized the study, OOJ designed the study, NOL participated in the field work, K-MOO participated in data collection. OEN prepared the initial draft of the manuscript. All authors contributed to the development of the final manuscripts and approved its submission.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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