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Original article

# Prevalence and Antibiotic Resistance Profile of *Streptococcus pneumoniae* isolated from Patients attending Selected Federal Hospitals in Parts of North Central Nigeria

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## ABSTRACT

The study investigated the prevalence and antibiotic resistance profile of *Streptococcus pneumoniae*, within the study area. The screening of 768 specimens collected from the study areas revealed that, 29.2% of the samples were positive for *Streptococcus pneumoniae*, while *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Moraxella catarrhalis* were identified in 10.2%, 14.8%, and 10% of the samples, respectively. Additionally, *Haemophilus influenzae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were observed in 4.4%, 19.9%, 8.2%, and 3.3% of the samples, respectively. The prevalence rate of *S. pneumoniae* within the study locations were 30.6%, 26.1%, 29.2% and 31.5% from UATH, Keffi, Bida and Lokoja centres respectively. The highest antibiotic resistance rates shown by *Streptococcus pneumoniae* were observed against the more frequently consumed antibiotics such as cefotaxime, ampicillin, tetracycline, co-trimoxazole, with average resistance rates of 53.8%, 53.7%, 53.7%, and 53% respectively. Conversely, the lowest resistance rates of 11.3%, 33% and 37.5% were observed against levofloxacin, cephalexin and linezolid, respectively. The study observed an average multidrug resistance rate (MDR) of 59.7% with the highest MDR rate of 69.02% recorded from Gwagwalada center. Analysis, of infection rates by age distribution, showed that the highest infection rate was among the pediatric patients, below 5 years (74.2%), followed by the age bracket above 65 years (66.4%). There was a higher infection rate among females (66.1%), compared to males (52.4%). Patients with tertiary education exhibited a slightly lower infection rate of 24.6%, in contrast to 32.9% and 27 % for those without formal education and with secondary education, respectively. Slightly higher infections rates were observed in the months of December and January. This was probably due to the cold, windy and drier weather condition, which encouraged sneezing by carriers and so increasing chances of the disease spreading to healthy persons.

**Keywords:** Prevalence, demographic, pathogens, susceptibility, antibiotic resistance

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## INTRODUCTION

*Streptococcus pneumoniae* is an important human pathogen that is a major cause of morbidity and mortality especially in children below five years, the elderly who are above sixty-five years of age and the immuno-compromised [1]. Younger people are more frequently colonized and serve as reservoirs for the pathogen and play a significant role in spreading the pathogen to healthy people, [2]. Colonization rate in children can be between 27% to 65% [3, 4].

The incidence of pneumococcal diseases in developing countries is significantly higher than in developed countries due to difference in economic and medical conditions [5,6]. The pathogen has been identified as one of the bacteria of international concern and it is responsible for a number of diseases such as bacteremia, meningitis, bacterial pneumonia, otitis media, bronchitis and sinusitis [6].

Infections caused by *S. pneumoniae* can be divided into two categories. These are mucosal infections such as otitis media and sinusitis as well as invasive infections such as septicemia, meningitis and pneumonia [7]. Bacterial pneumonia is one of the commonest pneumococcal infections and can again be divided into two groups depending on where the infection is contracted [4]. Similarly, pneumonia contracted from the community is referred to as community acquired pneumonia(CAP) while pneumonia that develops in an individual during or following a stay in the hospital or healthcare facility is called health care associated pneumonia(HAP), [4].

*Streptococus pneumoniae* can be transmitted through respiratory droplets from healthy individuals carrying the pathogens in their nasopharynx or from people with pneumococcal disease [8]. Several risk factors are believed to be associated with pneumococcal infections such as race, previous antibiotic therapy, age, immunodeficiency as well as daycare attendance [9]. The emergence of multi-drug resistant strains of the pathogen has made the treatment of pneumococcal infections more difficult [10]. Bacterial pneumonia is responsible for over one million deaths annually across the world with a higher percentage of these deaths occurring in developing countries such as Nigeria [5,6].

The first clinical diagnosis of penicillin-resistant *Streptococcus pneumoniae* was reported in New Guinea in 1967 while multi-resistant strains were first reported from South Africa in 1977 [11]. Experts worry that the phenomenon of multi-drug resistance might wipe out the gains achieved by use of antibiotics and take us back to the pre-antibiotic era [12]. Consequently, the effectiveness of antimicrobials has been hindered by the resurgence of multi-drug resistance among the pneumococci [10].

There are over 90 serotypes of *S. pneumoniae* which vary in their geographical distribution and pathogenicity but only about 20 are more frequently linked to pneumococcal infections [13,14]. Besides, over 60% of pneumococcal infections are caused by the 10 most common isolates [11]. There is evidence to indicate that significant gaps still exist in standard diagnostic procedures, technical capacity, surveillance and data sharing from hospital settings in Africa [15]. The rising cases of multi-drug resistance coupled with these gaps in local capacity, makes treatment of

pneumococcal infections sometimes ineffective especially in developing countries.

Multi-drug resistance (MDR) is a widespread global problem. Strains of *Streptococcus pneumoniae* resistant to up to three distinct classes of antimicrobials are considered as multi-drug resistant [16]. The extent of multi-drug resistance varies from region to region and may not be fully understood in developing countries such as Nigeria due to limited research and publications on the subject [15]. The introduction of Pneumococcal Conjugate Vaccines (PCVs) has led to a significant reduction in the incidence of pneumococcal diseases in countries where the vaccines have been fully incorporated into their national immunization programmes.

However, in Nigeria, the Pneumococcal Conjugate Vaccine is yet to be fully integrated into the National Policy on Immunization. This implies that effective treatment of pneumococcal infections especially those caused by resistant strains remains a challenge. To improve on the effectiveness of antibiotic therapy, there is the need for knowledge of local antibiotic resistance patterns from regular up-to-date surveillance. Unfortunately, data on these resistance patterns are either limited, unavailable or out-of-date.

The aim of the research was to determine the antibiotic resistance profile of *Streptococcus pneumoniae* isolated from patients attending selected federal tertiary hospitals across parts of North Central Nigeria

## MATERIALS AND METHODS

### Description of the Study Area

The multicenter study spanned four distinct geographical regions in North-Central Nigeria, encompassing three states and the Federal Capital Territory (FCT). The three states included Nassarawa, Kogi and Niger. The investigation was conducted at four federal hospitals, situated across the region: Federal Medical Center Keffi, Federal Medical Center Lokoja, Federal Medical Center Bida and the University of Abuja Teaching Hospital (UATH).

Keffi, situated in the current Nasarawa state, Nigeria, is a local government area with a population of approximately 85,000 residents, according to the 2016 census figures. The Federal Medical Center Keffi came into existence in April 2000, utilizing the premises of the former General Hospital Keffi, which was constructed in 1957. FMC Keffi was one of the 44 Federal Medical Centers announced by the Federal government in 1999 and sits at Latitude $8.84689^0$N and Longitude $7.88454^0$E. The modern settlement of Lokoja, as it stands today, has a population exceeding 190,000 inhabitants according to the 2016 census. Lokoja plays a crucial role as a major transit route connecting northern and southern Nigeria. The Federal Medical Center Lokoja was established on November 9, 1999, utilizing the premises of the former General Hospital Lokoja. It is situated at latitude $7.79688^0$N and longitude $6.74848^0$E.

The Bida emirate, ranks as the second-largest city in Niger state, boasts an estimated population of approximately 178,640 residents according to the 2016 census. The Federal Medical Center Bida came into existence in April 1997, taking over the premises of the former General Hospital Bida. Positioned at a longitude of

9.07380 N and latitude of 5.99850 E, the city serves as a vital healthcare hub.

The University of Abuja Teaching Hospital (UATH) is situated in Gwagwalada within the Federal Capital Territory of Nigeria. Gwagwalada has a population of 537,086 according to the 2016 census figures. Initially established as a specialist hospital in 1992. In September 2006, the hospital was elevated to the status of a Teaching Hospital for the University of Abuja. Located at latitude 8.95040N and longitude 7.06260E, the hospital Functions as a key referral and training center.

**Table 1 Coordinates of Study Locations.**

| Study Location | Collection Center | Sample Sources | Location Coordinates |
|---|---|---|---|
| Keffi, Nassarawa State. | FMC Keffi | Sputum, throat swab & nasal swab | $8.4689^0$N $7.88454^0$E. |
| Bida, Niger State. | FMC Bida | Sputum, throat swab & nasal swab | 9.07380 N 5.99850 E |
| Lokoja, Kogi State | FMC Lokoja | Sputum, throat swab & nasal swab | $7.79688^0$N $6.74848^0$E |
| Gwagwalada, Abuja | University of Abuja Teaching Hospital (UATH) | Sputum, throat swab & nasal swab | 8.95040N 7.06260E, |

## Study Population

The study population included 768 children and adults, including males and females attending the four Federal hospitals selected for the study. The age range of the patients were from 4 months to 87 years. This included out-patients and in-patients. The total number of samples collected for initial screening for prevalence of *Streptococcus pneumonia*e were 222, 168, 108 and 270 from Keffi, Bida, Lokoja and University of Abuja Teaching Hospital, Abuja, respectively, bringing to a total of 768 samples.

## Sample Size Determination

Sample size was determined using Kish Lisle equation, using average national prevalence rate as described by Iliyasu *et al*. (2017).

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$$n = z2p(1-p)/ d2$$

Where z = Z score for 95% confidence interval = 1.96, p=prevalence; 60%, d= margin of tolerable error = 5%. On imputing the values into the equation, the calculated sample size was approximately 369. However, this sample size was exceeded by the end of the study period, which increased the reliability of the data.

## Administration Questionnaire

A structured questionnaire was administered to obtain demographic information such as age, gender, location of residence, socio-economic status, previous respiratory tract infection, previous treatment or vaccination and so on. This was voluntary. Some additional information was also obtained from patient's medical records where possible, with the aid of laboratory and medical records staff.

## Determination of Frequency of Previous Antibiotic Intake by Patients

The structured questionnaire administered to the patients at the point of sample collection sought responses from patients to questions about history of antibiotic consumption prior to sample collection, on

the prevalence of antibiotic resistance. Data on history of antibiotic consumption were obtained for only 315 of the patients out of the 464 that tested positive to *S. pneumoniae*. Several reasons were responsible for this: refusal of some patients to disclose such information or were unable to recollect the details and some patients were either irregular or first-time visitors to the hospitals.

### Sample Collection and Processing

Samples, including sputum, nasal and throat swabs, were collected from consenting patients at the four federal medical hospitals using appropriate tools and storage containers such as disposable sterile swabs and sterile 50ml screw-cap centrifuge tubes, each labelled with the corresponding patient ID number. A loopful of the pure colonies was streaked on sterile blood agar plates, which were then incubated at 37°C for 24 hours [18].

The data were gathered over a 24-month period from June 2020 to May 2022, from both in-patients and out-patients, with measures taken to mitigate potential seasonal biases.

### Identification of Isolates of *Streptococcus Pneumoniae*

The identification of the *Streptococcus pneumoniae* isolates was based on morphological characteristics as well as biochemical tests. Colony morphology on agar plate was first observed (alpha-hemolytic, small, gray, and showing mucoid colonies) Isolates of *S. pneumoniae* were also identified by their susceptibility to optochin and also by using the bile solubility tests, following standard procedure [19].

### Optochin test

A few isolated colonies of *Streptococcus pneumoniae* were seeded on to a blood agar plate. A 6 mm optochin disk was placed in the streaked area and the culture incubated at $37^0$ C. After 24 hours of incubation, the zone of inhibition was measured where applicable. Where the zone of inhibition was 14 mm or more, the organism was identified as *S. pneumoniae* but where the zone of inhibition was less than 14 mm, further confirmatory tests were carried out for identification, using the bile solubility test [20].

### Bile solubility test

The bile (Sodium deoxycholate) test distinguishes *S. pneumoniae* from other alpha- haemolytic Streptococci. Sodium deoxycholate lyses pneumococcal cell walls, so *S. pneumoniae* is bile soluble while other alpha haemolytic Streptococci are bile-resistant.

The isolates tested were grown for 24 hours on sheep blood agar at $37^0$C at 5% $CO_2$ concentration. A few bacterial colonies from the overnight culture was transferred to 1.0 ml of 0.85 % saline to achieve turbidity in the range of 0.5-1.0 McFarland standard.

The suspension was divided equally into two tubes so that each tube contained 0.5 ml. To one of the 0.5 ml tubes 2% sodium deoxycholate was added and 0.5 ml of the 0.85 % saline was added to the other, both tubes were properly mixed. The tubes were incubated at $35^0$C after which the tubes were vortexed. Subsequently, the tubes were observed for any clearance of turbidity after 10 minutes up to a period of 2 hours while still in incubation. The disappearance of the turbidity in the bile tube but not in the saline control tube indicated a positive test. Partial clearance or partial solubility was not considered as a positive identification for *S. pneumoniae*. A standard strain of *S. pneumoniae* was used as a positive control [20].

### Susceptibility test (agar diffusion test)

Susceptibility of Streptococci strains to antimicrobial agents was determined by the agar diffusion test according to standard clinical procedures. A standard strain of *S. pneumoniae* was included in each run as control. The isolates tested against twelve of the commercially available antibiotics was classified as susceptible or resistant in accordance with standard procedures [21].

The following antibiotic disks were tested for all isolates: Ampicillin (20 mcg), cotrimoxazole (25 mcg), cephalexin (30mcg), tetracyclin (30 mcg), cefotaxime (30 mcg), ciprofloxacin (5 mcg), Levofloxacin (5mcg), Linezolid (30 mcg), cloxacillin (15 mcg), Roxithromycin (15mcg), Lincomycin (10mcg).and Gentamycin (10mcg).

## RESULTS AND DISCUSSION

### Prevalence of *S. pneumoniae* from clinical samples

The examination of 768 specimens from sputum, throat, and nasal swabs in specific study areas revealed the presence of various respiratory tract bacterial pathogens. Notably, 29.2% of the total samples were positive for *Streptococcus pneumoniae*, while *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Moraxella catarrhalis* were identified in 10.2%, 14.8%, and 10% of the samples, respectively. Additionally, *Haemophilus influenzae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were observed in 4.4%, 19.9%, 8.2%, and 3.3% of the samples, respectively

Overall, the prevalence rate of *S. pneumoniae* from the study was 29.2% of the total samples. Distribution according to sampled locations was 30.6%, 26.1%, 29.2% and 31.5% from UATH, Keffi, Bida and Lokoja respectively. The prevalence rate of *Streptococcus pneumoniae* (29.2%), recorded in this study is at variance with prevalence rates reported from other studies, such as 13.8% [9] and 21.4% [22].

The observed prevalence rate recorded in this study may be due to a number of factors which were also corroborated by the findings of other researchers. These included crowded living conditions of some of the patients [22], prior nasopharyngeal carriage [7], age range, cigarette smoking, sharing beds with carriers or infected persons [9], previous antibiotic intake before sampling [23], and airways problems such as asthma [9].

### Prevalence of *S. pneumoniae* from clinical Samples taken from FMC Keffi

From the Keffi center, *S. pneumoniae* had the highest prevalence rate (26.1%), followed by *Staphylococcus aureus* (22.1%), *Streptococcus pyogenes* (15.3%). The least prevalent were *Pseudomonas aeruginosa* (1.4%), Moraxella *catarrhalis* (5.4%) and *Haemophilus influenzae* (6.7%).

**Table 2: Prevalence of *S. pneumoniae* from clinical Samples taken from FMC Keffi**

| Respiratory bacterial pathogens | Sample source S | T | N | Total n=222 | Percentage % |
|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 28 | 15 | 15 | 58 | 26.1 |
| *Klebsiella pneumoniae* | 14 | 6 | 7 | 27 | 12.2 |
| *Streptococcus pyogenes* | 12 | 14 | 8 | 34 | 15.3 |
| *Moraxella catarrhalis* | 4 | 5 | 3 | 12 | 5.4 |
| *Haemophilus influenzae* | 2 | 6 | 7 | 15 | 6.7 |
| *Staphylococcus aureus* | 27 | 12 | 10 | 49 | 22.1 |
| *Escherichia coli* | 10 | 6 | 8 | 24 | 10.8 |
| *Pseudomonas aeruginosa* | 0 | 2 | 1 | 3 | 1.4 |

Key: S: sputum, T: throat swab, N: nasal swab, FMC: Federal Medical Center, UATH: University of Abuja Teaching Hospital.

### Prevalence of *S. pneumoniae* from clinical Samples taken from FMC Bida

From the Bida center, *S. pneumoniae* had the highest prevalence rate (29.2%), followed by *Staphylococcus aureus* (18.5%), *Streptococcus pyogenes* (18%).and *Klebsiella pneumoniae* (11.9%) The least prevalent were Haemophilus influenzae (2.3%) and *Pseudomonas aeruginosa* (2.3%).

**Table 3 : Prevalence of *S. pneumoniae* from clinical Samples taken from FMC Bida**

| Respiratory bacterial pathogens | Sample source S | T | N | Total n=168 | Percentage |
|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 25 | 12 | 12 | 49 | 29.2 |
| *Klebsiella pneumoniae* | 10 | 6 | 4 | 20 | 11.9 |
| *Streptococcus pyogenes* | 11 | 13 | 6 | 30 | 18 |
| *Moraxella catarrhalis* | 1 | 7 | 4 | 12 | 7.1 |
| *Haemophilus influenzae* | 0 | 3 | 1 | 4 | 2.3 |
| *Staphylococcus aureus* | 15 | 8 | 8 | 31 | 18.5 |
| *Escherichia coli* | 6 | 5 | 7 | 18 | 10.7 |
| *Pseudomonas aeruginosa* | 1 | 3 | 0 | 4 | 2.3 |

Key: S: sputum, T: throat swab, N: nasal swab, FMC: Federal Medical Center, UATH: University of Abuja Teaching Hospital.

### Prevalence of *S. pneumoniae* from clinical Samples taken from FMC Lokoja

From the Lokoja center, *S. pneumoniae* had the highest prevalence rate (31.5%), followed by *Staphylococcus aureus* (23.1%), *Streptococcus pyogenes* (13.9%). The least prevalent were *Haemophilus*

*influenzae* (1.8%) and *Pseudomonas aeruginosa* (2.8 %).

**Table 4: Prevalence of *S. pneumoniae* from clinical Samples taken from FMC Lokoja**

| Respiratory bacterial pathogens | Sample source S | T | N | Total n=108 | Percentage |
|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 18 | 8 | 8 | 34 | 31.5 |
| *Klebsiella pneumoniae* | 7 | 1 | 5 | 13 | 12.1 |
| *Streptococcus pyogenes* | 5 | 6 | 4 | 15 | 13.9 |
| *Moraxella catarrhalis* | 2 | 3 | 4 | 9 | 8.3 |
| *Haemophilus influenzae* | 0 | 1 | 1 | 2 | 1.8 |
| *Staphylococcus aureus* | 13 | 6 | 6 | 25 | 23.1 |
| *Escherichia coli* | 4 | 0 | 3 | 7 | 6.5 |
| *Pseudomonas aeruginosa* | 2 | 0 | 1 | 3 | 2.8 |

Key: S: sputum, T: throat swab, N: nasal swab, FMC: Federal Medical Center, UATH: University of Abuja Teaching Hospital.

**Prevalence of *S. pneumoniae* from clinical Samples taken from University of Abuja Teaching Hospital, Gwagwalada.**

From the Gwagwalada center, *S. pneumoniae* had the highest prevalence rate (30.6%), followed by *Staphylococcus aureus* (17.7%), *Moraxella catarrhalis* (16.3%) and *Streptococcus pyogenes* (13.1%). The least prevalent were *Haemophilus influenzae* (4. 8%) and *Escherichia coli* (5.2%).

**Table 5: Prevalence of *S. pneumoniae* from clinical Samples taken from University of Abuja Teaching Hospital, Gwagwalada.**

| Respiratory bacterial pathogens | Sample source S | T | N | Total n=270 | Percentage |
|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 42 | 21 | 20 | 83 | 30.6 |
| *Klebsiella pneumoniae* | 8 | 6 | 4 | 18 | 6.7 |
| *Streptococcus pyogenes* | 14 | 16 | 5 | 35 | 13.1 |
| *Moraxella catarrhalis* | 15 | 15 | 14 | 44 | 16.3 |
| *Haemophilus influenzae* | 3 | 6 | 4 | 13 | 4.8 |
| *Staphylococcus aureus* | 22 | 16 | 10 | 48 | 17.7 |
| *Escherichia coli* | 6 | 0 | 8 | 14 | 5.2 |
| *Pseudomonas aeruginosa* | 4 | 5 | 6 | 15 | 5.6 |

Key: S: sputum, T: throat swab, N: nasal swab, FMC: Federal Medical Center, UATH: University of Abuja Teaching Hospital.

### Susceptibility of *S. pneumoniae* isolates to selected antibiotics across study areas

The antibiotic sensitivity testing results for *Streptococcus pneumoniae* isolates revealed varying susceptibility and resistance levels. A relatively high resistance rate was observed against ampicillin, (65.7%, 58.2%), from Keffi and Gwagwalada study areas respectively. This was similar to the 57.2% resistance rate to penicillin reported from a study in Morocco [24] but was at variance with rates reported in other studies, such as 17.5% [25], 39.6% [26] and 98.9% [27].

Similarly, resistance to gentamycin was high at 66.4%, 75.6%, and 69.5% from Keffi, Bida and Gwagwalada study areas respectively. The observed 55.4% average resistance rate to roxithromycin (macrolide), was similar to the 59.6% reported in another study [25], for the macrolide erythromycin but different from the 39.9% and 96.6% reported by others [26,27]. Also, average resistance rate to tetracycline was found to be 53.7%, which aligned with a report of a study from India [28], that reported 54.2% resistance to tetracycline but differed from reports from other studies: 93.2% [29] and 100% [30]. The lowest resistance rates were recorded for Levofloxacin with 16.8%, 15.9%, 0 and 8.2% from Keffi, Bida, Lokoja and Gwagwalada study areas respectively. Additionally, there was relatively low resistance to cefotaxime and linezolid in all study areas. These results closely aligned with the zero resistance rate obtained in similar study [27]. The observed antibiotic resistance rate may be due to a number of factors which also aligned with findings of others, ranging from host factors, previous colonization or infection [7], previous antibiotic use [31], living in crowded conditions [22], and so on.

**Table 6: Susceptibility of *S. pneumoniae* isolates to selected antibiotics across study centres**

| Antibiotics | | Study Centres: UATH (n= 184) | FMC Bida (n= 82) | FMC Keffi (n= 143) | FMC Lokoja (n= 55) | Total | P-value |
|---|---|---|---|---|---|---|---|
| Ampicillin | R | 107(58.2) | 22(26.8) | 94(65.7) | 26(47.3) | 249 (53.7) | 0.000 |
| | S | 77(41.8) | 60(73.2) | 49(34.3) | 29(52.7) | 215(46.3) | |
| Co-Trimoxazole | R | 98(53.3) | 30(36.6) | 93(65.0) | 25(45.5) | 246(53.0) | 0.000 |
| | S | 86(46.7) | 52(63.4) | 50(35.0) | 30(54.5) | 218(47.0) | |
| Cephalexin | R | 61(33.2) | 30(36.6) | 39(27.3) | 28(50.9) | 158(34.0) | 0.032 |
| | S | 123(66.8) | 57(69.5) | 104(72.7) | 27(49.1) | 311(67.0) | |
| Tetracyclin | R | 124(67.4) | 30(36.6) | 78(54.5) | 17(30.9) | 249(53.7) | 0.000 |
| | S | 60(32.6) | 52(63.4) | 65(45.5) | 38(69.1) | 215(46.3) | |
| Cefotaxime | R | 55(29.9) | 22(26.8) | 40(28.0) | 25(45.5) | 142(30.6) | 0.080 |
| | S | 129(70.1) | 60(73.2) | 103(72.0) | 30(54.5) | 322(69.4) | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ciprofloxacin | R | 93(50.5) | 46(56.1) | 67(46.9) | 24(43.6) | 230(49.6) | 0.446 |
| | S | 91(49.5) | 36(43.9) | 76(53.1) | 31(56.4) | 234(50.4) | |
| Levofloxacin | R | 15(8.2) | 13(15.9) | 24(16.8) | 0(0.0) | 52(11.2) | 0.002 |
| | S | 169(91.8) | 69(84.1) | 119(83.2) | 55(100.0) | 412(88.8) | |
| Linezolid | R | 68(37.0) | 21(25.6) | 57(39.9) | 28(50.9) | 174(37.5) | 0.023 |
| | S | 116(63.0) | 61(74.4) | 86(60.1) | 27(49.1) | 290(62.5) | |
| Cloxacillin | R | 95(51.6) | 35(42.7) | 41(28.7) | 24(43.6) | 195(42.0) | 0.002 |
| | S | 89(48.4) | 47(57.3) | 102(71.3) | 31(56.4) | 269(58.0) | |
| Roxithromycin | R | 124(67.4) | 36(43.9) | 75(52.4) | 22(40) | 257(55.4) | 0.000 |
| | S | 60(32.6) | 46(56.1) | 68(47.6) | 33(60) | 207(44.6) | |
| Lincomycin | R | 103(56.0) | 23(28.0) | 52(36.4) | 21(38.2) | 199(42.9) | 0.000 |
| | S | 81(44.0) | 59(72.0) | 91(63.6) | 34(61.8) | 265(57.1) | |
| Gentamycin | R | 128(69.6) | 62(75.6) | 95(66.4) | 12(21.8) | 297(64.0) | 0.000 |
| | S | 56(30.4) | 20(24.4) | 48(33.6) | 43(78.2) | 167(36.0) | |

*$p \leq 0.05$ shows that there is a significant difference in the distribution of susceptible and resistant S. pneumoniae* across the study centres. Key: FMC: Federal Medical Centre, UATH: University of Abuja Teaching Hospital.

## Prevalence of multidrug resistance (MDR)

The results presented in table 7, showed that the highest MDR rate was recorded from Gwagwalada centre with 69.02%, followed by Keffi (59.4%) and Bida (54.9%). The lowest MDR rate was recorded at the Lokoja centre, with 36.4%. The 59.7% average MDR rate observed in this study aligned closely with MDR rates reported in studies carried out elsewhere in the world [26,29,32]. However, the findings of this research was at variance with findings of some other studies, that reported much lower MDR rates [22,25].

The moderately high MDR rate observed in this study was due to a number of factors. These include the inappropriate and frequent use of antibiotics sometimes without doctor's prescription recent hospitalization within 60 days prior to testing, antibiotic failure, circulation and consumption of sub-standard drugs as well as a relatively large percentage of children and the elderly in the study population. These findings were corroborated by similar reports from other studies [31,33]. Besides, the over reliance on medical history and physical examination for drug prescription, rather than accurate determination of the etiological agent and susceptibility pattern was found to be another factor that may lead to drug resistance.

**Table 7 Prevalence of multidrug resistance (MDR) Across study areas**

| Study Area | No of patients tested | No of patients whose isolates were multidrug resistant | Percentage of MDR (%) | p-value |
|---|---|---|---|---|
| FMC Keffi | 143 | 85 | 59.4 | |
| FMC Bida | 82 | 45 | 54.9 | |
| FMC Lokoja | 55 | 20 | 36.4 | 0.000 |
| UATH | 184 | 127 | 69.02 | |
| Total | 464 | 277 | 59.7 | |

*$p \leq 0.05$ shows that there is a significant difference in the distribution of MDR S. pneumoniae across the study areas. The average MDR for the study was 59.7.*

## Demographic characteristics of patients

The analysis of infection rates by age distribution, shows that the highest infection rate was among the pediatric patients, below 5 years (74.2%), followed by the age bracket above 65 years (66.4%). This aligned closely with reports from other studies [24,26,27]. It was also observed that then the age range of 5-14 years had an infection rate of 60.9% while the age range of 35 to 45 years had the lowest infection rate of 48%.

Analysis of gender, showed a higher infection rate among females (66.1%), compared to males (52.4%). This outcome was similar to the findings of a study from Morocco that reported that reported a 54.9% prevalence rate among female patients [24] but was at variance with another study that reported a higher prevalence rate of 57.8% among males as against the 42.2% among females [27].

**Table 8 Demographic Characteristics of Patients positive for *Streptococcus pneumoniae* infection**

| Age groups (years) : | Total Number of Isolates ( n=768) | Number of isolates positive for *S. pneumoniae* (n=464) | Prevalence rate (%) | No of Isolates with MDR | MDR Rate (%) |
|---|---|---|---|---|---|
| < 5 | 155 | 115 | 74.2 | 69 | 60 |
| 5-14 | 92 | 56 | 60.9 | 33 | 58.9 |
| 15-24 | 80 | 41 | 51.2 | 25 | 61 |
| 25-34 | 87 | 50 | 57.5 | 30 | 60 |
| 35-44 | 75 | 36 | 48 | 21 | 58.3 |
| 45-54 | 78 | 41 | 52.6 | 25 | 61 |
| 55-64 | 82 | 46 | 56.1 | 27 | 58.7 |
| > 65 | 119 | 79 | 66.4 | 47 | 59.5 |
| p-value | | | 0.001 | | 0.247 |
| Gender: | | | | | |
| Male: | 319 | 167 | 52.4 | 98 | 58.7 |
| Female: | 449 | 297 | 66.1 | 179 | 60.3 |
| p-value | | | 0.001 | | 0.010 |

*$p \leq 0.05$ shows that there is a significant difference in the distribution of S. pneumoniae* among age groups and sex across the study areas. *S. pneumoniae infection was significantly higher in female ($p<0.001$)*

## Educational status and prevalence of Streptococcal infections

From the study, it was observed that patients with tertiary education exhibited a reduced infection rate of 24.6%, in contrast to 32.9% and 27% for those without formal education and with secondary education, respectively. The correlation between lower educational status and higher incidence of pneumococcal infections have been earlier reported by others [34]. This disparity in infection rates with those with higher educational status was attributed to a heightened awareness of disease prevention measures and more willingness to visit a hospital when early signs of infection is apparent [35]. However, contrary to expectation individuals with only primary education displayed the lowest infection rate at 15.5%, this was not surprising as children of this age are still under the care of adults.

**Table 9 Educational status and Prevalence of Pneumococcal Infections.**

| Educational Status ( n=441) | Total Number of Patients (Age > 25) | No of Patients positive for S. pneumoniae | Overall Prevalence rate (%) | p-value |
|---|---|---|---|---|
| Primary | 76 | 39 | 15.5 | 0.000 |
| Secondary | 108 | 68 | 27 | |
| Tertiary | 100 | 62 | 24.6 | |
| No formal | 157 | 83 | 32.9 | |
| Total | 441 | 252 | 100 | |

*S. pneumoniae infection was significantly higher in subjects within no formal education ($p<0.05$) than in any other subject with education*

## Variations in prevalence rates of Streptococcal Infections according to months of the year.

The rate of infection was analyzed over a 12-month period to assess the possible impact of monthly or seasonal bias on the outcome of prevalence data. No significant difference in infection rates was observed overall. However, slightly higher rates were observed in the months of December and January which agrees with reports of other studies [26]. This was attributed to the cold, windy and drier weather condition, which encouraged sneezing by carriers and so increasing chances of the disease spreading from carriers or infected persons to healthy persons. However, this did not align with the findings of another study [29], which reported higher prevalence rate in the months of March, April and May (spring), with the lowest rates in the months of September, October and August (autumn).

**Table 10 Monthly variation in Prevalence of Streptococcal infections**

| Month | No of isolates Tested N=768 | No of isolates positive for *S. pneumoniae.* N=464 | Percentage of isolates positive to *S. pneumoniae.* (%) | p-value |
|---|---|---|---|---|
| January | 82 | 49 | 10.5a | |
| February | 68 | 41 | 8.8a | |
| March | 73 | 44 | 9.5a | |
| April | 60 | 36 | 7.8a | |
| May | 45 | 27 | 5.8a | |
| June | 66 | 40 | 8.6a | 0.000 |
| July | 53 | 32 | 6.9a | |

| | | | |
|---|---|---|---|
| August | 56 | 34 | 7.3a |
| September | 46 | 28 | 6.0a |
| October | 69 | 42 | 9.1a |
| November | 71 | 43 | 9.3a |
| December | 79 | 48 | 10.3b |
| Total | 768 | 464 | |

*Interpretation: S. pneumoniae infection was significantly higher December compared to all other month (p=0.000).*
Interpretation: Each subscript letter denotes a subset of *S.pnuemoniae* categories whose column proportions do not differ significantly from each other at the .05 level.

## Impact of previous antibiotic intake on the prevalence of MDRSP among patients.

Results indicated that 70.2% of patients had taken antibiotics three months before sample collection, with higher resistance rates observed for commonly consumed antibiotics such as ampicillin (53.7%), co-trimoxazole (53%), tetracycline (53.7%), and cloxacillin (42.02%). Notably, patients had lower exposure to ciprofloxacin, levofloxacin, and linezolid and corresponding resistance rates of 49.6%, 11.3% and 37.5% respectively were comparatively lower. This was comparable to 31% average resistance rate among patients who took antibiotics three months prior to testing, in a study carried out in Morocco [24].

However, while the study observed a 64% resistance rate for gentamycin *in vitro,* none of the patients had a history of gentamycin intake, 3 months before sample collection (Table 9). This implies that other risk factors such as exposure to hospital environment, previous infection with MDR pathogens and so on, may have been responsible [36]. The positive correlation between specific antibiotic use and multidrug resistance was also reported in other studies [37,38].

Increased antibiotic consumption may be due to increased access to pharmaceutical drugs, evading prescription from doctors, circulation of sub-standard antibiotics, patients not following recommended doses strictly as well as taking antibiotics for purposes other than what they are meant for. An example of an antibiotic which was widely circulated and consumed during the Covid 19 era was azithromycin [38].

Frequent exposure to antibiotics can lead to antibiotic resistance as a result of transposon transfer and selective pressure [39]. These resistance strains mostly emerge through adaptation to antibiotic pressures resulting from interactions with the antibiotics and other pathogens. while naturally residing in host respiratory tract. Antibiotic selective pressure increases spread of antibiotic resistance by reducing susceptible bacteria and allowing resistant bacteria to multiply and spread in the community. This is because the bacteria may have had a random change in their DNA through mutations, that equips them to survive in the presence of the antibiotics and therefore outgrow non-resistant bacteria. Interaction with other pathogens may also lead to horizontal transfer of antibiotic resistance genes from those that have already acquired resistance [40].

**Table 11: Previous Antibiotic Intake and Rate of multidrug Resistance**
(N= 315: number of patients with information on previous antibiotic intake)

| Antibiotic Consumed | Months since last antibiotic consumption (months) (n/%) | | | | Average resistance Rates | P-value |
|---|---|---|---|---|---|---|
| | 1-3 | 4-6 | 7-9 | 10-12 | | |
| Ampicillin | 34 (15.4) | 7 ( 5.8) | 11 (9) | 13 (15.9 ) | 53.7 | |
| Co-Trimoxazole | 26 ( 11.8) | 13( 10.8) | 16 (13) | 5 ( 6.1 ) | 53.0 | |
| Cephalexin | 16 (7.2) | 12 (10) | 9 (7.4 ) | 0 ( 0 ) | 33.0 | |
| Tetracyclin | 25( 11.3) | 10(8.3) | 7 ( 5.7) | 0 ( 0 ) | 53.7 | |
| Cefotaxime | 32 ( 14.5) | 12 (10 ) | 18 (14.8 ) | 14 (17.1 ) | 53.8 | |
| Ciprofloxacin | 0 ( 0) | 0 ( 0) | 6 ( 5 ) | 6 ( 7.3 ) | 49.6 | |
| Levofloxacin | 11 ( 5) | 10 ( 8.3) | 10 ( 8.2) | 9 (11 ) | 11.3 | |
| Linezolid | 11 (5) | 15 (12.5 ) | 9 ( 7.4 ) | 5 ( 6.1 ) | 37.5 | 0.690 |
| Cloxacillin | 33 ( 14.9 ) | 19 (15.8 ) | 12 (9.8 ) | 23 ( 28 ) | 42.02 | |
| Roxithromycin | 17 ( 7.7) | 19 (15.8) | 7 ( 5.7 ) | 7 (8.5 ) | 49.6 | |
| Lincomycin | 16 ( 7.2) | 3 (2.5 ) | 17 (13.9 ) | 0 ( 0 ) | 42.9 | |
| Gentamycin | 0 (0) | 0 (0) | 0 ( 0 ) | 0 ( 0 ) | 64.0 | |
| Total | 221 | 120 | 122 | 82 | | |

*There is no correlation between consumption rates of the antibiotics within 1-3 months of sample collection with average resistance rates* observed *for the respective antibiotics (0.690).* Average MDR Rate obtained from the study: 59.7%

## CONCLUSION

Overall, the prevalence rate of *Streptococcus. pneumoniae* observed from the study was 29.2 compared to other respiratory tract pathogens identified. However, the prevalence rate of *S. pneumoniae* within the sampled locations were 30.6%, 26.1%, 29.2% and 31.5% from UATH, Keffi, Bida and Lokoja respectively.

Analysis of the average resistance rates to the various antibiotics tested, showed that the highest antibiotic resistance rates were against gentamycin, ampicillin, co-trimoxazole and tetracycline, with average resistance rates of 64.0%, 53.7%, 53% and 53.7% respectively. Again, the highest susceptibility rates or lowest resistance of *S. pneumoniae* was to levofloxacin, with an average of 11.3%. Resistance of against cephalexin and linezolid was relatively lower at 33% and 37.5% respectively.

The study found that some positive correlation existed between previous antibiotic intake and antibiotic resistance. 70.2% of the patients were found to have consumed antibiotics three months prior to sample collection. This corresponded to the significantly high resistance rates to some of the antibiotics, as previously discussed. While the average MDR obtained from this study was 59.7%, it was observed that the patients were least exposed to ciprofloxacin (0%), levofloxacin (5%), linezolid (5%) and gentamycin (0%), three months to sample collection. This equally aligned to relatively lower resistance rates observed for ciprofloxacin, levofloxacin and linezolid respectively, compared to other antibiotics

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