Journal of Basics and Applied Sciences Research (JOBASR)
ISSN print: 3026-9091
Volume 2(1) March 2024
DOI: https://doi.org/10.33003/jobasr-2024-v2i1-38

Prevalence and antibiotic susceptibility pattern of *Streptococcus pneumoniae* amongst residents of Obong Ntak in Etim Ekpo Local Government of Akwa Ibom State, Nigeria

Anosike, I. K.[1]*, Egbomuche, R. C.[1], Etang, U. E.[1,2], Akpan, N. G.[2] and Abasiubong, V. N.[1]
[1]Department of Microbiology, Faculty of Natural and Applied Sciences, Obong University, Obong Ntak, Etim Ekpo, Akwa Ibom State, Nigeria.
[2]Institute for Biomedical Research and Innovations, College of Health Sciences, University of Uyo.
*Corresponding Author Email: anosikeignatius@gmail.com

ABSTRACT
Streptococcus pneumonia is among the major causative agents of respiratory tract infections and colonizes the nasopharyngeal cells of asymptomatically healthy individuals. Prevalence and antibiotics sensitivity of *Streptococcus pneumonia* amongst residents in a rural setting was evaluated. A total of 250 nasal swab samples (125 males and 125 females) were collected using sterile swab sticks. Administration of open-ended questionnaires, culture of the samples, identification of isolates, antimicrobial sensitivity test and determination of prevalence rate were all done using standard protocols. Out of the 250 samples collected, 35 were positive for *S. pneumonia* giving an overall prevalence of 14%. Based on sex, the prevalence rates were 15% and 20% for males and females, respectively. Prevalence rates of 12.5%, 13.7%, 12.7% and 25% were recorded for age groups of 10-20, 21-40, 41-60 and >60 years, respectively. The presence of *S. pneumonia*e based on the health status of the participants showed that the prevalence rates were 20% and 11.70% for clinically ill and clinically healthy individuals, respectively. The resistance profile to the test antibiotics were 22.9%, 22.9%, 11.4%, 100%, 100%, 34.3%, 34.3%, 100%, 54.3% and 77.1% respectively to perfloxacin, septrin, ciprofloxacin, amoxacilin, zinacef, gentamicin, erythromycin, ampliclox, streptomycin and rocephin**.** Ciprofloxacin was the most effective antibiotic followed by perfloxacin and septrin. Our isolates showed 100% multi-drug resistance and is a cause for concern.

Keywords:
Prevalence,
Streptococcus pneumonia,
Antimicrobial susceptibility,
Community setting.

INTRODUCTION

*Streptococcus pneumoniae***,** also referred to as pneumococcus is a non-spore forming Gram-positive bacterium (Chenoweth, 2009). It occurs in pairs (diplococci) or often lancet-shaped, alpha haemolytic and encapsulated. Based on the polysaccharide of the capsule, at least 84 serotypes have been identified (Mandell *et al*., 2010). It is implicated in respiratory tract infections (RTIs) and colonizes the nasopharyngeal cells of asymptomatically healthy individuals (Bogaert *et al*., 2004). It is found in the nasopharynx and throat of humans, and 40-70% of humans are carriers of the organism (Sleeman *et al*., 2005). It is a normal flora of healthy adults and children (Chenoweth, 2009). It is also reported to exhibit a commensal relationship with humans, and about 27–65% of children and less than 10% of adults are carriers. Young children, adults over 65 years of age, and people with impaired immune system are among the most susceptible. Older people are especially at risk of death from this disease (Weiser *et al*., 2018).

The organism is usually transmitted from one person to another through coughing, sneezing or direct contact with articles soiled with sputum or nasal discharges (Weiser *et al*., 2018).The bacterial transmission is facilitated by viral RTI which destroys the respiratory epithelial cells, increasing its load, penetration and disease manifestation (Weiser *et al*., 2018). It can be carried more frequently in crowded environments (e.g. day care centers, army barracks, religious pilgrims, etc.) and poorly ventilated areas (Chenoweth, 2009). In adults, the incidence increases during the peak of winter and towards the middle of the summer as a result of closer living conditions (Kim *et al*., 1996). The infection may lead to complications such as pneumonia, bacteraemia, sinusitis, meningitis, and otitis media (Martson *et al*., 1997; Arai *et al*., 2011). Severe cases may lead to chronic conditions such as deafness, blindness, coma and finally death (Weiser *et al*., 2018). Morbidity and mortality rates

due to pneumococcal infections are generally becoming common more especially with the increasing prevalence of the multidrug resistant strains (Lindsay *et al*., 2016).

Antibiotic resistant *S. pneumoniae* is increasing worldwide, principally affecting beta-lactams and macrolides. Selection pressure due to antibiotics exposure is one of the drivers of multi-drug resistance (MDR) amongst the pathogens (Lindsay *et al*., 2016). Resistance to the beta lactam antibiotics is pervasive and complex amongst these pathogens (Lindsay *et al*., 2016). Wang *et al.* (2019) observed resistance rates that ranged from 66.7 to 95.80% by *S. pneumoniae* to their used antibiotics. Furthermore, they also reported resistance rates of 86.9% and 1.4% and 8.2% and 18.1%, to penicillin and ceftriaxone respectively amongst non-meningitis and meningitis isolates. Most importantly, 46.1% of invasive clinical isolates were multidrug resistant. Resistance amongst these pathogens is further compounded by the emergence of non-vaccine multidrug-resistant *Streptococcus pneumonia* (Revathy *et al*., 2019). Of particular concern is the emergence of serotype 15A which has MDR potentials (Büyükcam *et al*., 2017; Bastiaens *et al*., 2018; Nakano *et al*., 2019).

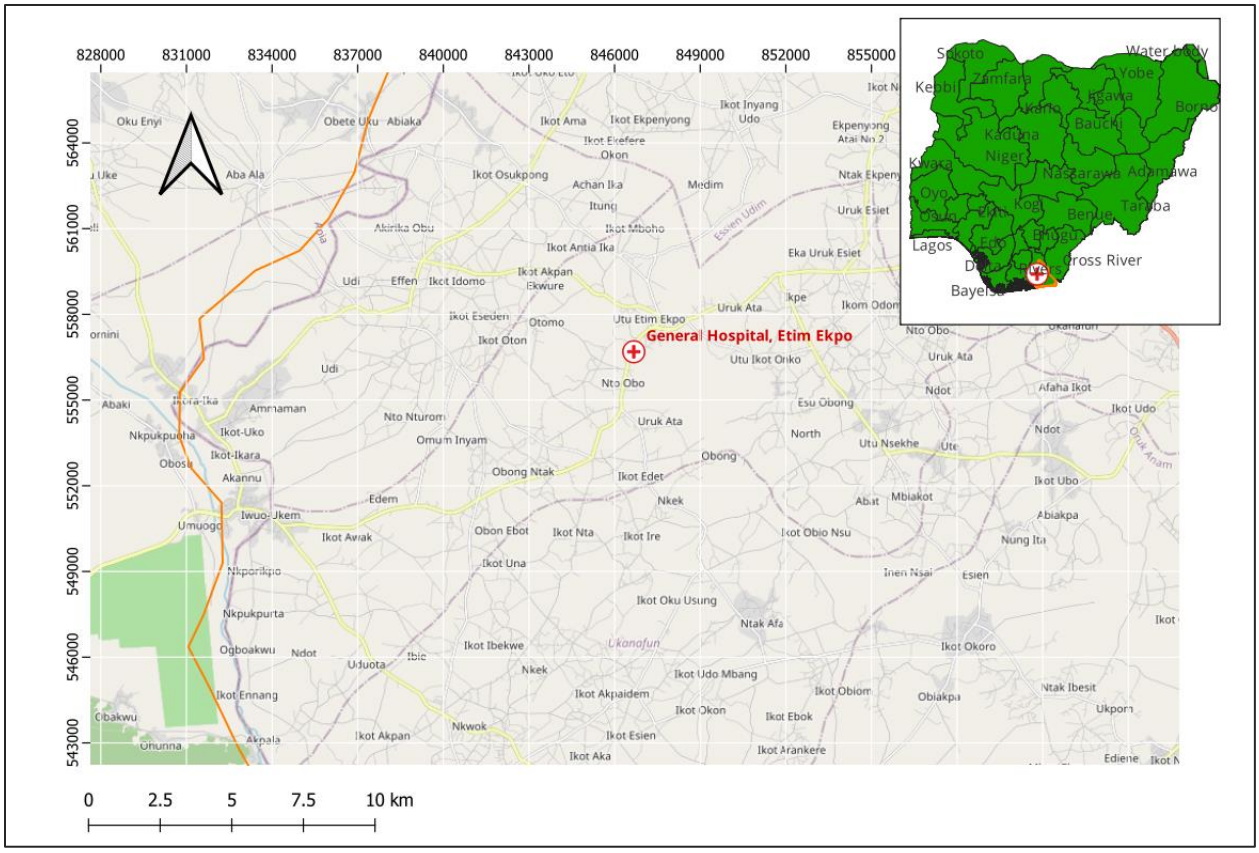
Streptococcus pneumoniae has continued to remain an important pathogen of man right from its discovery in the late 17th century worldwide. Pneumococcus accounts for 30-70% of community-acquired pneumonia (CAP) cases requiring hospitalization (Fang *et al*., 1990). CAP is a pneumonia acquired outside hospital settings. The onset of symptoms of pneumococcal pneumonia is often sudden with high fever, chest pain, painful cough, respiratory difficulty and blood tinged sputum (Crossley & Lippincott, 2004). If therapy is given early in the infection, development of lung consolidation may be prevented. The pathogen is estimated to kill around one million people worldwide every year (Scott, 2007). World health organization (2007) estimated that mortality due to pneumococcal infection every year is 1.6 million, including approximately 1 million children under 5 years of age, and casualties are greatest in the developing countries. Sub-Saharan African and South Asia together bear the highest brunt of deaths from pneumonia (WHO, 2022).

Studies in Nigeria have shown that pneumococcal infection accounts for 50, 54.5, and 60% of CAP cases in Zaria, Enugu, and Kano, respectively (Musa *et al*., 2008). In Kano, pneumococci have been shown to account for 46.4% of CAP, meningitis and bactaeremia (Iliyasu, 2011). In a more recent study carried out in North western part of Nigeria, 302 cases of community acquired pneumonia, bacteraemia and meningitis were screened, out of which 51.7%, 30.4% and 21.1% were pneumococcal pneumonia, pneumococcal meningitis and pneumococcal bacteraemia respectively, with overall mortality rate of 12.9%. Moreover, 69.7% of the patients had co-morbidity conditions of human immunodeficiency virus infection and chronic heart disease, which were identified as risk factors for the mortality including the multi-drug resistant *Pneumococci* isolated (Garba *et al*., 2015). In another report, *Pneumococcus* was found to be the most predominant cause of community-acquired sporadic meningitis in Enugu (Ozumba, 1994). In a study in Ibadan, Nigeria, eight out of the nine cases with culture-proven pneumococcal meningitis resulted to death of the patients (Falade *et al*., 2009). Similar community-based prevalence studies are lacking in south-south region of Nigeria. Thus, we carried out a cross-sectional study to determine its prevalence and antibiotics susceptibility to routinely used antibiotics in a community setting.

MATERIALS AND METHODS

Study area

The study was carried out in Obong Ntak, Etim Ekpo local government of Akwa Ibom state, Nigeria. It has a total land mass of 183.3 Km^2 and it is geographically situated on the latitude 5º1’0” North, longitude 7º37’0” East in Utu Etim Ekpo. The state is located in the south-south region of Nigeria and has varying climate with cold dry season (harmattan) from October to February, warm wet season from March to May and a cold wet season from June to September. The major occupation of the inhabitants is agriculture (Okonkwo &Oguamanam, 2013).



Experimental design and ethical approval

The population under study was Obong Ntak residents, and the study design was a cross sectional study, a type of observational study in which outcomes and exposures are measured at the same time using a section of the population (Setia, 2016). The experiment was designed to compare the result obtained from males to that from the females, putting into account the parameters such as the health status, sex and age groups. Ethical approval was obtained from Obong University Research Directorate.

Inclusion and exclusion criteria

The criteria used in recruiting the participants in this study were Obong Ntak residents, up to 10 years of age and who gave their consent to fill our questionnaire. Both the healthy and sick individuals were recruited. People less than 10 years of age were excluded from the study as the experiment was primarily designed for the adults and the adolescents.

Design and administration of questionnaires

Open ended questionnaire meant to obtain their sex, age and clinical status was designed using open-ended question design. Participants with chronic illness such as diabetes and hypertension were regarded as clinically ill while those with none were regarded as clinically healthy.

Sample collection

Sample collection was done using simple random sampling method. This study was conducted from April to October, 2019. A total of 250 nasopharyngeal swab samples were aseptically collected from 125 males and 125 females using sterile swab sticks. The swabs were appropriately labeled and immediately transported to the laboratory for further analysis.

Isolation of *Streptococcus pneumoniae*

The samples were inoculated on blood agar, and incubated at 37ºC for 24 hours at 5-10% CO2-enriched atmosphere. Discrete, mucoid, grayish colonies with α-haemolysis and greenish pigmentation were observed on some blood agar plates after the incubation. These α-haemolytic reactions were interpreted as incomplete clearing around the colonies, and were scored as positive reactions indicating the presence of *S. pneumoniae*. The isolates were confirmed by the cultural characteristics, Gram staining, biochemical tests, capsular swelling test, and optochin sensitivity test, following standard procedures previously described (Holt *et al.*, 1995; Cheesbrough, 2009). In addition, the isolates were also identified using API and ID 2 test kits (Biomerieux)

Antibiotic susceptibility test

This was carried out using disc diffusion method originally described by Bauer *et al.* (1960) and guidelines previously reported (CLSI, 2012). Representative isolates were first adjusted to 0.5 McFarland standard (1.5 X 108 CFU/ml) and then inoculated on sterile Mueller-Hinton agar supplemented with 5% sheep blood. After 3-5 minutes, antibiotic discs were placed on the inoculated agar plates, and the plates incubated at 37ºC for 24 hours. The antibiotics were Perfloxacin (30µg/disc), Septrin (30µg/disc), Ciprofloxacin (30µg/disc), Amoxacilin (30µg/disc), Zinnacef (20µg/disc), Gentamicin (30µg/disc), Erythromycin (10µg/disc), Ampiclox (30µg/disc), Streptomycin (30µg/disc) and Recephin (25µg/disc). The zones of inhibition were measure and interpreted as susceptible, intermediate or resistant using guidelines previously reported (Sham *et al*., 2001; CLSI, 2012; Srinivasan *et al*., 2012).

RESULTS AND DISCUSSION

Following the microbial culture from the 250 swab samples collected, *S. pneumoniae* was isolated from 35 samples, giving an overall prevalence of 14%. According to sex, the prevalence rates were 15% and 20% for males and females respectively. A total of 70 samples were obtained from clinically ill (sick) individuals and 14 of them were positive with 20% prevalence, while 21 out of the 180 samples collected from the clinically healthy individuals were positive with 11.7% prevalence (Table 1).

Table 2 shows the prevalence according to age of the respondents. Of the 80 samples collected from people within the age group of 10-20 years, 10 were positive for *S. pneumonia* giving a prevalence of 12.5%. A total of 95 samples were collected from people within the age group of 21-40 years and 13 were positive with a prevalence of 13.7%. Also, for the age group of 41-60 years, 55 samples were collected and 7 tested positive for *S. pneumonia* giving a prevalence of 12.7%. For the respondents > 60 years of age, 25% prevalence was obtained (n = 5/20).

Tables 3, 4 and 5 show the susceptibility and resistance profile of the test isolates. Table 3 indicates the resistance and susceptibility patterns of all 35 isolates used for sensitivity. None of the isolates was 100 sensitive to the test antibiotics. Table 4 gives a summary of the MDR isolates and the result indicates that all the isolates displayed varying forms of MDR. None of the isolates showed resistance to 2 antibiotics, however, they all showed resistance to at least three antibiotics and some even showed resistance to 10 (n=1). A total of 12 and 8 isolates showed resistance to 5 and 4 antibiotics respectively. Table 5 shows their percentage susceptibility profile. The susceptibility patterns revealed that 88.6% of the organisms was susceptible to ciprofloxacin, 77.1% susceptible to perfloxacin and septrin, 65.7% susceptible to gentamicin and erythromycin, 45.7% susceptible to streptomycin, and 22.9% susceptible rocephin. However, amoxacilin, zinnacef and ampiclox did not inhibit the growth of any of the isolates. In other words, 100% of the isolates was resistant toamoxacilin, zinnacef and ampliclox. Others were 77.1% resistant to rocephin, 54.3% resistant to streptomycin, 34.3% resistant to gentamicin and erythromycin, 22.9% resistant to Perfloxacin and septrin, and 11.4% resistant to ciprofloxacin. There was no intermediate resistance by the isolates.

Table 1: Prevalence of *S. pneumoniae* in the studied population

Parameter	Positive	Negative	Total
1. Gender			
Male	15 (12%)	110	125
Female	20 (16%)	105	125
Total	35 (14%)	215	250
2. Health Status			
Clinically ill	14 (20%)	56	70
Clinically healthy	21 (11.7%)	159	180
Total	**35 (14%)**	**215**	**250**

Table 2: Prevalence of *S. pneumoniae* according to age groups studied

Age group (years)	Positive	Negative	Total
10 – 20	10 (12.5%)	70	80
21 – 40	13 (13.7%)	82	95
41 – 60	7 (12.7%)	48	55
>60	5 (25%)	15	20
Total	**35 (14%)**	**215**	**250**

Table 3: The Antibiotic susceptibility and resistant profile of *Streptococcus pneumonia* isolates

Isolates	PEF	SXT	CPX	AM	ZN	GEN	ERY	APX	STR	RCN
P1	S	S	S	R	R	S	S	R	S	S
P2	S	S	S	R	R	S	S	R	S	R
P3	S	S	S	R	R	S	S	R	R	R
P4	R	R	S	R	R	R	R	R	R	R
P5	R	R	R	R	R	R	R	R	R	R
P6	S	S	S	R	R	S	R	R	R	S
P7	S	S	S	R	R	R	S	R	S	R
P8	R	R	S	R	R	R	S	R	R	R
P9	R	R	S	R	R	S	S	R	S	R
P10	S	S	S	R	R	S	R	R	S	R
P11	S	S	S	R	R	S	S	R	S	R
P12	R	R	S	R	R	S	S	R	R	R
P13	S	R	S	R	R	S	S	R	R	R
P14	S	S	S	R	R	R	S	R	S	R
P15	S	S	S	R	R	S	S	R	R	S
P16	R	S	R	R	R	S	S	R	S	R
P17	S	S	S	R	R	S	R	R	S	R
P18	R	S	S	R	R	R	S	R	R	R
P19	S	S	S	R	R	S	S	R	R	S
P20	S	S	S	R	R	S	R	R	S	S
P21	S	R	R	R	R	S	S	R	S	R
P22	S	S	S	R	R	R	R	R	R	R
P23	S	S	S	R	R	R	S	R	R	S
P24	S	S	S	R	R	S	S	R	R	S
P25	S	S	S	R	R	S	R	R	S	R
P26	R	S	S	R	R	S	R	R	S	R
P27	S	S	S	R	R	R	R	R	R	R
P28	S	S	S	R	R	S	S	R	S	R
P29	S	S	S	R	R	S	S	R	R	R
P30	S	S	S	R	R	S	S	R	R	R
P31	S	R	R	R	R	R	S	R	S	R
P32	S	S	S	R	R	R	S	R	R	R
P33	S	S	S	R	R	S	R	R	S	R
P34	S	S	S	R	R	S	R	R	R	R
P35	S	S	S	R	R	R	S	R	R	S

P1 – P35 = *Streptococcus pneumonia* isolates, S = Susceptible, R = Resistant, PEF= Perfloxacin, SXT= Septrin, CPX= Ciprofloxacin, AM= Amoxacillin, ZN= Zinnacef, GEN= Gentamicin, ERT= Erythromycin, APX= Ampiclox, STR= Streptomycin and RCN= Rocephin.

Table 4: MDR isolates and the number of antibiotics

Isolates	Number of antibiotics	Total isolates (n =35)
P1	3	1
P2, P11, P15, P19, P20, P24, P28	4	7
P3, P6, P7, P10, P14, P17, P23, P25, P29, P30, P33, P35	5	12
P9, P13, P16, P21, P26, P32, P34	6	7
P12, P18, P22, P27, P31	7	5
P8	8	1
P4	9	1
P5	10	1

Table 5: Percentage susceptibility of the isolates

Antibiotics	Susceptible	Intermediate	Resistant
Perfloxacin(30µg)	27(77.1%)	-	8(22.9%)
Septrin (30µg)	27(77.1%)	-	8(22.9%)
Ciprofloxacin (30µg)	31(88.6%)	-	4(11.4%)
Amoxacilin (30µg)	-	-	35(100%)
Zinnacef (20µg)	-	-	35(100%)
Gentamicin (30µg)	23(65.7%)	-	12(34.3%)
Erythromycin (10µg)	23(65.7%)	-	12(34.3%)
Ampiclox (30µg)	-	-	35(100%)
Streptomycin (30µ)	16(45.7%)	-	19(54.3%)
Recephin (25µg)	8(22.9%)	-	27(77.1%)

Discussion

In our study, *S. pneumonia* is prevalent in Obong Ntak, and possibly, other parts of the state. Bogaert et al. (2004) reported *S. Pneumoniae* to be among the major bacteria that cause respiratory tract infections, and the organism colonizes the nasopharynx and throat of asymptomatically healthy individuals. The 14% prevalence in this study is lower than that reported by Sleeman *et al* (2005) which states that 40-70% of humans are carriers of the organism. The reason for the low occurrence could be attributed to the fact that children below 10 years of age were excluded in this study. Pneumococcus mostly affects children, the elderly, and people with low immunity. The adolescents and the middle aged individuals have stronger and well developed immunity against the infection. However, the result is higher than a previous study which states that *S. pneumoniae* is normally found in 5 -10% of healthy adults (Chenoweth, 2009). It is also higher than the report by Weiser *et al*. (2018**)** which states that less than 10% of adults are carriers of *S. pneumonia*. *It should be noted that both the sick and the healthy individuals were considered in this study instead of 100% healthy individuals, and this is expected to have contributed to the higher prevalence.*

Pneumococcal infections occur mostly in people with impaired immune system. Most often, the illness affects people of all ages with debilitating diseases, including persons with diabetes, malignancy, liver disease, chronic obstructive pulmonary diseases and glucocorticoid therapy. The higher percentage (20%) of *S. pneumoniae* among the clinically ill participants as compared to the clinically healthy (11.7%) individuals is in line with the fact that people with impaired immune system are the most susceptible, and at risk of the infection. Diseases of the respiratory system, respiratory tract injury, damage to epithelial cells, excess secretion of mucus by type I hypersensitivity reaction, inhibition of phagocytosis and cough reflex by drug intoxication which enhances aspiration of foreign materials, circulatory abnormalities such as pulmonary congestion, sickle cell anaemia, malnutrition and debility are generally regarded as the predisposing factors of *S. pneumonia* (Ojo, 2009). The 25% prevalence recorded among the people greater than 60 years of age, which is the highest prevalence among the age groups in this study shows that the disease affects the elderly people more than the adolescents and middle aged.

Epidemics generally occur in crowded or close contact areas and poor ventilated areas (Chenoweth, 2009). The incidence peaks between April and October in tropical countries, more especially during the peak of rainy season, high humidity and low ambient temperature. This coincided with the period during which samples were collected. In temperate countries, the incidence increases during the peak of the winter and towards the middle of the summer as a result of closer living conditions (Kim *et al*., 1996). Our findings also show that pneumococcal infection occurs more in females than males. There may be no substantial reason for this. However, women are more prone to hormonal imbalance than the males because of their periodic sexual cycles, and this generally results in immunosuppression. Newborn babies and infants are easily infected from their parents, more especially, their mothers. Iliyasu *et al* (2011**)** reported that pneumococci account for 46.4% of community acquired pneumonia infections, meningitis, and bactaeremia more especially in young children.

Most of the pneumococcal isolates showed resistance to the antibiotics tested. In other words, they are multidrug resistant strains. Moreover, antibiotics such as amoxacilin, zinnacef and ampiclox did not inhibit the growth of any of the isolates. Also, Rocephin and streptomycin were not effective to the majority of the test isolates. However, Ciprofloxacin was the most effective antibiotic for these isolates, followed by Perfloxacin and septrin. Multiple drug resistant pneumococci have been reported in different parts of Nigeria (Emele, 2000; Habib *et al*., 2003; Iliyasu *et al*., 2011). Misuse and widespread use of antimicrobials are reported to be a driver of MDR pneumococcal strains (Kraemer *et al*., 2019).

Wang *et al* (2019) in their observational study in mainland China obtained a MDR prevalence rate of 46.10% (n = 6132) which was lower than our finding of 100%. Furthermore, they also obtained resistance rates of 95.8%, 95.2%, 93.6%, and 66.7%, respectively to clindamycin, erythromycin, tetracycline, and

trimethoprim/sulfamethoxazole. Compared to our finding, the resistance rates ranged from 11.4 (ciprofloxacin) to 100% (zinacef, amoxacilin and amoxacilin). In another study, *S. pneumonia* isolates obtained from a 20-month old patient showed resistance to erythromycin, co-trimoxazole, tetracycline, and chloramphenicol, as well as to penicillin, ceftriaxone, and cefotaxime. In their study Toda *et al* (2018) examined 4534 isolates obtained from children and adults for antimicrobial resistance. These isolates showed resistance to penicillin, cephalosporins, carbapenem and macrolides amongst children and adults in their study. Our MDR isolates were obtained across all age cadre and they showed resistance to these similar classes of antibiotics. In an earlier study a lower MDR prevalence of 31.6% than our findings was obtained. Like our study, almost all their isolates showed varying degrees of resistance that ranged from ampicillin (5.3%) to trimethoprim/sulfamethoxazole (78.9%). This pathogen is capable of causing outbreaks in hospital settings. Bastiaens *et al* (2018) reported nosocomial outbreak of MDR of *S. pneumoniae* serotype 15A.

CONCLUSION

Our findings show a high rate of occurrence of pneumococcal infection in Obong Ntak, Etim Ekpo local government of Akwa Ibom state. Also, the 14% prevalence seen in this study area suggests that pneumococcal infection is widespread in Akwa Ibom state and possibly Nigeria. The work also reveals that multi-drug resistant strains are prevalent which can lead to increased morbidity and mortality among the infected people. This therefore, calls for immediate responses by Government and other agencies to put in place measures to prevent the spread of this pathogen especially at community level.

REFERENCES

Arai, J., Hotomi, M., Hollingshead, S. K., Ueno, Y., Briles, D. E. &Yamanaka, N. (2011). *Streptococcus pneumoniae* isolates from middle ear fluid and nasopharynx of children with acute otitis media exhibit phase variation. *Journal of Clinical Microbiology*,*49*, 1646-1649.

Bastiaens, G. J. H., Cremers, A. J. H., Coolen, J. P. M.,Nilesen, M. T., Boeree, M. J., Hopman, J & Wertheim, H. F. L. (2018). Nosocomial outbreak of multi-resistant *Streptococcus pneumoniae* serotype 15A in a centre for chronic pulmonary diseases.*Antimicrob Resistance Infect Control*,*7*,158 (2018). https://doi.org/10.1186/s13756-018-0457-3

Bauer,A. W., Kirby, W. M. N., Sherries, J. C. &Turck, M. (1960). Antibiotics susceptibility testing by a Standardized single disk method. *American Journal of clinical pathology*,*36*, 493-496.

Bogaert, D., De Groot, R. &Hermans, P. W. M. (2004).*Streptococcuspneumoniae* colonization: the key to pneumococcal disease. *Lancet Infectious Disease*, *4*, 144-154.

Büyükcam, A.,Güdücüoğlu, H., Karaman, K., Gürbüz, V., Aliyev, E., Kara, A.&Ceyhan, M. (2017). Invasivepneumococcal infection due to serotype 15A after the pneumococcal conjugate vaccine implementation in Turkey.*Human Vaccine Immunotherapy*,*13*(8), 1892-1894.

Cheesbrough, M. (2009). *Medical laboratory manual for tropical countries, microbiology (ELBS)*, New York. 2nd edition, pp. 62-70.

Chenoweth, C. E. (2009)."Streptococci." APIC Text of Infection Control and Epidemiology. Washington, D.C. *Association for Professionals in Infection Control and Epidemiology*, *71*, 1-71.

Clinical Laboratory Standard Institute (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. Clinical Laboratory Standard Institute. Wayne, Pennsylvania, USA Magiorakos.

Crossley, K. B. & Lippincott, W. W. (2004). "Streptococci" Hospital Epidemiology and Infection Control. Third Edition Philiadelphia. Pp. 523-524.

Emele, F. E. (2000). Etiologic spectrum and pattern of antimicrobial susceptibility in bacterial meningitis in Sokoto, Nigeria. *Acta Paediatric*,*89*(8), 942-6.

Falade, A. G., Lagunju, I. A., Bakare, R. A., Odekanmi, A. A. &Adegbola, R. A. (2009).Invasive pneumococcal disease in children aged, 5 years admitted to 3 urban hospitals in Ibadan, Nigeria. *Clinical Infection Disease*,*48*, 190-6.

Fang, G. D., Fine, M., Orloff, J.&Arisumi D. (1990). New and emerging etiologies for community-acquired pneumonia with implications for therapy: A prospective multicenter study of 359 cases. *Medicine (Baltimore)*, 69(5), 307-16.

Garba, I., Abdulrazaq, G. H., Aminu, B. M. & Mohammad, M. B. (2015). Epidemiology and Clinical Outcomes of Community Acquired Pneumococcal Infection in North-West Nigeria. *Sub-Saharan African Journal of Medicine*,*2*,79-84.

Habib, A. G., Nwokedi, E. E., Ihesiulor, I. U., Mohammed, A. & Habib, Z. G. (2003). Widespreadantibiotic resistance in savannah Nigeria. *African Journal Medical of Science,32*, 303-305.

Holt *et al* (1995). Bergey's manual of determinative bacteriology. 9th Edition, Maryland,

Iliyasu, G., Habib, A. G. & Ahmed, M. (2011). Clinical pattern and profile of invasive pneumococcal infection in Aminu Kano Teaching Hospital. National Postgraduate Medical College Nigeria. Pneumococcal infection in Nigeria: Preparing for the vaccine. Sub-Saharan African *Journal of Medicine vaccine, 1*,15-19.

Kim, P. E., Musher, D. M., Glezen, W. P., Rodriguez-Barradas, M. C., Nahm, W. K. & Wright, C. E. (1996). Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. *Clinical Infectious Diseases,22*, 100-106.

Kraemer, S. A., Ramachandran, A., & Perron, G. G. (2019). Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy. *Microorganisms*, *7*(6), 180. https://doi.org/10.3390/microorganisms7060180

Lindsay, K., Lesley, M. G., Sara, T. &Bernard, B. (2016). Biological and Epidemiological Features of Antibiotic-Resistant *Streptococcus pneumoniae* in Pre- and Post-Conjugate Vaccine Eras: A United States Perspective. *Clinical Microbiology Reviews,29*(3), 525-552.

Mandell, G. L., Gordon, D. R., John, E. B. & Raphael, D. (2010). "*Streptococcus Pneumoniae*." Mandell, Douglas, and Bennett's Principles and Practice of *Infectious Diseases*, 7th ed, New York: Elsevier/Churchill Livingstone.2010

Martson, B. J., Plouffe, J. F., File, T. M.,*et al*. (1997). Incidence of Community-acquired pneumonia requiring hospitalization: results of population based active surveillance in Ohio.he Community-Based Pneumonia Incidence Study Group.*Archines Internal Medicine, 157*, 170-918.

Musa, B. M., Tijjani, B. M., Okpapi, J. U., Borodo, M. M., Babashnai, M. & Shehu, Y. (2008). *et al*. Bacterial isolates and Antibiotic Sensitivity in Community Acquired Pneumonia. *Nigeria Medical Journals, 49*, 63-66.

Nakano, S., Fujisawa, T., Ito, Y., Bin, C, et al. (2019). Whole-genome sequencing analysis of multidrug-resistant serotype 15A *Streptococcus pneumoniae* in Japan and the emergence of a highly resistant Serotype 15A-ST9084 clone.*Antimicrobial Agents Chemotherapy, 63*(5), e02579-18.

Ojo, M. O. (2009). Manual of Pathogenic Bacteria and Fungi. Banola Multi Project Limited. P.O. Box19927 University of Ibadan Post Office, Ibadan, Nigeria.2009.

Okonkwo, E. E. & Oguamanam, C. C. (2013). Traditional Crafts and Tourism Development and Promotion in Etim Ekpo Local Government of Akwa Ibom State, Nigeria. *Research on Humanities and Social Sciences,* 3(6), 139-147.

Ozumba UC. Changing pattern of acute bacterial meningitis in Enugu, Nigeria. East Africa Medical Journal 1994; 71: 300-303.

Revathy, A., Hema, R., Rohaidah, H., Fairuz, A., Nazirah, S.&Norazah, A. (2019). Multidrug-resistant *Streptococcus pneumoniae* causing invasive pneumococcal disease isolated from a paediatric patient. *International Journal of Infectious Diseases* 2019; 90: 219-222.

Scott, J. A. G. (2007). The preventable burden of pneumococcal disease in the developing world. *Vaccine, 25*, 2398-2405.

Setia, M. S. (2016). Methodology Series Mdoule 3: Cross Sectional Studies. *Indian Journal of Dermatology, 61*(3), 261-264.

Sham, D. F., Thomsberry, C, Mayfield, D. C., Jones, M. E. &Karlowsky, J. A. (2001). Multidrug resistant urinary tract isolates of *Escherichia coli*: Prevalence and patient demographics in united states in 2000. *Antimicrob Agents Chemother, 45*(5), 1402-1406.

Sleeman, K. L., Daniels, L., Gardiner, M., Griffith, D., Deeks, J. J. &Dagan, R. (2005). Acquisition of *Streptococcus pneumoniae* and nonspecific morbidity in infants and their families: A cohort study. *Pediatrics Infectious Disease Journal, 24*, 121-127.

Srinivasan, A. P., Carey, A., Carmeli, R. B., Falagas, Y. M. E.,*et al*. (2012). Multidrug resistance, extensively drug resistance and pandrug-resistance bacteria: An international expert proposal for interim standard definations for acquired resistance. *Clin Microbiol Infection, 18*, 268-281.

Toda, H., Satoh, K. & Komatsu, M. (2018). Laboratory surveillance of antimicrobial resistance and multidrug resistance among Streptococcus pneumoniae isolated in the Kinki regionof Japan - Comparison of the prevalence

of drug-resistant strains before and after introduction of conjugated pneumococcal vaccine. *Journal of Infection and Chemotherapy*, 24, 171-176.

Wang, C. Y., Chen, Y. H., Fang, C., et al (2019). Antibiotic resistance profiles and multidrug resistance patterns of Streptococcus pneumoniae in pediatrics. *Medicine*, *98*(24), 1-7.

Weiser, J. N., Ferreira, D. M. & Paton, J. C. (2018). Streptococcus pneumoniae: transmission, colonization and invasion. *Nat Rev Microbiol. 16*(6), 355-367. doi: 10.1038/s41579-018-0001-8.

World Health Organization (2007). Pneumococcal conjugate vaccine for childhood immunization-WHO position paper. *Weekly Epidemiological Records*82, 93-104.

World Health Organization (2022). Pneumonia. https://www.who.int/news-room/fact-sheets/detail/pneumonia. Accessed 20/05/2022.