Prevalence of Drug-Resistant
Strains of *Streptococcus Pneumoniae* in Abakaliki

**[1]Iroha Ifeanyichukwu Romanus, [2]Okafor Florence Njideka,
[1]Nwakaeze Emmanuel Amobi, [1]Oji Anthonia Egwu, [1]Afiukwa Felicitas Ngozi,
[2]Nwosu Okechukwu Kenneth, [3]Ayogu Thomas Eze and [4]Oladimeji Sikiru**

[1]Department of Applied Microbiology, Faculty of Biological Sciences,
Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria
[2]Department of Microbiology,
Federal University Teaching Hospital Abakaliki, Nigeria
[3]Department of Chemical Pathology,
Faculty of Clinical Medicine, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria
[4]Department of Food Science and Technology,
School of Technology Institute of Management and Technology, P.M.B. 01079, Enugu

ABSTRACT

Streptococcus pneumoniae is a major cause of illness such as pneumonia, meningitis, bacteremia and otitis media in children and the elderly. The emergence of drug-resistant strains threatens to complicate the management of these diseases. We conducted a hospital-based surveillance for drug-resistant Strep. Pneumoniae in outpatients with pneumococcal infection in Abakaliki Ebonyi State Nigeria. Between 2003-2005 from January through December, a cross-sectional study was conducted in Ebonyi State University Teaching Hospital (EBSUTH) Abakaliki to assess the prevalence of drug resistant strains of *Streptococcus pneumoniae* isolated from sputum samples of patients with pneumococcal infections attending the outpatient clinics. A total of 305 sputum samples of patients with clinically diagnosed pneumonia were collected and inoculated on 5% sheep-blood agar, incubated at 35°C for 24 h in 5-10% CO_2. Colonies were Gram-stained; alpha-hemolysis colonies were tested with a 6mm optochin disk followed by bile solubility test. Susceptibility testing panels of the following antibiotics: penicillin, ciprofloxacin, clavulanic acid/amoxicillin, septrin, erythromycin, gentamycin, clarithromycin, cefotaxime and cefuroxime were tested against isolated strains of *Streptococcus pneumoniae* using disc diffusion method. Of the 305 sputum samples collected 30 (9.8%) of *S. pneumoniae* was isolated from patients within the age range of 20-40 years, 140 (45.9%) from patients within 41-60, 135(44.2%) from patients within 60 years and above. Susceptibility studies showed that the highest resistance was 182 (59.6%), for penicillin followed by septrin 156 (51.2%), erythromycin 120(39.3%), clavulanic/amoxicillin 118(38.7%) cefotaxime 114(37.4%) clarithromycin 100(32.7%), ciprofloxacin 94(30.8%), gentamycin 75(24.6%), cefuroxime 70(22.9%) and ceftriaxone 69(22.6%). The prevalence of *S. pneumoniae* resistance was relatively high and we suggest that proper antibiotics use should be adopted to avert development of multi-drug resistant strains in future.

Keywords: Prevalence, *S. Pneumoniae*, Resistance, Sputum

1. INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is the most common cause of community acquired pneumonia, meningitis Invasive pneumococcal disease is defined as isolation of *S. pneumoniae* from a normally sterile site which most frequently affects children less than 2 years old, adult at least 65 years

old and immunocompromised individuals (Lynch and Zhanei, 2009a). Invasive pneumococcal disease is defined as isolation of *S. pneumoniae* from a normally sterile site which most frequently affects children less than 2 years old, adult at least 65 years old and immunocompromised individuals (children or adults) (Naheed *et al*., 2009). Globally, an estimated 1.6 million people including 1 million children less than 5 years old die of invasive pneumonia disease annually (Lynch and Zhanei, 2009b).

In the past, approximately 80 percent of patients hospitalized with bacteremic pneumococcal infections died of their illness (Peter *et al*., 2008). With effective antimicrobial agents, mortality has decreased but remains at nearly 20% for bacteremic diseases in elderly adults (Lynch and Zhanei, 2009a; Ramdani-Bouguessa and Rahal, 2003). In the United States of America over $4 billion is spent in treatment of pneumonia and other related diseases and emergency of drug resistant strains of *S. pneumoniae* will make these common infection more difficult to treat (Spika *et al*., 1991; Pallares *et al*., 1987; Viladrich *et al*., 1991; Michael, 1994). Following the initial detection of penicillin non-susceptible *S. pneumoniae* in a few geographic regions (e.g., South Africa, India, Australia, Spain) in the 1970s, resistance to penicillin and other antibiotics classes has spread worldwide (Kiran *et al*., 2010; Lynch and Zhanel, 2009b). However, the incidence of *S. pneumoniae* resistance varies markedly among countries or regions (e.g., Spain, France, Asia) but remains low (<5%) in some countries (e.g., Finland, Sweden, Germany) (Kiran *et al*., 2010). Rates of *S. pneumoniae* resistance in the United States and Canada are in the moderate range (Wierzbowski *et al*., 2007; 2009). Prior antibiotic use is the dominant risk factor associated with drug resistant pneumococci (Kiran *et al*., 2010). Here in Nigeria, information on epidemiology of *S. pneumoniae* resistance is scare; hence the present study was designed to determine the prevalence of drug resistance strains of *S. pneumoniae* isolated from sputum samples of adult visiting outpatients clinics of EBSUTH Abakaliki, Ebonyi State Nigeria.

2. MATERIALS AND METHODS

2.1. Collection of Clinical Specimen (Sputum)

Between 2003-2005, a total of 305 sputum samples of adults attending the outpatient clinic of Ebonyi State University teaching hospital Abakaliki were collected. The sputum samples were collected from patients with symptoms of lower respiratory tract infection especially cough and who was clinically diagnose of having respiratory tract infection. A case of pneumococcal infection was defined by the isolation of *S. pneumoniae* from sputum samples. Sputum samples of patients devoid of *S. pneumoniae* but was diagnosed of upper respiratory infection were not included in the study. Patients were also provided with questionnaire to identify risk factors for drug resistance *S. pneumoniae* acquision which includes previous hospitalization, respiratory diseases, intravenous drug use, use of antibiotics for any care within the past years, chronic illness and tuberculosis infections. Consent of patients was obtained before samples were collected.

2.2. Isolation of *S. Pneumoniae* from Sputum Sample

Patient's sputum samples were collected and inoculated on 5% sheep-blood agar and incubated at 35°C for 24 h in 5-10% CO_2. Colonies were Gram-stained; alpha-hemolysis colonies were tested with a 6 mm optochin disk in sheep-blood agar dish followed by bile solubility test. Further identification and characterization was carried out using standard microbiology technique (Cheesbrough, 2006).

2.3. Susceptibility Studies

Susceptibility testing panels that includes antibiotics used in EBSUTH in treating patients with pneumonia which includes penicillin (10 μg), ciprofloxacin (5 μg), clavulanic acid/amoxicillin (20/10 μg), septrin (25 μg), erythromycin (15μg), gentamycin (10 μg), clarithromycin (25 μg), cefotaxime (30μg) and cefuroxime (30 μg) were tested against isolated strains of *S. pneumoniae* using disc diffusion method. Briefly, A Mueller-Hinton agar plate was prepared using manufacturers instruction, test organisms equivalent to 0.5 McFarlane equivalent standard was inoculated on the surface of sterile agar plate and was allowed for 15 min to pre-diffuse, then antibiotics disc as mentioned above was placed on the agar with sterile forceps. This was incubated for 18-24 h at 37°C, after which the inhibition zone diameter was taken with a transparent meter rule to the nearest millimeter. Susceptibility result was interpreted as resistance or susceptible or intermediate according to the definitions of the National Committee for Clinical and Laboratory Standard Institute.

3. RESULTS

From 2003-2005, 305 cases of pneumonia disease were reported in EBSUTH in the outpatient clinic, majority of the isolates were from male patients 203 (66.5%).

Table 1. Percentage of prevalence of *S. pneumonaie*

Year range of patients	Percentage of occurrence of *S. pneumonaie*	Clinical specimen
20-40 years	30(9.8%)	Sputum
41-60 years	140(45.9%)	Sputum
60 and above	135(44.2%)	Sputum
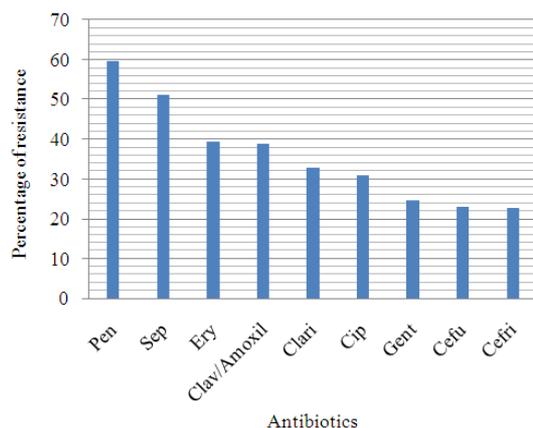
Fig. 1. Percentage of antibiotics resistance of *S. pneumoniae* isolated from sputum samples of outpatient. Key: Pen: Penicillin, Sep: Septrin, Ery: Erythromycin, Clav/Amoxil: Clavulanic Acid/Amoxicillin, Clari: Clarithromycin, Cip: Ciprofloxacin, Gent: Gentamycin, Cefu: Cefuroxime, Cefri: Ceftriaxone.

The year range of patients involved in the study was from twenty to sixty years and above. 30(9.8%) of *S. pneumoniae* was isolated from patients within the age range of 20-40 years, 140(45.9%) from patients within 41-60, 135(44.2%) from patients within 60 years and above (**Table 1**). Susceptibility studies showed that the highest resistance was 182(59.6%), for penicillin followed by septrin 156(51.2%), erythromycin 120(39.3%), clavulanic/amoxicillin 118(38.7%) cefotaxime 114(37.4%) clarithromycin 100(32.7%), ciprofloxacin 94(30.8%) gentamycin 75(24.6%), cefuroxime 70(22.9%) and ceftriaxone 69(22.6%) **Fig. 1**. Sex, age, prior antimicrobial therapy for the current problem or within the last 6 months and underlying conditions like TB did not predict or explain their resistance pattern.

4. DISCUSSION

The result obtained in the present study described the epidemiology period of one of the two major hospitals in Abakaliki, Ebonyi State in which low susceptibility to

different classes of antibiotics was observed especially with penicillin, a common drug of choice for treatment of pneumonia. The hospital were this study was carried out represent this town population because it is the only University teaching hospital which serves as a referral hospital for patients with serious medical problems in Ebonyi state. Infections caused by *Streptococcus pneumoniae* such as otitis media, sinusitis, bronchitis and meningitis, which have led to a number of deaths especially in children and the elderly are a reason for worldwide concern. The overall prevalence of pneumonia infection that occurred within our study period (2003-2005) was low. Previous antibiotic exposure has been documented as a risk factor for antibiotics resistance in many studies (Kaplan and Mason, 1998) but this could not be confirmed in our study. We could not demonstrate any relationship between the occurrence of resistant strain and the other risk factors previously described such as extreme age, hospital-acquired infections, probably due to small sample size. There are reports of high incidence of pneumonia in children and adults from different parts of the world (Hofman *et al.*, 1995). Highest prevalence of resistant strains of *S. pneumoniae* was observed with penicillin in both adult and children. Such findings have been reported in and outside Nigeria (Jacobs *et al.*, 1978; Ramdani-Bouguessa and Rahal, 2003; Simberkoff *et al.*, 1986) and such was observed in our study. Recent studies of adults and children in a variety of communities have found high proportions of penicillin-resistant pneumoncocci. Discovery of penicillin was one of the major medical advances of the 20th century, being indicated for the treatment of community pneumonia caused by *Streptococcus pneumoniae*. Nevertheless, in the beginning of the 21st century it has been losing its role in the treatment of pneumonia due to the crescent increase of antimicrobial resistance (Barlett *et al.*, 1998). Amoxicillin and clavulanic acid alone or associated macrolide can be used as an alternative drug of choice in the treatment of *Streptococccus pneumoniae* infections, although the optimal therapy for infections with drug-resistant pneumococci is not well defined. Studies have suggested that cephalosporins or high dose penicillin may be effective in patients with non-meningeal bacteremic infections (Tan *et al.*, 1992; Garcia-Leoni *et al.*, 1992).

There are report that children six years of age were more likely than older children and adults to be infected with cefotaxime-resistant or multi-resistant isolates. However, we found a disturbingly high incidence of drug resistant pneumococcal infections among adults. Our

data suggest that recommendations for empirical therapy are needed for pneumococcal infections in adults as well as children, particularly in communities in which the prevalence of drug resistant is high. The geographic variation in the prevalence of drug-resistant strains of pneumococci in Nigeria highlights the importance of community-based monitoring of pneumococcal susceptibility to antimicrobial agents to guide therapy. In addition, the increased prevalence of drug-resistant *S. pneumoniae* emphasizes the critical need for preventive strategies in populations at risk from serious pneumococcal infections.

Frequent and prophylactic use of antimicrobial drugs has been associated with a risk of drug-resistant pneumococcal infections but such was not observed in our study as all the risk factors checked could not be associated with the prevalence of multi-drug resistant in *S. pneumoniae*. Despite a low incidence of pneumococcal infection in Abakaliki particularly in patients between the ages of 20-40 years, all age groups was found to be associated with drug-resistant *S. pneumoniae*. Patients within the age range of 60 years and above were also associated with an increased rate of drug resistant *S. pneumoniae*. Rural residence and poverty may be responsible for poor health status and little or no access to medical care. As a result, people tend to see the roadside chemist/pharmacy dealers and procure cheap drugs without doctor's prescription because there is no restriction or guidelines to the use of antibiotics in Nigeria. A critical component for the control of drug-resistant *S. pneumoniae* will be community-wide educational programs for clinicians and the public on the importance of appropriate antibiotic use.

The vast majority of *S. pneumoniae* isolates had a relatively high resistant to all the antibiotics tested namely; penicillin, cefotaxime, septrin, erythromycin, clavulanic acid/amoxicillin, cefotaxime, clarithromycin, ciprofloxacin, gentamycin, cefuroxime and ceftriaxone. With the continuing spread of drug-resistant strains of pneumococci, treatment options will become more limited and prevention measures will become critical.

5. CONCLUSION

This study emphasizes the importance of antimicrobial-susceptibility testing of all *S. pneumoniae* infection in both children and adults in Abakaliki, Ebonyi State as well as a crucial need for community-based programs of surveillance for drug-resistant pneumococcus to aid clinicians in their choice of therapy

for pneumococcal infections. The prevention of infections with *S. pneumoniae* will require strategies to encourage judicious antibiotics use and recommendation for the treatment and prevention of pneumococcal infections must be addressed by health care and public health agencies. Our data suggest an urgent need for consensus guidelines to prevent development of multi-drug resistant strains of *Streptococcus pneumoniae* in this region in future.

6. REFERENCES

Barlett, J.G., R.F. Breiman, L.A. Mandell and T.M. File, Jr., 1998. Community-acquired pneumonia in adults: Guidelines for management. The Infectious Diseases Society of America. Clin. Infect. Dis., 26: 811-838. PMID: 9564457

Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, ISBN-10: 0521676312, pp: 440.

Garcia-Leoni, M.E., E. Cercenardo, P. Rodena, J.C.B.D. Quiros and D. Martinez-Hernandez *et al.*, 1992. Susceptibility of *Streptococcus pneumoniae* to penicillin: A prospective microbiological and clinical study. Clin. Infect. Dis., 14: 427-435. PMID: 1554828

Hofman, J., M.S. Cetron, M.M. Farley, W.S. Banghman and R.R. Fackeam *et al.*, 1995. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. N. Engl. J. Med., 333: 481-486. PMID: 7623880

Jacobs, M.R., H.J. Koornhof, R.M. Robins-Browne, C.M. Stevenson and Z.A. Vermark *et al.*, 1978. Emergence of multiply resistant pneumococci. N. Engl. J. Med., 299: 735-340. PMID: 29219

Kaplan, S.L. and E.O. Mason, Jr., 1998. Management of infections due to antibiotic-resistant *Streptococcus pneumoniae*. Clin. Micrbiol. Rev., 11: 628-644. PMID: 9767060

Kiran, C., B. Gurung, C. Mukhopadhyay and I. Bairly, 2010. Reporting emerging resistance of *Streptococcus pneumoniae* from India. J. Glob. Infect. Dis., 2: 10-14. DOI: 10.4103/0974-777X.59245

Lynch, J.P. and G.G. Zhanel, 2009a. *Streptococcus pneumoniae*: Epidemiology, risk factors and strategies for prevention. Semir. Respir. Crit. Care Med., 30: 189-209. PMID: 19296419

Lynch, J.P. and G.G. Zhanel, 2009b. *Streptococcus pneumoniae*: Does antimicrobial resistance matter? Semir. Respir. Crit. Care Med., 30: 210-238. PMID: 19296420

Michael, S.S., 1994. Drug-resistant pneumococcal infections in the United States: A problem for clinicians, laboratories and public health. JAMA, 271: 1875-1876. DOI: 10.1001/JAMA.1994.03510470079039

Naheed, A., S.K. Saha, R.F. Breiman, F. Khatun and W.A. Brooks *et al*., 2009. Multihospital surveillance of pneumonia burden among children aged <5 years hospitalized for pneumonia in Bangladesh. Clin. Infect. Dis., 48: S82-S89. PMID: 19191623

Pallares, R., F. Gudiol, J. Linares, J. Ariza and G. Rifi *et al*., 1987. Risk factors and response to antibiotic therapy in adults with bacteremic pneumonia caused by penicillin-resistant pneumococci. N. Engl. J. Med., 317: 18-22. PMID: 3587309

Peter, K., O. Hytikainen, P. Ruutu, J. Ollegren and J.P. Nnorti, 2008. Invasive pneumococcal infections among persons with and without underlying medical conditions: Implications for prevention strategies. BMC Infect. Dis., 8: 96-96. PMID: 18647385

Ramdani-Bouguessa, N. and K. Rahal, 2003. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* Isolated in Algiers, Algeria. Antimicrbio. Agents Chemother., 47: 834-826. DOI: 10.1128/AAC.47.2.824-826.2003

Simberkoff, M.S., M. Lukaszewski, A. Cross, M. Al-Ibrahim and A.L. Baltch *et al*., 1986. Antibiotic-resistant isolates of *Streptococcus pneumoniae* from clinical specimens: A cluster of serotype 19A organisms in Brooklyn, New York. J. Infect. Dis., 153: 78-82. PMID: 3941289

Spika, J.S., R.R. Facklam, B.D. Plikaytis and M.J. Oxtoby, 1991. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States, 1979-1987. The pneumococcal surveillance working group. J. Infect. Dis., 163: 1273-1278. PMID: 2037792

Tan, T.Q., E.O. Manson Jr. and S.L. Kaplan, 1992. Systemic infections due to *Streptococcus pneumoniae* relatively resistant to penicillin in a children's hospital: Clinical management and outcome. Pediatrics, 90: 928-933. PMID: 1437436

Viladrich, P.F., F. Gudiol, J. Linares, R. Pallaries and I. Sabate *et al*., 1991. Evaluation of vancomycin for therapy of adult pneumococcal meningitis. Antimicrob. Agents Chemother., 35: 2467-2472. PMID: 1810180

Wierzbowski, A.K., F. Tailor, K. Nichol, J.A. Karlowsky and D.J. Hoban *et al*., 2009. Antimicrobial-resistant *Streptococcus pneumoniae* in Canadian hospitals: Results from the 2007 CANWARD study. Can. J. Infect. Dis. Med. Microbiol., 20: 37A-42A.

Wierzbowski, A.K., K. Nichol, N. Laing, T. Hisanaga and A. Nikulin *et al*., 2007. Macrolide resistance mechanisms among *Streptococcus pneumoniae* isolated over 6 years of Canadian Respiratory Organism Susceptibility Study (CROSS) (1998 2004). J. Antimicrob. Chemother., 60: 733-740. PMID: 17673477