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Abstract

The clinical isolate *Streptococcus pneumoniae* is a major cause of illness such as pneumoniae, meningitis, bacteremia and otitis media in children and the elderly. The emergence of drug-resistant strains threatens to complicate the management of these diseases. An hospitalbased and community-based surveillance for drug-resistant *Streptococcus pneumoniae* in outpatients with respiratory infection in Abraka, Delta State Nigeria was conducted. Between August 2014 to October 2014, a cross-sectional study was conducted in Abraka general hospital and Abraka community to assess the prevalence of drug resistant *Streptococcus pneumoniae* isolated from sputum samples of patients with cough and catarrh (respiratory tract infection). A total of 125 sputum samples of patients with respiratory tract infection were collected and inoculated on 5

Index terms— pneumonia; resistance; susceptibility; prevalence.

1 I. Introduction

The discovery of antibiotics and their general use had transformed the patterns of disease and death in many countries (James, 2006 & Sekowska, 2002). Many diseases that once caused high mortality such as tuberculosis, pneumonia and septicaemia became controllable and surgical infections reduced. These successes could hardly be measured when clinical antimicrobial resistance emerged (Knothe, 1983) leading to treatment failures. But this antimicrobial resistance was to a single antibiotic at the time, so many researchers (Al-Jasser, 2006) are of the view that the appearance of multi drug resistant pathogens is a recent phenomenon, which has become a worldwide problem.

There are currently 90 known serotypes, with the ten most common causing greater than 60% of worldwide invasive disease (CDC, 2003a). *Streptococcus pneumoniae* is a normal inhabitant of the human upper respiratory tract. The bacterium can cause pneumoniae usually of the lobar type, paranasal sinusitis and otitis media, or meningitis, *Streptococcus pneumoniae* is currently the leading cause of invasive bacteria disease in children and the elderly. Pneumonia is a disease of the lung that is caused by a variety of bacteria including streptococcus, Staphylococcus, pseudomonas, Haemophilus, Chlamydia and Mycoplasma, several viruses and certain fungi and protozoans. The disease may be divided into two forms; bronchial pneumonia is most prevalent in infants, young children and aged adults. It is caused by various bacteria, including *Streptococcus pneumoniae* and Lobar pneumonia which is more common in younger adults. A majority (more than 80%) of the cases of lobar pneumonia are caused by *Streptococcus pneumoniae*, lobar pneumonia involves all of a single lobe of the lungs, wherein the entire areas of involvement tends to become a consolidated mass, in contrast to the spongy texture of lung tissue. (Kenneth, 2003).

Pneumococcal pneumonia is the most common form of pneumococcal infection in adults, and accounts for approximately 36% of community-acquired pneumonia and 50% of hospital-acquired pneumonia. The period of time from infection to presentation of signs and symptoms is short, lasting only one to three days. Mortality rates range from 5-7%, and may be higher in the elderly (CDC, 2003b).

Transmission of *Streptococcus pneumoniae* occurs via respiratory droplets from healthy persons carrying the organism in the naso-pharynx or from person with pneumococcal disease. Following exposure, the organism may

establish itself in the nasopharynx of its new host usually resulting in asymptomatic colonisation. The organism can be carried for a period of weeks to months. However sometimes, the newly acquired pneumococcus evades host defensive mechanisms and causes illness (Buttler et al 1993).

Antibiotics from different classes can be used in treatment. These are chosen according to the host (age, allergy, localisation of the infection and the epidemiology of antibiotics resistance of pneumococcus strains). Aminopenicillins and penicillins are widely used for the treatment of pneumococcal infection and they are the first line anti-microbial in many countries to treat acute community acquired pneumonia. (Barlett, et al., 2004). β -Lactam inhibits the peptidoglycan synthesis of the bacteria cell wall by forming a covalent bond with the active site of penicillin-binding proteins leading to hydrolysis of the bacteria. Mutations results in a decrease affinity to β -lactam. With the widespread use antibiotics, starting in the 1940s with penicillin, there has been a steady rise in the number of resistant serotypes or strains of drug resistant *Streptococcus pneumoniae*. The first clinical diagnosis of penicillin-resistant *S. pneumoniae* appeared in the 1967 in New Guinea, and multi-drug resistant strains appeared in South Africa in 1977 (Tomasz, 1997).

The CDC reports that annually there are an estimated 175,000 hospitalized cases of pneumococcal pneumonia, 50,000 cases of pneumococcal bacteremia and 3,000-6,000 cases of pneumococcal meningitis (CDC, 2003a). Additionally in children less than 5 years there are estimated 5 million case of otitis media every year in this country. Due to the severity of the disease caused by invasive *S. pneumoniae* and the continued rise of antibiotic resistance, several vaccines have been developed in the United State.

The increasing prevalence of resistance to established antibiotics among key bacterial respiratory tract pathogens such as *Streptococcus pneumoniae* is a major healthcare problem in Delta State. However, the increasing prevalence of antibiotics resistance bacterial therefore poses a significant problem in selection of drug of choice for treatment of the infection.

Amazingly, the phenomenon of bacteria resistance is now threatening to take us back to a preantibiotics era. It has been reported that the growing phenomenon of antibiotics resistance is caused by the use and abuse of antibiotics.

The overall aim of study is to investigate the prevalence of resistance strains of *Streptococcus pneumoniae* to oxacillin, ofloxacin and rifampicin (fluoroquinolones) and contribute to the existing body of knowledge on the antimicrobial efficacy of antibiotics against the bacteria.

2 II. Materials and Methods

3 a) Collection of clinical specimen (sputum)

Specimen collection was carried out from August to October 2014, a total of 125 sputum samples of adults with upper respiratory tract infection in Abraka was collected. The sputum samples were collected from patients with symptoms of lower respiratory tract infection especially cough and who was clinically diagnosed of having respiratory tract infection. A sterile flexible swab was inserted into the patient's nostrils until it touches the posterior wall of the nasopharynx. After performing rotator movements for 5 seconds so that secretion could be absorbed, the swab was removed and submerged on Amies medium without charcoal and taken to the microbiology laboratory at room temperature. A single collection was performed for each patient.

4 b) Isolation of streptococcus pneumonia from sputum samples

Sputum samples was streaked onto nutrient agar plate and incubated at 35-37 °C for 24 hours and plates that showed growth of organism was further plated on blood agar prepared with tryptose blood agar base (Difco, Becton Dickson and company, Sparks MD USA) Supplement with 5% of defibrinated sheep blood.

5 c) Identification of test micro-organism

The identification of the test organisms by appropriate cultural methods and gram staining was done following standard microbiological procedures.

6 d) Biochemical reactions

i. Bile solubility test 1. Preparation of Bile salt 2% bile salt was prepared by dissolving 2g in 100mls of sterilized normal saline, making a solution of bile salt.

7 Inoculum preparation

Bacterial inoculum was prepared by inoculating 0.25mls of 24 hours overnight culture into sterilized test tubes.

0.25mls of the bile salt was added into broth test tube. 0.25mls of normal saline was dispensed into another test tube to serve as the control. 0.25mls of broth organism was added into both test tubes and incubated for 2 hours. A clear solution indicated a positive test for *Streptococcus pneumoniae*.

8 e) Susceptibility studies

Susceptibility testing panels that include antibiotics used in treating patients with pneumonia in Abraka which includes Ofloxacin, Rifampicin and Oxacillin were treated against isolated strains of *Streptococcus pneumoniae* using disc diffusion method.

Briefly a Mueller-Hinton agar plate was prepared following manufacturer's instruction, test organisms equivalent to 0.5 McFarlane equivalent standard was inoculated on the surface of sterile agar plate and was allowed for 15mins to prediffuse, then antibiotics disc as mentioned above was placed on the agar plates with sterile forceps and incubated for 18-24hours at 37°C after which the inhibition zone diameter was taken in millimetre. Susceptibility result was interpreted as resistance or susceptible or intermediate according to the definitions of the national committee for clinical and laboratory standard institutes.

Other biochemical test such as Carbohydrate Fermentation, Iodine and Coagulase were carried out as described by Monica 2002.

9 III. Results

10 a) Identification of collected test micro-organisms

The identification test carried out on the clinical isolates as presented in Table ??1 showed that *Streptococcus pneumoniae* presented a characteristic alpha haemolytic reaction (greenish zone of inhibition) on blood agar which differentiates *Streptococcus pneumoniae* from the group A (beta haemolytic) *Streptococcus*, but not from commercial alpha haemolytic (viridans).

Motility test results showed that *Streptococcus pneumoniae* is non-motile. Catalase test results showed that they are catalase negative (they lack catalase) and fermentation test result showed they ferment glucose to lactic acid. Gram-staining reaction test results showed that they are gram positive showing a lancet shaped cocci (elongated cocci with a slightly pointed outer curvature), they were seen as pairs of cocci (diplococci) but they also occur singly and in short chains.

In bile testing, results showed that they undergo lysis by bile salt (eg deoxycholate). Virtually all clinical isolates of pneumococci harbour the autolysis and undergo deoxycholate lysis.

11 b) Antimicrobial sensitivity test results i. Population of *streptococcus pneumoniae* isolated

From Table ??1, the prevalence rate of *Streptococcus pneumoniae* carries among the adult studied was 22.4% (28/125). Eighteen out of seventyfive male adult (24%) and 10 out of 50 female adults (20%) had positive cultures for *Streptococcus pneumoniae*.

A significant difference was found when the positive result of pneumococcal isolation was arranged according to age group ($p=0.0005$), with a higher rate of isolation in the adult aged from 20 -25 years (31%; 20/65). Among 40 adults, from 26 -30 years old (13%, 8/60) had positive cultures for pneumococcus. 60 patients were treated with antimicrobial drugs in the last month with a higher rate of isolation is (25%).

12 c) Antimicrobial sensitivity result

The results of penicillin susceptibility tests of pneumococci using rifampicin and oxacillin, determined that of 28 specimens of confirmed *Streptococcus pneumoniae* all were resistant to rifampicin and oxacillin. All isolates were sensitive to ofloxacin which gave a mean zone of inhibition of 18.68 and standard deviation of 2.75 (18.68 ± 2.75) with percentage inhibition of 74.3%.

13 IV. Discussion

The result obtained in the present study described the epidemiology period in Abraka, Delta state where the prevalence of adults colonized with nasopharyngeal *Streptococcus pneumoniae* was 22.4% from which 24% male adults and 20% female adults are for *Streptococcus pneumoniae*. This study confirmed that age is an important factor for pneumococcal isolation with high prevalence of isolates in the age group between 20-25 years (31%) and low prevalence of isolates in the age group between 26-30 years (13%) which is agreement with Austrian (1986).

According to ??orer et al.2001, the alterations in the mucosa of the respiratory tract of allergic patients can result in impaired mucociliary activity, predisposing to bacteria colonization in this site. The interpretation of susceptibility of the drugs used in this study showed that pneumococcal resistance rate was higher with rifampicin and oxacillin as they showed no zone of inhibition indicating no susceptibility and a high susceptibility (74.3%) was observed in ofloxacin.

Previous antibiotic exposure has been documented as a risk factor for antibiotic resistance in many studies and also, antibiotic therapy may increase the rate of isolation of penicillin-resistant nasopharyngeal pneumococci (Kaplan and Mason 1998). This fact may explain the high prevalence of oxacillin resistant pneumococcal strains found in this study, since 60(48%) of the 125 adults had been treated with antimicrobial drugs in the month prior to the study.

The high rate of resistance to rifampicin and oxacillin found in this study is similar to that described in other studies in and outside Nigeria ??Jacobs et al., 1978).

Ofloxacin can be used as an alternate drug of choice in the treatment of *Streptococcus pneumoniae* infections, although the optional therapy for infections with drug-resistant pneumococci is not well defined.

There are reports that children 6years of age were more likely than older children and adults to be infected with multi-resistant isolates. However in this study, a disturbingly high incidence of drug resistant pneumococcal infections among adults was found.

Although the overall prevalence of pneumococcal infections in Abraka community is low 22.4% recommendations for empirical therapy are needed 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 *Streptococcus pneumoniae* emphasizes the critical need for preventive strategies in populations at risk from serious pneumococcal infections.

Frequent and prophylactic use of antimicrobial drugs has also been associated with a risk of drug resistant pneumococcal infections and such was observed in this study of 125 adult sputum collected 44 adults had not been treated with antibiotics prior to the collection while 60 adults had been treated and 21 adult cases not reported. A significant difference was observed with adults who had been treated with antibiotics prior to sputum collection and those who had not been treated. Of the 28 *Streptococcal pneumonia* isolated, 15 (25%) out of 60 adults who had had antibiotics prior to collection was positive for *Streptococcus pneumonia* while 10 out of 44 adults who had not had antibiotic prior to collection of sputum were positive. (10; 16%). Despite a low incidence of pneumococcal infection in Abraka especially in patience between the ages of 26 -31 years, age groups of 20 -31 years was found to be associated with drug-resistant *Streptococcus pneumoniae*. Rural residence and poverty may be responsible for poor health states 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 *Streptococcus pneumoniae* will be community wide educational programs for clinicals and the public on the importance of appropriate antibiotic use.

The vast majority of *Streptococcus pneumoniae* isolates had a relatively high resistant to rifampicin and oxacillin. The continuing spread of drug resistant strains of pneumococci treatment options will become more limited and prevention measures will become critical.

From this study, possible reasons for this low prevalence in populations infected with *Streptococcus pneumonia*, include the fact that the survey only adults, the subjects were enrolled for a very short time, winter (a period of frequent respiratory illness) was not the season of enrolment, and the fact that human genetic traits may play a role.

The identification and confirmatory tests carried out on the organism to establish their identity yielded results that were in agreement with previous studies. For example *Streptococcus pneumonia* appeared as alpha haemolytic when cultured in blood agar, colonies characteristically produced an alpha (green) hemolysis.

Solubility in bile salt agar solution, also in agreement with previous studies, *Streptococcus pneumonia* fermented glucose producing acid and gas, were coagulase negative and purple gram-positive cocci in clusters and single when viewed from the microscope.

Antimicrobial susceptible test is an essential technique used in pharmacology to determine the efficacy of novel antimicrobial agent against microorganism.

Susceptibility studies was carried out using oxacillin, rifampicin and ofloxacin against isolated strains of *Streptococcus pneumoniae*. The experiment was replicated twice and an average zone of inhibition reading was taken. Studies were conducted to investigate the prevalence of *Streptococcus pneumoniae* infection in adults in Abraka community and also to investigate the prevalence of resistance of *Streptococcus pneumoniae* to oxacillin, rifampicin and ofloxacin and to know the potential of these listed drugs as a broad spectrum antimicrobial agent in the treatment of nasopharyngeal infection.

The data collected were subjected to analysis, mean deviation, standard deviation and percentage deviation of the zones of inhibition exhibited by ofloxacin as no zone of inhibition was observed with rifampicin and oxacillin indicating non-sensitivity towards *Streptococcus pneumoniae*. This study emphasizes the importance of antimicrobial susceptibility testing of all *Streptococcus pneumoniae* infection in adults in Abraka, as well as crucial need for community based programs of surveillance for drug resistant pneumococcus to aid clinicians in their choice of therapy for pneumococcal infections.

From the study the following conclusion can be drawn;

The overall prevalence of *Streptococcus pneumoniae* infection in Abraka community is low.

? The standard antimicrobial agent ofloxacin was significantly more effective than rifampicin and oxacillin. ? Rifampicin and oxacillin antibiotics are not sensitive to *Streptococcus pneumoniae* as the bacteria were totally resistant. ? The study data suggest an urgent need for consensus guidelines to prevent development of multi-drug resistant strains of *Streptococcus pneumoniae* in this region in future.

14 ? The prevalence of fluoroquinolone resistance in

Streptococcus pneumoniae is low.

15 V. Recommendation

From the result obtained the following recommendations are made;
 ? Ofloxacin should be used as a first-line drug alone or in combination to treat pneumococcal infection as well as infection of the upper respiratory tract in Abraka metropolis. ? Strategies to encourage judicious antibiotics use should be implemented this will enhance prevention of infections with *Streptococcus pneumoniae*.

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Identification Tests		Results
1	Cultural Characteristics	Colonies produce a zone of green (alpha) haemolysis when cultured on blood agar. They grow as glistening colonies on agar about 1mm in diameter
2	Motility Tests	They are non-motile
3	Staining Reactions	They appear as dark purple gram positive cocci occurring in pairs, singly or short chains.
4	Biochemical Reactions	
I	Coagulase	Negative (They lack coagulase)
Ii	Indole	Negative (They lack indole)
Iii	Sugar Fermentation	Show a yellow colour with bubbles in a tube (they ferment glucose to lactic acid.
5	Bile Solubility Test	Show a clear solution in the tube containing 2.5ml of bile salt and 2.5ml of broth culture of organism.

Figure 1: Table 3 . 1 :

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Variable	Negativity, N (%)	Positive, P (%)
Population	97 (77.6%)	28 (22.4%)
Sex		
Female	40 (80%)	10 (20%)
Male	57 (76%)	18 (24%)
Age		
20 -25 Years	45 (69%)	20 (31%)
26 -30 Years	52 (86%)	8 (13%)
Use of Antibiotics (prior to sputum collection)		
No		
Yes	34 (84%)	10 (16%)
Not Reported	45 (75%)	15 (25%)
	18 (86%)	3 (14%)

Figure 2: Table 3 . 2 :

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