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Original Research Article

Serotypes and Antibiotic Susceptibility of *Streptococcus pneumoniae* Isolated from Adults with Lower Respiratory Tract Infection in Jos, Nigeria

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Background: ***Streptococcus pneumoniae*** **causing lower respiratory tract infection remains a global challenge, particularly when it involves drug resistant isolates. It is a cause of morbidity and mortality in developing countries including Nigeria. Several studies documented the prevalence, serologic and molecular types, antibiotic susceptibility patterns; as well as the herd effect following vaccination. This study thus determined the prevalence of the Heptavalent vaccine serotype of** ***Streptococcus pneumoniae*** **in adult patients and the antibiotic susceptibility pattern.** ***Methods:*** **A cross sectional study involving 91 adult patients with clinical features of lower respiratory tract infection were recruited for this study. Serologic typing as well as antibiotic susceptibility testing were carried out on the** ***Streptococcus pneumoniae*** **isolates.** ***Results:*** **Four (4.4%) of the 91 patients had** ***Streptococcus pneumoniae*** **isolated from their clinical samples. Only one (14.3%) of the heptavalent vaccine serotype (serotype 23F) was found. All the four** ***Pneumococci*** **were susceptible to Ceftriaxone, Levofloxacine, Trimethoprim- sulfamethoxazole and Amoxicillin-clavulanic acid but varying susceptibility to Erythromycin and Tetracycline.** ***Conclusion:*** **Heptavalent vaccine serotype of** ***Streptococcus pneumoniae*** **causes infection in adults. Early detection and appropriate antibiotic susceptibility will enhance proper management of patient. Vaccination of children will reduce infection due to the vaccine serotypes in adult population.**

Keywords: *Streptococcus pneumoniae,* Heptavalent vaccine, Antibiotics, Serology.

INTRODUCTION

Streptococcus pneumoniae remains an important bacterial cause of lower respiratory tract infection responsible for, marked, morbidity and mortality in both developing and industrialized countries (Chih-Jung et al., 2007). Colonization of the nasophrynx by *Streptococcus pneumoniae* serves as an important reservoir for community-wide spread of these pathogens and a key factor preceding pneumococcal diseases (Chih-Jung et al., 2007; Pichichero and Casey, 2007).

The virulence of the organism is due to its possession of polysaccharide capsule that enables it to resist phagocytes hence evading host immune response. Based on capsular polysaccharides, the *pneumococci* are classified into at least 90 serologic types (Hausdorff et al., 2005; Chih-Jung et al., 2007; Pichichero and Casey, 2007). The prevalence and the distribution of serotypes differ significantly among isolates, from one geographic region to the other; from asymptomatic carriers versus those from patients with invasive pneumococcal diseases[3]. Despite the large number of serologic types only very small numbers are implicated in infections (Hausdorff et al., 2005; Aguiar et al., 2008).

The 7-valent *pneumococcal* polysaccharide –CRM_{19}-protein conjugate vaccine (PCV-7) was introduced in several countries and recommended to expanded risk groups followed by the universal vaccination program. This vaccine provides protection against invasive pneumococcal infections caused by serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, and reduces nasopharyngeal carriage of these serotypes after vaccination (Black et al., 2000). In a prospective study by Lexau et al., in the United States, it was observed that mass vaccination of children resulted in a herd effect, shown by the reduction in the

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incidence of invasive diseases among adults. The PCV-7 includes the most resistant serotypes, with hopes that vaccination effectively controls the spread of resistant clones (Lexau et al., 2005; Stephens et al., 2005). Several other studies particularly in the United States, showed that the reduction of vaccine serotypes in asymptomatic carriage and invasive pneumococcal dieases was countered by replacement with non vaccine serotypes(Laurent et al., 2009; Miller et al., 2011; Ladhani et al., 2013).

The objectives of this study were to determine the prevalence of Hepta valent vaccine serotypes of *Streptococcus pneumoniae* in adult patients with lower respiratory tract infection, to describe the antibiotic susceptibility of all the *pneumococci.*

METHODS

This prospective study was carried out among adults with lower respiratory tract infection after written informed consents were obtained from them. Blood and sputum samples were obtained from all the patients. Three sets of blood were collected for culture. Culture of both blood and sputum from each patient was carried out. Identification of *S.pneumoniae* isolates was confirmed by optochin sensitivity and bile solubility test.

The preliminary typing using polyvalent typing sera (Debendiagnostics Ltd, United Kingdom) allowed to determine the pneumococcal isolate. Complete serotyping was performed at the National Veterinary Research Institute (VOM, Nigeria) using latex particles sensitized with antisera (monovalent typing sera) purchased from Debendiagnostics Ltd, United Kingdom. The panel of monovalent sera used allowed to determine the heptavalent vaccine serotypes (4,6B,9V,14,18C,19F and 23F). 10ul of pneumococcal overnight growth culture adjusted to 1Mc Farland turbidity standard using a nephelometer was mixed with 10ul of latex reagent on a glass slide and rocked for 10seconds. The presence of agglutination of the latex particles read with the naked eye within 10 seconds was considered as positive reaction. This procedure was carried out for every *S. pneumoniae* isolated.

The antimicrobial susceptibility testing was performed using the disc diffusion method (tetracycline, trimethoprim-sulfamethoxazole, erythromycin, levofloxacine). MIC determination was by E test method (ceftriaxone, amoxycilline-clavulanic acid, Oxoid U.K). Data were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement (CLSI, 2010).

RESULTS

Of the 91 adult patients enrolled, 4 (4.4%) had *Streptococcus pneumoniae* isolated. (Table1). Out of the four pneumococcal isolates, two were from age group 21-30years (6.7%) while age groups 31-40years and 41-50 years, both had one isolate each accounting for 4.2% and 10% respectively.

Based on the sex of the study population, three (75%) of the *S. pneumoniae* isolates were obtained from males while the remaining (25%) from a female. (Table2). Out of the four *S. pnuemoniae* isolates obtained, only one (14.3%) of the heptavalent vaccine serotype was found (Fig1). All of the four *S. pneumoniae* isolates were susceptible to Ceftriaxone, Levofloxacine and Trimethoprim-sulfamethoxazole; Amoxicillin-clavulanic acid, but varying susceptibility to Erythromycin and Tetracycline. (Table4).

DISCUSSION

Results from this study showed that lower prevalence (4.4%) of streptococcal infection is seen among adult patients. Similarly, low values were reported by Agwu *et al*., (6.4%) in Edo State, Nigeria and 1.4% by Fashae *et al*., among children in Ibadan, Nigeria (Fashae et al., 2002; Agwu et al., 2006). Higher values were reported in several other studies (Adelaye et al., 2008; Po-Ren et al., 2009). The antimicrobial abuse, socioeconomic status of the study population may be possible factors implicated in the low prevalence of *Streptococcus pnuemoniae* infection in this study.

In this study, *Pnuemococcus* was isolated more from males (75%) than females (25%). This is similar to findings in a study by Francisco and his colleagues in Alicante, Spain (Francisco *et al*., 2008). The *pnuemococcal* isolates were all obtained from patients in the age range 21-50years. This contradicts findings by Regev-Yochay *et al*, which showed high prevalence of *pneumococcal* infections at the extremes of life (Regev-Yochay *et al*., 2004).

By virtue of the predisposing factors to infection, the most prevalent was alcohol intake (16.5%) followed by cigarette smoking (12.1%). A reversal of this finding was observed by Francisco *et al*., in Spain. These differences may be due to geographic locations of the study. Alcohol abuse is known to impair muco-ciliary clearance just like cigarette smoke (Francisco *et al*., 2008). Cigarette smoke enhances bacterial adherence, and disrupts the respiratory tract. These factors, alone or together can explain the increased risk of pneumococcal disease in smokers. A causative role of smoking in the pathogenesis of pneumococcal infections seems highly plausible. Indeed, a case control study found an increased risk of *pneumococcal* infection among current and former smokers (Regev-Yochay *et al*., 2004; Francisco *et al*., 2008).

Based on the heptavalent vaccine serotype, only one (25%) was obtained in this study (i.e serotype 23F). This serotype is associated with invasive and non invasive *pneumococcal* infection in children, the elderly and non elderly adults. This serotype was the third common serotype in a study in Germany (Regev-Yochay *et al*., 2004; Aguiaret al., 2008). This difference in the serotype prevalence might be due to the differences in the age group studied, the geographic location, genetic predisposition, as well as the number of the studied population.

Report by the CNRP in 2006, confirmed a high prevalence of serotype 7F in adult population. This increase in the frequency of the serotype 7F in invasive infections among adults was described in Portugal (Regev-Yochay *et al*., 2004; Adelaye et al., 2008). The PCV-7 related serotypes represented 40.6 % of *S. pnuemoniae* isolated from adults' invasive diseases.[8,17,18] Laurent *et al* reported a higher prevalence of serotype 19A in a molecular capsular typing study in France (Crew – Brown et al., 1997; Regev – Yochay et al., 2004; Laurent et al., 2009).

Based on the susceptibility pattern of *Streptococcus pnuemoniae* isolate to antibiotics, all were susceptible to Ceftriaxone, Levofloxacine, Amoxicillin-clavulanic acid and Trimethoprim- sulfamethoxazole; but varying susceptibility to Erythromycin and Tetracycline. Varying susceptibility to Erythtomycin was also reported by Laurent *et al* in a molecular capsular typing carried out in France (Crew – Brown et al., 1997; Regev – Yochay et al., 2004; Laurent et al., 2009).

Table 1. Prevalence of *Streptococcus pneumoniae* infection among adult patients with lower respiratory tract infection in Jos, Nigeria

Age group (years)	Total no Tested	*S. pneumoniae* isolates	Percent (%)
11– 20	9	0	0
21 – 30	30	2	6.7
31 – 40	24	1	4.2
41 – 50	10	1	10
51 – 60	9	0	0
61 – 70	5	0	0
71 – 80	2	0	0
81 – 90	1	0	0
91 – 100	0	0	0
101 – 110	1	0	0
Total	**91**	**4**	**4.4**

χ^2 =2.17 p = 0.975

Table 2. Distribution of *Streptococcus pneumoniae* isolates by sex of adult patients with lower respiratory tract infection in Jos, Nigeria

Sex	*S. pneumoniae* isolates	Percent (%)
Female	1	25
Male	3	75
Total	**4**	**100**

Table 3: Distribution of some predisposing factors to *Streptococcus pneumoniae* infection among adult patients with lower respiratory tract infection in Jos, Nigeria

Sex (male)	54%
Age (years)	37.4
Cigarette smoking	12.1%
Alcohol intake	16.5%
HIV	3.3%

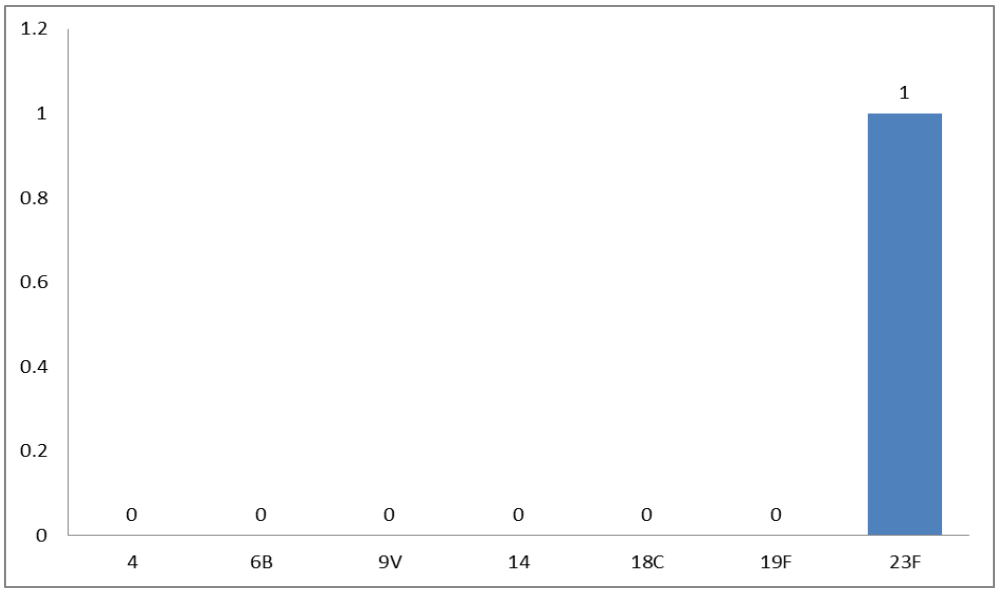

Fig 1. Distribution of Heptavalent vaccine serotype/serogroup of *Streptococcus pneumoniae* among adult patients with lower respiratory tract infection in Jos, Nigeria

Table 4. Antibiotic susceptibility pattern of *Streptococcus pneumoniae* among adult patients with lower respiratory tract infection in Jos, Nigeria

Antibiotics	%Sensitive	%Resistant
Ceftriaxone	100	0
Trimethoprin-sulfamethoxazole	100	0
Levofloxacin	100	0
Amoxicillin-clavulanic acid	100	0
Erythromycin	75	25
Tetracycline	50	50

They reported a high resistance of serotype 19A to Erythromycin and suggested that if serotype 19A would be added to a new *pnuemococca*l vaccine (PCV) formulation, the incidence of antibiotic resistance among *pnuemococci* would fall. It was reported that the emergence of non-vaccine serotypes has been observed in countries with low PCV-7 vaccine up take, such as Spain (Crew – Brown et al., 1997; Hicks et al., 2007; Griffin et al., 2013).

Finally the high cost of molecular investigation did not permit us to carry out a highly reliable molecular typing to compare with the conventional serotyping which is still acceptable the world over. These results confirm the presence of heptavalent vaccine serotypes as agents of disease in adults.

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CONFLICT OF INTEREST

We declare no conflicts of interest.

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