

Serotype Distribution Pattern of *Streptococcus Pneumoniae* Isolates from Invasive Infections at a University Teaching Hospital in Northern Nigeria

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Abstract

Background: Infections with *Streptococcus pneumoniae* are endemic worldwide. It is a public health problem and responsible for 1.6 million of 8.8 million annual deaths of children under 5 years of age, with 50% occurring in sub-Saharan Africa. This descriptive study was done to determine the prevalent *S. pneumoniae* serotypes responsible for infections at Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria. **Materials and Methods:** Clinical specimens of blood, cerebrospinal fluid, and aspirates from abscess, ear swab, throat swab, pus and sputa were collected over a period of 18 months from 420 patients with pneumonia, meningitis, septicemia, and otitis media. Specimens were cultured on 5% defibrinated sheep blood agar and chocolate agar. Incubation was done aerobically in a CO₂-enriched atmosphere at 37°C for 18–24 h. Isolates of *S. pneumoniae* were identified by standard biochemical techniques using Gram reaction, catalase test, Optochin disc, and bile solubility tests. Antimicrobial susceptibility was determined by the modified Kirby–Bauer disc diffusion method with Mueller–Hinton agar supplemented with 5% sheep blood. Serotyping was done using the slide agglutination method (Denka Seiken Co. Ltd., Japan). The serotype final results were recorded as matching, discordant, or nontypeable. **Results:** A total of 420 patients participated in this study, in which 227 were males (54%) and 193 were females (46%). Participants' ages ranged from 2 days to 85 years. *S. pneumoniae* isolates were mainly from blood 12 (52.2%) and sputum 6 (26.1%). Samples with most isolates were from the pediatric age group of 15 years (65.2%). The serotypes identified were 6, 19, and 20 which were all from blood, as none of the strains from sputum was typeable. **Conclusion:** The major *S. pneumoniae* serotypes found in this study were 6, 19, and 20.

Keywords: Capsule, serotype, *Streptococcus pneumoniae*, Northern Nigeria

INTRODUCTION

Infections with *Streptococcus pneumoniae* are important causes of morbidity and mortality worldwide.^[1] *S. pneumoniae*, also known as the *Pneumococcus*, is a primary cause of community-acquired pneumonia, meningitis, and otitis media in infants and children.^[2] Globally, 4 million deaths occur in children under 5 years of age from pneumonia each year, and *S. pneumoniae* accounts for 20%–40%.^[3] In developing countries, approximately 2 million children under 5 years of age die yearly of acute respiratory infection, which is the main cause of death in this age group.^[4] The ten countries with the highest burden of pneumococcal infection are in Africa and Asia, together they account for two-thirds of the reported cases worldwide.^[5] Nigeria has the third highest disease burden after India and China and accounts for 5% of the global burden.^[6]

Studies in Nigeria have shown that pneumococcal infection accounts for 50%, 54%, and 60% of community-acquired pneumoniae cases in Zaria, Enugu, and Kano, respectively.^[7-9] Onyemelukwe and Greenwood reported an overall mortality of 39% in the 1980s in Zaria.^[10]

The polysaccharide capsule on the outermost surface of most clinical strains of *S. pneumoniae* is the major virulence factor involved in evasion of the host immune system.^[11] Based on

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How to cite this article: Suleiman MR, Ejembi J, Giwa FJ, Jimoh O, Suleiman AO, Olayinka AT. Serotype distribution pattern of *Streptococcus Pneumoniae* isolates from invasive infections at a university teaching hospital in Northern Nigeria. *Ann Trop Pathol* 2018;9:145-9.

Access this article online

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DOI:
10.4103/atp.atp_33_18

structural differences in capsular polysaccharides, 90 serotypes of *S. pneumoniae* have been identified, including serogroups 1, 4, 5, 6, 9, 14, 18, 19, and 23, which are the cause of approximately 80%–90% of invasive disease in the world.^[2,11] Globally, they account for 30%–70% of community-acquired pneumoniae cases requiring hospitalization.^[12] The World Health Organization estimates that 1.6 million people, including up to 1 million children <5 years of age, die of pneumococcal infection annually.^[13]

Globally, out of the known 90 serotypes, about 20 serotypes account for >80% of invasive pneumococcal infection in all age groups. In addition, 13 serotypes have been implicated in >75% of invasive pneumococcal infection in children.^[14] The dominant serotypes associated with invasive pneumococcal infections worldwide include 14, 4, 1, 6A, 6B, 3, 8, 7F, 23F, 18C, 19F, and 9V.^[15-17] The existing serotypes in West Africa are types 1–10, 12, 14, 18, 19, 23, 25, 45, and 46.^[18] A study in Zaria, Nigeria, by Onyemelukwe and Greenwood in 1980, reported serotypes 1, 2, 3, 5, 18, 19, and 41 as the prevalent serotypes.^[10] Different serotypes exhibit differences in attack rates and colonization, case fatality rates, and clinical expression of disease.^[19]

This study aimed to determine the prevalent serotypes of *S. pneumoniae* isolates in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. Determining prevailing serotypes in a locality is important to evaluate the efficacy of relevant vaccines in order to reduce morbidity and mortality caused by *S. pneumoniae* infections.

MATERIALS AND METHODS

Study area and design

This descriptive study was carried out at the Medical Microbiology Department, ABUTH, Zaria. The hospital is located in the northwestern part of Nigeria and serves as a major referral center for the north central part of the country. It offers specialist inpatient and outpatient care for all age groups, across various specialties.

Sample size calculation

Clinical specimens were collected from a total of 420 patients over a period of 18 months. The sample size was determined using the Fisher's formula.^[20]

Sample collection

Sputum, cerebrospinal fluid, blood cultures, throat swab, and ear swab specimens were collected from patients with pneumonia, meningitis, septicemia, and otitis media, respectively, from various wards in Paediatrics, Medicine, Ear, Nose, and Throat units of ABUTH, Zaria.

Sample processing

Specimens collected were cultured on both 5% defibrinated sheep blood and chocolate agar at a temperature of 37°C for 18–24 h.^[21,22] The chocolate agar was incubated in a CO₂-enriched atmosphere using a candle extinction

jar.^[23] Isolates of *S. pneumoniae* were identified by standard microbiological techniques.^[24] Isolates were stored in skimmed milk and frozen at –80°C.

Antimicrobial susceptibility testing

Susceptibility to antimicrobials was determined by the modified Kirby–Bauer disc diffusion method using Mueller–Hinton agar supplemented with 5% sheep blood and antibiotic discs manufactured by Oxoid, UK.^[24] Erythromycin 15 µg, co-trimoxazole 25 µg, ciprofloxacin 5 µg, ceftriaxone 30 µg, cefuroxime 30 µg, chloramphenicol 30 µg, and oxacillin 1 µg were used. The results of the antimicrobial susceptibility testing were interpreted using the 2010 Clinical and Laboratory Standard Institute guidelines [Table 1].^[25]

Serotyping

Isolates were serotyped by the slide agglutination method using the Denka kit by Denka Seiken Co. Ltd., Japan, with batch number E2-E003/R01 10-10.

These isolates were tested one at a time first with the polyvalent antisera and then with the respective monovalent antisera included for the polyvalent serotypes that showed agglutination.

Visual agglutination that occurred within 1 min was interpreted as being a positive reaction; no agglutination was interpreted as a negative result. Positive and negative controls from the kit were processed alongside with the isolates according to the manufacturer's instruction. The serotype obtained was used for analysis and final results were recorded as matching, discordant, or nontypeable.

Ethical approval

Ethical clearance was obtained from the Health Research Ethical Committee of ABUTH, Zaria.

RESULTS

A total of 420 patients participated in this study, of which, 227 (54%) were males and 193 (46%) were females. Their ages ranged from 2 days to 85 years and 23 (5.4%) were identified as *S. pneumoniae* [Figure 1].

Three different serotypes were identified in this study: 6, 19, and 20 belonging to polyvalent serotypes 2, 8, and 5,

Table 1: Antibiotic susceptibility pattern of the isolates to the different antibiotics

Antibiotics (µg)	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)
Erythromycin (15 µg)	16 (69.6)	3 (13)	4 (17.4)
Co-trimoxazole (25 µg)	13 (56.5)	2 (8.7)	8 (34.8)
Oxacillin (1 µg)	7 (30.4)	-	16 (69.6)
Chloramphenicol (30 µg)	20 (90.9)	2 (4.8)	1 (4.3)
Ciprofloxacin (5 µg)	22 (95.7)	-	1 (4.3)
Cefuroxime (30 µg)	8 (34.8)	-	15 (65.2)
Ceftriaxone (30 µg)	12 (52.2)	-	11 (47.8)

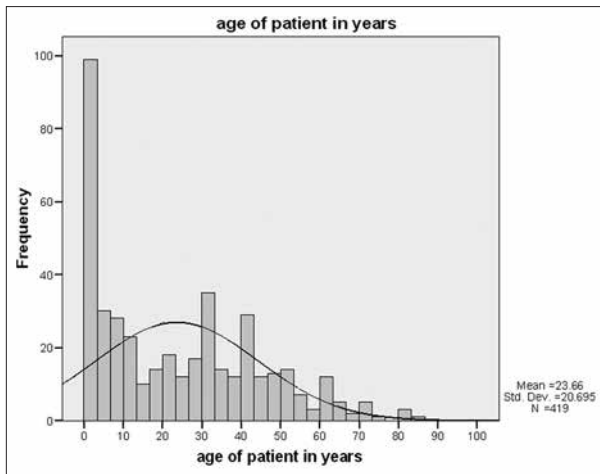


Figure 1: Age distribution of respondents

	Sputum	Blood	Throat	Total (%)
6	0	4	0	4 (17)
19	0	2	0	2 (9)
20	0	2	0	2 (9)
Nontypeable	8	6	1	15 (65)

	Frequency (%)
Sex	
Male	9 (39)
Female	14 (61)
Age	
<10	13 (57)
11-20	1 (4)
21-30	2 (9)
31-40	4 (17)
41-50	1 (4)
51-60	1 (4)
>60	1 (4)
Occupation	
Neonate	4 (17)
Pupils	13 (56)
Housewives	3 (13)
Traders	3 (13)
Civil servant	1 (4.34)
Type of invasive device	
No invasive device	11 (48)
Urinary catheter	12 (52)
Previous antibiotic use	
Yes	8 (35)
No	15 (65)
Associated immunosuppression	
Yes (HIV)	3 (13)
No	20 (87)

HIV: Human immunodeficiency virus

respectively. Serotype 6 was the most prevalent and was obtained from blood isolates. All the strains obtained from sputum were nontypeable [Tables 2 and 3].

DISCUSSION

Three different serotypes were found in this study and all were obtained from blood cultures. They were serotypes 6, 19, and 20. The most frequent was serotype 6. Studies on *S. pneumoniae* serotypes have been carried out in Nigeria and different parts of the world.^[26-28] A study done in the 1980s in Zaria and Malumfashi found serotypes 1, 3, 5, 2, 18, 19, and 4.^[10] Serotypes 6 and 20 found in this study were not obtained then. This may be as a result of the emergence of resistant strains. A similar study done in Lagos in 2006 had 17 isolates with serotypes 23F, 19F, 6A, and 14 identified.^[29] This is similar to what was found in this study, although the Lagos study used only nasopharyngeal swab samples while sputum and blood were used in this study. Nasopharyngeal carriage of *Pneumococcus* is very frequent in small children, particularly those in day-care centers and other crowded settings because they are prone to colonization with a single or a few serotypes within a few weeks or months.^[30-32] Some of the serogroups prevalent in children are 6, 14, 19, and 23, which also show antimicrobial resistance, probably resulting from prior antimicrobial exposure.^[33]

A study done in Ibadan, Nigeria, on invasive pneumococcal disease in children <5 years admitted in three urban hospitals found three major serotypes: 5, 19F, and 4.^[34] These serotypes however were different from that obtained from Lagos and Zaria studies. The distribution of pneumococcal capsular serotypes found in nasopharyngeal carriage and invasive pneumococcal disease was found to vary by age, geography, and socioeconomic status,^[35-37] which could explain these differences. In a study done in West Africa, which included Gambia, Nigeria, and Ghana, serotypes 5, 3, and 1 were found.^[38] Interestingly, in the USA and Europe, at the beginning of the 20th century, serotypes 1 and 2 accounted for up to 65% of lobar pneumonia cases and 75% of serotypes 1, 2, 3, and 5 of invasive serotypes.^[39-42] Today, serotypes 2 and 5 are almost never isolated in Western countries and serotype 1 occurs below 10%.^[43-46] In a study done in Turkey on healthy schoolchildren, the frequent serotypes were 1, 6, 19, 23, 20, and 17,^[47] similar to those found in this study. These serotypes are commonly involved in invasive pneumococcal diseases, highlighting the importance of nasopharyngeal colonization in the development of serious community infections. In Hungary, the dominant serotype strains was 19, which was also found in this study.^[48] While in France, serotypes 14 and 23 were the most common.^[49] These serotypes were different from those obtained from this study. The serotype distribution pattern seen in different countries today may indicate different susceptibilities to different capsular polysaccharides in hosts living under different socioeconomic conditions. In this study, *S. pneumoniae* isolates from sputum were nontypeable. Nontypeable *Pneumococcus* are often found in colonization

and are due to absence of capsules.^[50] This is similar to findings in Lagos by Oduyebo *et al.* where some of the nasal swabs were nontypeable.^[29] This is also similar to a study done in China by Zhao, out of 112 *S. pneumoniae* isolates obtained from sputum cultures and serotyped using the Quellung test, 7% were also nontypeable.^[51] In a similar vein, a study carried out by Hanage *et al.* in Finland on nasopharyngeal swabs and middle-ear fluid of Finnish children among the nontypeable *S. pneumoniae* strains, 70% belonged to a pneumococcal lineage which had lost its capsular locus.^[52] Nontypeable *S. pneumoniae* isolates are frequently isolated from nonsterile sites, but are rarely isolated from sterile sites.^[53] Nontypeable *S. pneumoniae* isolates have been reported for many years.

CONCLUSION

The major *S. pneumoniae* serotypes found in this study were 6, 19, and 20. The findings are important because vaccines exist against these serotypes, so including such vaccines in National Programme on Immunization will reduce morbidity and mortality. Surveillance for laboratory-confirmed pneumococcal disease should also be enhanced.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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