

ORIGINAL ARTICLE

ANTIMICROBIAL RESISTANT PROFILE OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM THE NASOPHARYNX OF SECONDARY SCHOOL STUDENTS IN JOS, NIGERIA

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Abstract

Background: Antimicrobial resistance in *Streptococcus pneumoniae* has compromised the effectiveness of therapy for pneumococcal diseases and asymptomatic nasopharyngeal carriers play an important role in transmission of resistant strains.

Method: Eighty-eight volunteer students attending 2 secondary schools in Jos, Nigeria were involved in this study to determine the antimicrobial resistant profile of *Streptococcus pneumoniae* isolated from the nasopharynx. The study population consisted of males and females between the ages of 15 – 25 years. Nasopharyngeal swab samples were analyzed for the presence of *S. pneumoniae* using standard bacteriological methods.

The isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method.

Results: *S. pneumoniae* was isolated from 37(42.04%) of the 88 samples. Isolates showed the highest resistance of 12 (32.43%) to erythromycin and lowest resistance of 4(10.81%) to ciprofloxacin. The resistance profiles for the 26(70.27%) penicillin-sensitive and 11(29.72%) penicillin-resistant isolates were similar. Both exhibited varying degrees of resistance to several groups of antimicrobials. However, isolates found to be resistant to penicillin showed a higher degree of resistance to other antimicrobial agents.

Conclusion: This study has shown that some secondary school students are carriers of multiple antibiotic-resistant *S. pneumoniae*.

Key words: Antimicrobial, resistant, *Streptococcus pneumoniae*

Résumé

Fond: Résistance antimicrobienne dedans *Streptocoque pneumoniae* a compromis l'efficacité de la thérapie pour les maladies pneumococcal et les porteurs nasopharyngaux asymptomatiques pour jouer un rôle important par transmission des contraintes résistantes.

Méthode: Des étudiants volontaires d'Eighty-eight allant à 2 écoles secondaires en Jos, Nigéria ont été impliqués dans cette étude pour déterminer le profil résistant antimicrobien de *Streptocoque pneumoniae* d'isolement dans le nasopharynx. La population d'étude s'est composée des mâles et des femelles entre les âges de 15 - 25 ans. Des échantillons nasopharyngaux de tige ont été analysés la présence de *S. pneumoniae* employer des méthodes bactériologiques standard.

Les isolats ont été soumis à la susceptibilité antimicrobienne examinant en utilisant la méthode de diffusion de disque.

Résultats: *S. pneumoniae* a été isolé dans 37 (42.04%) des 88 échantillons. Les isolats ont montré la résistance la plus élevée de 12 (32.43%) à l'érythromycine et la plus basse résistance de 4 (10.81%) au ciprofloxacine. Les profils de résistance pour les 26 (70.27%) pénicilline-sensibles et 11 (29.72%) isolats résistants à la pénicilline étaient semblables. Tous les deux ont montré des degrés variables de résistance à plusieurs groupes d'antimicrobiens. Cependant, les isolats avérés résistants à la pénicilline ont montré un degré plus élevé de résistance à d'autres agents antimicrobiens.

Conclusion: Cette étude a prouvé que quelques étudiants d'école secondaire sont des porteurs de résistant aux antibiotiques multiple *S. pneumoniae*.

Mots clés: Antimicrobien, résistant, *Streptocoque pneumoniae*

Streptococcus pneumoniae is the etiologic agent of many diseases. The spectrum of infections caused by this bacterium ranges from otitis media and bacteremia to pneumonia and meningitis.¹ The human nasopharynx is the primary reservoir for *S. pneumoniae* and the main source of person to person transmission.²

Strains of *S. pneumoniae* were usually considered to be sensitive to penicillins before the 1960s and routine antibiotic susceptibility testing were not performed in hospitals.³ The first clinically significant isolate not susceptible to penicillin was reported in 1967 from Australia.⁴ Since then penicillin-resistant *S. pneumoniae* has been reported worldwide with increasing frequency^{5, 6} and multiple antibiotic resistant strains have also been reported.^{3, 7}

S. pneumoniae is a leading cause of morbidity and mortality especially in children, the elderly and the immunocompromised, therefore its antimicrobial resistance is of major concern.⁸ Since the nasopharynx is the primary source of pneumococci the antimicrobial resistance profile of strains isolated from there can be used to predict antimicrobial resistance that may arise in clinically significant isolates. Therefore, it has been used to assess antibiotic resistance among pneumococci from different population groups.⁹ Hence the following study was undertaken to determine the antimicrobial resistant pattern of *S. pneumoniae* isolated from the nasopharynx of selected secondary school students in Jos, Nigeria.

Materials and Methods

Study population

The study population consisted of 88 secondary school students between 15 and 25 years. All participants were apparently healthy without respiratory tract infections and were currently not using antibiotics at the time of sample collection. Informed consent was obtained from each student who participated in the study.

Specimen collection

Nasopharyngeal swab specimens were collected using a sterile disposable wooden tongue depressor

to depress the tongue. A sterile cotton swab stick was placed 1 to 1.5 in. into the nasopharynx. The specimens were collected by a single investigator. All specimens collected were then transported immediately to the laboratory for analysis. The specimens were processed within 1 to 3 hours after collection.

Processing of specimens

Swab specimens were inoculated on tryptic soy agar plates containing 5% sheep blood and incubated at 37°C in 5 – 10% CO² (Candle extinction jar) for 24-48hours. The isolates were identified according to guidelines given by Duguid and Ross¹⁰ which included Gram stain, catalase test, optochin sensitivity test and bile solubility test.

Susceptibility of isolates to antibiotics was determined on Mueller-Hinton agar plates containing 5% sheep blood using the Kirby-Bauer disc diffusion technique.¹¹ Susceptibility tests were performed from a bacterial inoculum whose turbidity was equivalent to that of a McFarland standard of 0.5. The inoculum was produced from a pure culture of the isolate to be tested. *Staphylococcus aureus* NCTC No. 6571 and *Escherichia coli* NCTC No. 10418 were used as controls.

The inocula were spread over the surface of the agar plates and the antibiotic (Antec Diagnostics, UK) discs were placed on the plates and incubated at 37°C in 5 – 10% CO² overnight. The diameter of the zone of inhibition for each test antibiotic was measured and sensitivity or resistance estimated by comparing with zone-diameter interpretive standard according to that recommended by the National Committee for Clinical Laboratory Standards (NCCLS).¹²

Discs containing 1mcg oxacillin was used to determine penicillin resistance.¹² Other antibiotics tested were ciprofloxacine (10mcg), chloramphenicol (10mcg), gentamicin (30mcg), tetracycline (10mcg), erythromycin (10mcg), and ampicillin (10mcg).

Results

A total of 88 nasopharyngeal swab specimens were collected and analyzed in this study. Results indicate that *Streptococcus pneumoniae* was isolated

from 37(42.04%) of the samples. Eleven (29.72%) of *S. pneumoniae* isolated were found to be resistant to penicillin and 26 (70.27%) sensitive.

Twelve (32.43%) of the isolates were resistant to erythromycin, 11(29.72%) to oxacillin and ampicillin 10(27.02%) to tetracycline, 8(21.62%) to gentamicin and chloramphenicol and 4(10.81%) were also resistant to ciprofloxacin (Table 1).

The invitro antimicrobial resistant pattern for the penicillin-resistant *S. pneumoniae* strains is as follows; 6(54.54%) of the isolates were resistant to ampicillin, 4(36.36%) to erythromycin, 3(27.27%) to tetracycline and chloramphenicol, 2(18.18%) to gentamicin and 1(9.09%) to ciprofloxacin (Table 2).

In Table 3 the invitro antimicrobial resistant profile for the penicillin-sensitive *S. pneumoniae* isolates is shown. Eight (30.77%) were resistant to erythromycin and 7(26.92%) to tetracycline.

Table 1. Invitro antimicrobial resistant profile of 37 *S. pneumoniae* strains isolated from nasopharyngeal samples

| Antimicrobial agent (Concentration in µg) | No. of resistant strains (%) |
|--|---------------------------------|
| Erythromycin (10) | 12(32.43) |
| Oxacillin (1) | 11(29.72) |
| Ampicillin (10) | 11(29.72) |
| Tetracycline (10) | 10(27.02) |
| Gentamicin (30) | 8(21.62) |
| Chloramphenicol (10) | 8(21.62) |
| Ciprofloxacin (10) | 4(10.81) |

Table 2. Invitro antimicrobial resistant profile of 11 penicillin-resistant *S. pneumoniae* strains isolated from nasopharyngeal samples

| Antimicrobial agent (Concentration in µg) | No. of resistant strains (%) |
|--|---------------------------------|
| Ampicillin (10) | 6(54.54) |
| Erythromycin (10) | 4(36.36) |
| Tetracycline (10) | 3(27.27) |
| Chloramphenicol (10) | 3(27.27) |
| Gentamicin (30) | 2(18.18) |
| Ciprofloxacin (10) | 1(9.09) |

Table 3. Invitro antimicrobial resistant profile of 26 penicillin sensitive *S. pneumoniae* strains isolated from nasopharyngeal samples

| Antimicrobial agent (Concentration in µg) | No. of resistant strains (%) |
|--|---------------------------------|
| Erythromycin (10) | 8(30.77) |
| Tetracycline (10) | 7(26.92) |
| Gentamicin (30) | 6(23.07) |
| Ampicillin (10) | 5(19.23) |
| Chloramphenicol (10) | 5(19.23) |
| Ciprofloxacin (10) | 3(11.53) |

Discussion

The present study showed a 29.72% prevalence rate for penicillin resistant *Streptococcus pneumoniae*. This is similar to the 25% reported for 7 United States regions¹³ but lower than the 41% reported from Taiwan¹⁴ and 42.9% from Ekpoma, Nigeria.³ Several factors have been reported to be related to carriage and transmission of penicillin resistant. *S. pneumoniae* however, the most important factor is probably recent antibiotic use. Other risk factors for resistant pneumococcal carriage include young age, attendance at day care centers and Human immunodeficiency virus (HIV) infections in some populations.^{15,16}

Resistance to antibiotics of at least 3 different groups has been defined as multiple drug resistance.¹⁷ Even though the rate of multiple antibiotic resistance was not very high in this study when compared with those in some other countries, at least 21% of our isolates showed multiple drug resistance. The relatively high frequency of resistance of our isolates to penicillin and other antibiotics limits the choice of antimicrobials that can be used for the treatment of pneumococcal infections.

The antimicrobial susceptibility profiles for both penicillin – sensitive and resistant strains of *S. pneumoniae* were similar. Both exhibited varying degrees of resistance to several groups of antimicrobials. However, isolates found to be resistant to penicillin showed a higher degree of resistance to other antimicrobial agents. This agrees with reported findings that pneumococcal isolates resistant to penicillin are likely more resistant to macrolides, cephalosporins, tetracyclines, as well as some other antimicrobials.¹⁸ Penicillin resistance among pneumococci is mediated by changes in penicillin – binding proteins which is found on the surface of the bacteria and not by beta-lactamases.¹⁸ Penicillin – resistant *S. pneumoniae* has been reported world wide^{5,6} with increasing frequency, however rates of resistance depend on many variables.¹⁸ For both penicillin – sensitive and resistant isolates, the highest resistance rates were recorded for erythromycin and the lowest for ciprofloxacin. These rates are however lower than those reported for the Ekpoma study.³ Resistance to macrolides is very common in staphylococci and streptococci but prevalence varies from location to location.¹⁹ Varying rates of resistance to quinolones (even same generation) by *S. pneumoniae* have been reported.³ Therefore, the usual practice in some health establishments where susceptibility test is carried out on one quinolone and another is used for treatment either due to cost constraints or availability should be discontinued.³

None of the antibiotics tested were effective against all our isolates which makes antibiotic susceptibility testing imperative for all strains of

pneumococci isolated from the clinical setting before treatment. The existence of multiple resistance poses a greater threat for the management of diseases caused by this bacterium.¹⁷

Several strategies for example, simultaneous multiple drug therapy, which have been helpful in limiting the development of resistance in pathogens such as HIV and *Mycobacterium tuberculosis* may not be effective with *S. pneumoniae* because within-host development of resistance during treatment is not common in this bacterium.¹

Some successes have been recorded in reducing resistance by judicious antibiotic use in the United States and Europe.¹ This strategy may not be very effective in a developing country like Nigeria where antibiotics are sold without prescription over the counter.

A newer strategy which seems to be very promising is the use of the conjugate pneumococcal vaccine which is reported to be very effective in preventing both pneumococcal disease and carriage of resistant serotypes included in the vaccine.²⁰

In conclusion, this study has shown that multiple antibiotic resistant *S. pneumoniae* can be isolated from the nasopharynx of some secondary school students in Jos. In addition none of the antimicrobials tested were effective against all isolates hence antimicrobials susceptibility testing should be conducted before treatment of diseases caused by *S. pneumoniae*.

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