RESISTANCE PATTERNS OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM THE UPPER RESPIRATORY TRACT OF PERSONS ATTENDING VARIOUS CLINICS OF A UNIVERSITY TEACHING HOSPITAL IN LAGOS, NIGERIA - A preliminary study.

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Abstract

The upper respiratory carriage rate, serotypes and resistance patterns of *Streptococcus pneumoniae* in persons attending four clinics of the Lagos University Teaching Hospital (LUTH) were determined. Nasal swab specimens were collected from a total of 372 persons, 175 of whom were males and 177 were females. Their ages ranged from 14 weeks to 65 years. The upper respiratory carriage rate found in the total population of both adults and children was 9.9%/ but the rate was highest in children less than 5 years (8.6%). Among the 17 isolates that were available for serotyping, there was no significant serotype, though resistant serotypes like 23F, 19F, 6A and 14 were identified. Initial oxacillin screening for penicillin resistance revealed that 12 out of 36 isolates were clearly sensitive, but combined with the result of Etest, penicillin resistance was found to be 68%. Susceptibility testing by disc diffusion revealed that 80.5% of isolates were sensitive to ceftriaxone and 94.4% to chloramphenicol. Sixty one percent were sensitive to erythromycin while 94.4% were resistant to co-trimoxazole and 80.5% to tetracycline. Except for amoxicillin and cefotaxime which showed high activity, sensitivity pattern by Etest was found to be similar to that of disc diffusion. The result of this study makes it possible to formulate hypothesis for a larger study. In this study, the carriage rate of *S. pneumoniae* probably ranged from 26% to over 18.6% depending on the study population. If the limitations of this study are excluded in a larger study, the rate must likely be higher. Also, penicillin resistance in carriage strains would be up to 68%, probably higher, but may be intermediate, so penicillin could still be useful for treatment of pneumonia and probably otitis media, but not meningitis. There is reason to watch out for increased resistance to penicillin, cephalosporin and erythromycin. Most pneumococcal isolates would likely be resistant to tetracycline and co-trimoxazole.
Introduction

Streptococcus pneumoniae, normal flora of the nasopharynx is an important agent of both community and hospital acquired pneumonia. It is also implicated in otitis media, sinusitis and meningitis (1,2). Till 1978, S. pneumoniae was generally susceptible to penicillin, but penicillin resistant pneumococci (PRP) and multiply antibiotic resistant strains are increasingly being reported from all over the world (3,4). PRP are highly resistant to penicillin and as many seven classes of antibiotics like aminoglycosides, tetracycline, macrolide, chloramphenicol, rifampicin and co-trimoxazole (5).

This increasing resistance has led to a change of treatment protocols and diagnostic guidelines in the affected countries. Guidelines have been developed by the World Health Organisation for the treatment of PRP infections and to differentiate between highly resistant, moderately resistant strains and strains with low level of resistance. The site of infection is also taken into consideration in the guidelines. Treatment of life threatening infections like meningitis requires the use of extended spectrum cephalosporins likeceftriaxone while for otitis media, amoxicillin clavulanate would be recommended (6).

Though many serotypes of pneumococci have been associated with antibiotic resistance, some like 23F, 19F, 14 and 6A are more often reported than others. Those common serotypes are also associated with invasive disease (6,7). Many of these serotypes are carried by healthy persons who have been shown to be important reservoirs of S. pneumoniae and high carriage rates apart from favoring dissemination also precede disease in infected individuals (8). In Nigeria PRP has not been documented as a problem and there is therefore no new guideline on the treatment of pneumococcal infections. This situation is largely due to the fact that very few laboratories have consistently isolated S. pneumoniae in the past 10 year mainly because of widespread antibiotic abuse (9). This study was therefore carried out as a preliminary study to determine the carriage rates of serotypes and resistance patterns of S. pneumoniae in patients attending selected outpatient clinics in the Lagos University Teaching Hospital.

Methodology

Patients and Methods

Between March and December 2004, nasal swabs were collected from all adults and children who attended the following 5 LUTH clinics: Child health and immunization clinic, Ear, nose and throat clinic, Staff clinic and pediatrics clinic. The specimen was collected each time patients attended the clinic, regardless of their complaints. The only exclusion criterion was consumption of antibiotics in the previous 2 weeks. Ethical clearance for the study was obtained from the Ethics and Research committee of the Lagos University Teaching Hospital and informed consent was obtained from the participants or their parents.

Procedure

The nasal specimen was collected with a sterile swab (sterlina). This was immediately inoculated on Columbia agar base (Oxoid) to which 5-7% sheep blood and 5mcg/ml gentamicin had been added. Incubation was in air in 5- 10% CO2 at 37°C for 24 hrs. Alpha haemolytic gram-positive diplococci were tested for sensitivity to optochin and bile solubility. Optochin sensitive and bile soluble isolates were identified as Streptococcus pneumoniae (10).
Optochin susceptibility test.

Optochin disk was applied to a quarter of sheep blood agar plate that has been streaked with a few colonies of alpha haemolytic streptococci isolated. Culture plates were then incubated at 35°C in 5-10% CO₂. A zone >14 mm with a 5 μg 6 mm diameter disk was indicative of inhibition and identified isolates as S. pneumoniae. Isolates with smaller zones of inhibition were then subjected to bile solubility test.

Bile solubility test

0.5 ml of 2% sodium deoxycholate was added to 0.5 ml of a (0.5 Mc Farland) saline suspension of the isolate. Incubation was at 35°C for up to 2 hours. A clearing in the presence of deoxycholate indicated a positive bile solubility test, which identified the organism as S. pneumoniae.

Storage

Isolates were stored in skim milk tryptone glucose glycerol broth (STGG) at -70°C until antibiotic sensitivity and serotyping were performed.

Oxacillin screening

This was used to identify isolates susceptible to penicillin and select isolates for resistance testing. One μg oxacillin disk was used.

Isolates were considered sensitive to penicillin if the zone of inhibition was >20 mm. For isolates with zones < 20 mm, a test was performed to confirm whether they were actually resistant to penicillin (10).

Sensitivity testing

Sensitivity testing was by Disc diffusion and Etest methods in accordance with the manufacturer’s instructions and interpretations of antimicrobial susceptibility results were in accordance with Clinical and Laboratory Standards Institute (formerly NCCLS) (11). Antibiotics included in the test were penicillin G, amoxicillin, chloramphenicol, Cefotaxime, Ceftriaxone, and tetracycline. Apart from oxacillin, antibiotics tested by disc diffusion included cotrimoxazole (25 μg), tetracycline, ceftriaxone, erythromycin, and chloramphenicol (30 μg) (11).

Serotyping of isolates was undertaken at Professor Richard Adegbola’s laboratory at the Medical Research Council Laboratories in The Gambia. It was carried with capsular and factor-typing sera (Staten’s Serum Institute, Copenhagen, Denmark) using the Neufeld (Quellung reaction) method (12).

RESULTS

Nasal swab specimens were collected from a total of 372 patients. One hundred and thirty samples came from the Children’s Health and Immunization clinic, 128 from the Ear, Nose, and throat clinic, 86 from the Staff clinic, and 28 from the Pediatrist clinic. One hundred and ninety-five of the patients were males while 177 were females. Their ages ranged from 14 to weeks to 65 years (Table 1).

Streptococcus pneumoniae was isolated from 36 (9.9%) out of the 372 specimens collected. Carriage rate was highest in children less than 5 years. Rates reduced with age till 25 years as shown in Table 1. More females (13.5%) were colonized compared to males (6.1%). This difference was statistically significant at 0.05 level. Most isolates came from the immunization clinic and the least number from the staff clinic.