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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 (18.6%). 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 6.8%. 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 2.6% to over 18.6% depending on the study population. If the limitations of this study are excluded in a larger study, the rate most likely will be higher. Also, penicillin resistance in carriage strains would be up to 6.8%, 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, cephaloporin 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 pnuemococci (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 other beta lactams, tetracycline, macrolides, chloraphenicol, rifampicin and co-trimoxazole (3).

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 cephalosoporins like ceftriaxone while for otitis media, amoxicillin clavuanate would be recommended[5].

Though many serotypes of pneumococci have been associated with antibiotic resistance, some like 23F, 19F, 14 and 6A are more often reported than others. These 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.pneumoiae* in the past 10 years 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 4 LUTH clinics: Child health and immunization clinic, Ear, nose and throat clinic, Staff clinic and paediatric 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 (sterilin). 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% CO_2 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 streptococcus isolated. Culture plates were then incubated at 35°C in 5-10% CO_2. A zone > 14mm with a 5 ug (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. 1mcg oxacillin disk was used.

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

Sensitvity 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 Etest were penicillin G, amoxicillin, chloramphenicol, Cefotaxime, Cotrimoxazole, and tetracycline. Apart from oxacillin, antibiotics tested by disc diffusion included co-trimoxazole(25ug), tetracycline, ceftriaxone , erythromycin and chloramphenicol (30ug) (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 (Statens Serum Institute, Copenhagen Denmark) using the Neufield (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 Pediatric 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 (Table1)

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.

Oxacillin screening showed that only 12 (33.3%) out of 36 isolates were clearly sensitive to penicillin (table2). E-test performed on 17 of the 24 isolates which had equivocal penicillin sensitivity revealed that two were resistant to penicillin. One had an intermediate resistance with an MIC of 0.125u/ml and the other was fully resistant (table3)

Susceptibility pattern of all 36 isolates as determined by disc diffusion is shown on (table2).Many isolates were sensitive to ceftriaxone (80.5%) and chloramphenicol (94.4%). Sixty one percent were sensitive to erythromycin while the majority were resistant to cotrimoxazole (94.4%) and tetracycline (80.5%).Except for amoxycilljn and cefotaxime, which showed high activity, sensitivity pattern by E-test was found to be similar to that of disc diffusion(tables 2&3).

There was no predominance of any serotype though resistant serotypes like 23F, 19F, 6A and 14 were identified.

TABLE 1; CARRIAGE RATES OF *S.PNEUMONIAE* IN PATIENTS ATTENDING SOME LUTH CLINICS

AGE GROUP(YEARS)	NUMBER OF PATIENTS	NUMBER COLONISED	CARRIAGE RATE
0-5	134	25	18.6%
6-10	32	4	12.5%
11-15	26	1	3.8%
16-20	17	1	5.8%
21--25	24	1	4.1%
26-30	27	1	3.7%
>30	112	3	2.6%
TOTAL	372	36	9.9%

TABLE 2; ANTIMICROBAL SENSITIVITY PATTERN OF 36 CARRIAGE STRAINS OF *S.PNEUMONIAE* BY DISC DIFFUSION METHOD

ANTIMICROBIAL AGENT	NO.(%) SENSITIVE	INTERMEDIATE SENSITIVITY (%)	NO. (%) RESISTANT
Oxacillin	12(33.3)	-	24(66)
Chloramphenicol	34(94.4)	1(27)	1(2.7)
Erythromycin	22(61.1)	11(30.8)	3(8.3)
Tetracyline	4.(11.1)	3(8.3)	29(80.5)
Ceftriaxone	29(80.5)	7(19.4)	-
Cotrimoxazole	1(2.7)	1(2.7)	34(94.4)

Table 3: Sensitivity of *S. pneumoniae* by E-test

Antibiotic	No.(%) tested	No. (%) sensitive	Intermediate resistance (%)	No.(%) resistant
Penicillin G	17	15 (88.2)	1 (5.88)	1 (5.88)
Amoxycillin	17	17 (100)	-	-
Chloramphenicol	17	17 (100)	-	-
Cefotaxime	16	15 (93.73)	-	1 (6.25)
Co-trimoxazole	17	4 (23.52)	2 (11.64)	11 (64.7)
Tetracycline	17	5 (29.41)	-	12 (70.58)

Discussion

The carriage rate of 9% found in this study is quite low, probably because it includes rates for both children and adults. Highest rates are usually found in children while very low rates are found in adults (13,14). As expected, the rate in this study was highest in children less than 5 years. However this rate of 18.6 % is still low when compared with a similar study carried out in Ghana in 2002, in which, a much higher rate of 51% was reported[15]. This finding is not surprising because the population studied in Ghana was 6 months to 2 years, the age group that has been found to have the highest carriage rate all over the world (13,14,16,17). In our study, the lower carriage rate found children in the immunization clinic is bound to be an under-estimate and may be attributed to the fact that nasal rather than nasopharyngeal samples were collected. Unfortunately, the mini wire loop required for collecting material from the posterior pharynx, recommended for carriage studies by the WHO was not available for our study hence the use of nasal swab (18). Nasal swabs even though not ideal have been found in some studies to be more efficient than oropharyngeal swabs for isolation of *S. pneumoniae* in the upper respiratory tract (19).

Initial oxacillin screening for penicillin resistance revealed that 12 out of the 36 isolates obtained were clearly sensitive to penicillin while 24 had equivocal penicillin sensitivity which had to be confirmed by quantitative methods. Due to storage difficulty E-test was performed on only 17 of these and only two out of the 17 were eventually found to be resistant to penicillin. If the results of Oxacillin screening and Etest were combined then only two out of 29 isolates were resistant to penicillin making the resistance rate 6.8%. Anecdotal reports suggest that high rates of penicillin resistant pneumococci exist in Nigeria and a limited survey carried out in 1978 put the prevalence at 20% (20). Out of the two penicillin resistant strains, one had a high level resistance, while the other had intermediate resistance. Infection with highly resistant isolates has to be treated with cephalosporins. In case of intermediate resistant isolates, serious potentially life threatening infections like meningitis or septicaemia must be treated with cephalosporins, however pneumonia and otitis media can be treated with high dose penicillin (5,21,22). All the penicillin resistant isolates were sensitive to amoxicillin, It is recommended that amoxycillin sensitivity of oxacillin resistant isolates be routinely confirmed by E test as oxacillin resistance is said to be generalizable for all beta lactams. Fortunately studies around the world, have shown that most oxacillin resistant isolates remained sensitive to amoxycillin (23,24).

Of concern in this study is the reduced sensitivity to erythromycin (22 out of 36 sensitive), which has been a valuable drug for pneumococcal infections especially as an alternative for persons allergic to penicillin. This finding has to be confirmed and monitored because of increasing global emergence and spread of Macrolide resistance[25].

There is still a high level of sensitivity to chloramphenicol, although it is does not provide adequate therapy for penicillin resistant pneumococcal meningitis because despite in –vitro susceptibility chloramphenicol has been associated with significant therapeutic failures in meningitis caused by penicillin – resistant pneumococci. This is thought to be due to loss of autolysins in penicillin – resistant strains resulting in chloramphenicol being bacteriostatic rather than bactericidal against such strains (26,27). So far, most isolates are still sensitive to the third generation antibiotics which is recommended for the therapeutic management of meningitis caused by penicillin resistant Pneumococci, but it is obvious that resistance is developing with >6% of isolates showing high level resistance. This trend needs to be monitored as resistance to cephalosporins is expected to rise with a rise in penicillin resistance since they share a similar mechanism of resistance (28).

The high level of tetracycline and co-trimoxazole resistance is consistent with the high level of penicillin resistant pneumococci and this situation has been in reported other parts of the world. In such settings a widespread and indiscriminate use of antibiotics, which is also found in Nigeria, is associated with high carriage rates and high level of antibiotic resistance. Rationale use of antibiotics when introduced in such settings led to reduction in antibiotic resistance and carriage rates (29).

Only 17 isolates were available for complete sensitivity testing. Unfortunately 19 were lost because of storage problem. Ideally *S. pneumoniae* should be stored at –70°C. This study is ongoing and -70°C freezer has since been purchased. Another major limitation of the study was the small sample size, a result of the exclusion criteria of

antibiotic use in the previous 2 weeks. This however should be seen as reflecting the reality of unregulated antibiotic use in Lagos. Nevertheless, in Nigeria where there are very few data, the results of this study enable one to formulate hypothesis for further studies.

In this study, the carriage rate of *S. pneumoniae* probably ranged from 2.6% to over 18.6% depending on the study population. If the limitations of this study are excluded in a larger study, the rate most likely will be higher. Also, penicillin resistance in carriage strains would be up to 6.8%, 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 not only for increased penicillin resistance but also reduced cephaloporin and erythromycin sensitivity. Furthermore, most pneumococcal isolates would likely be resistant to tetracycline and co-trimoxazole

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References

1. Scott JA, Hall AJ, Muyodi C, Lowe B, Ross M. *et al.* Aetiology, outcome and risk factors for mortality among adults with acute pneumonia in Kenya. Lancet 2000. 355 (9211): 1225 - 30
2. Broome CV, Facklam RR. Epidemiology of Clinically Significant Isolates of *Streptococccus pneumoniae* in the United States. Rev Infect Dis1981;3: 277-281
3. Jacobs MR. Emergence of Multiple Resistant Pneumocci. Lancet 1978;11:734 -740
4. Casal J. Antimicrobial Susceptibility of Streptococcus: Serotype distribution of Penicillin Resistant Strains in Spain. Antimicrob Agents Chemother 1982; 22: 222-5
5. Jacobs MR. Treatment and Diagnosis of Infectious Diseases caused by drug resistant *Streptococcus pneumoniae*. Clin Infect Dis. 1992; 15: 119-127.
6. Characterization of penicillin Resistant Streptoccus pneumoniae Invasive paediatric Isolates Recovered in six Latin Ameerican Counties: An Overview Microl Drug Resist 1998 (4) 195 -207.
7. Memish Z.A Balkhy H.H; Shibl A M et al Streptoccal pneumonia in Saudi Arabia: Antibiotic Resistance and Serotype of recent Clinical isolate. International Journal of Antimicrobial Agent 2004; 23: (1) 32-38.
8. Austrian R. some aspects of the pneumococcal carrier state J Antimircob chemother 1986; 18: 35
9. Ogunsola FT, Kesh CN, Odugbemi Tolu Anticirobial Resistance in Nigeria; An overview . Nigeria Quarterly Journal of hospital Medicine 1997, 7(1) ;:57-
10. PopovicT., Aellp G .and facklam R. Laboratory Method for the Diagnosis of Meningitis Caused by Neisseria Meningitis, Streptoccus Pneumonia and Haemohilus influenzae world Health Organisation communicable Disease Surveilance and Response WHO/ CDS/ CSR/ EDC / 99.7
11. Clinical and Laboratory Standards Institute (formerly NCCLS). Performance Standard for Antimicrobal disk Susceptibility testing 5th ed:

Approved standard NCCLS Document M2-A5 Villanova pa NCCLS 1993.

12. Klugman KP, Koornhof HJ Drug Resistant patterns and Serogroups or Serotypes of Pneumococcal isolates from Cerebrospinal fluid or Blood, 1979-1986. J infect Disk 1988; 158: 956-964.

13. Appelbaum PC, Gladkova c Hryniewkz wetal carriage of antibiotic - resistant streptoc ccus pneumoniae by children in eastern and central Europe – a multi centre study with the use of standardized methods . Clinical Disk 1996, 23 : 712 -17

14. Skill S.A, leach AJ and currie BJ streptoccus pneumoniae carriage and penicillin cceftriaxone resistant in hospitalized children in Darwin. Australian and New /ealand Journal medicine 1996; 26 (3): 391 -5

15 Denno Dm. Frimpong E, Gregory M and steele RN Nasophrynageal carriage and susceptibility patterns of streptoccus pneumoniae in Kumasi. Ghana. West African Journal of medicine 2002 : 21 (3) 233-236.

16. Gray B. M, Dillon H.C. Jr, Epidemiological Studies of Streptococcus pneumoniae in Infants . Antibody to types 3, 6,14 and 23 in the first two years of life. Journal of Infectious disease 1988(158) 948-955

17. Henderson E.W., Gilligan P.H., wait K., Goff d.A Nasopharyngeal Carriage of Antibiotic Resistant pneumococci by Children in Group day care J. Infect Dis. 1988(157)256-263.

18 O'Brien KL Nohynek H and the WHO Pneumococcal Vaccine Trials Carriage Working Group. Report from a WHO Working Group: Standard Method for Detecting Upper Respiratory carriage of *Streptococcus pneumoniae*. Paediatr Infect Dis J, 2003; 22:133-40.

19. Capending MR, Nohynek H, Sombrero LT, Pascual LG, Sunico ES, Esparar GA, Esko E, Leinonen M, Ruutu p. Evaluation of Sampling sites for detection of Upper Respiratory tract Carriage of Streptococcus pneumoniae and Haemophilus influenzae among healthy Phillipino infants. J Clin Microbiol 1995; 33(11): 3077 -3079.

20. Hansman D. Chloramphenicol resistant pneumococci in West Africa(letter). Lancet 1978; i: 1102 - 1103

21 Hoberman A, Marchant CD, Kaplan SL, Feldman S. Treatment of acute otits media. Consensus recommendations. Clin Paediatr 2002:4: 373-90

22. Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell CD et al. for the American Thoracic Society. Guidelines for the Management of adults with Community acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy and prevention. Am J Respir Crit Care Med. 2001; 163: 1730 - 54

23. Dagan R, Greenbers D, Lieberman A etal. *S pneumoniae* carriage in children with acute otitio media treated with high dose a moxycillin /clavulanate or Azithromycin in the presence of antibiotic resistant *S pneumoniae* . 43rd inter science conference on Antimicobial Antimicrobial Agents and chemotherapy

Chicago USA, 14 -17 september 2003. Abstract (2-942)

24. Brook land Gober AE; Eradication of Streptococcus pneumonaie in the nasopharyngeal flora of children with acute otitis media after amoxicillin –clarulenate therapy. Antimicrobial Agents and Chemotherapy 2004; 48;1419-1421.

25. Farrel DJ, Jenkins SG, Brown SD, Patel M, Lavin BS, Klugman KP. Emergence and Spread of Streptococcus pneumoniae with erm (B) and mef (A) Resistance. Emerging Infectious Diseases 2005;11(6) 851 -858.

26. Friedland JR and khngman kp: Recurrent penicillin resistant memingitis after chloramphenicol therapy. Pediatr infect Dis J 1991; 10: 705-07

27. Friedland R and Klugman KP failure of chloramphenicol therapy in penicillin – restant pneumococcal meningitis. Lancet 1992; 339: 405-08.

28. Markiewicz z, Tomask A. Variation in penicillin Binding protein of penicillin resistant clinical isolates of Pneumococci . Clinical Microbiology 1989; (27) 405 – 4109.

29. Schrag ST, Beall B, Dowell SF. Limiting the spread of Resistant prumococci . Biological and Epidemiologic Evidence for the Effective one of Atternative interventions, clinical and diagnostic Laboratory Immunology 2002; 9 (16) : 1328-1331