Nasopharyngeal carriage and susceptibility patterns of *Streptococcus Pneumoniae* in Kumasi, Ghana

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Summary

Penicillin resistant *Streptococcus pneunmoniae* poses an increasing problem in paediatrics, particularly in less developed countries. Outside of South Africa, little is known about *S. pneumoniae* susceptibilities in Sub-Saharan Africa. The objective of this study was to determine the prevalence of pneumococcal colonization and antimicrobial susceptibility among children in urban Ghana.

Methods

Nasopharyngeal pneumococcal colonization was examined in 311 children attending a polyclinic for sick children and an immunization clinic in Kumasi, Ghana. Isolates were tested for antibiotic susceptibility to penicillin, tetracycline, erythromycin, chloramphenicol, cefuroxime, cefotaxime, ceftriaxone, and trimethoprim-sulfamethoxazole.

Results

Over half (51.4%) of subjects were colonized with S. pneumoniae and 17% of isolates were resistant to penicillin, all demonstrating intermediate resistance. S. pneumoniae strains were also frequently resistant to trimethoprim-sulfamethoxazole and tetracycline, less so to chloramphenicol and cefuroxime and were almost uniformly sensitive to cefotaxime, cefriaxone and erythromycin.

Conclusions

Our study shows a high rate of pneumococcal nasopharyngeal colonization and a concerning level of penicillin resistance although at a less alarming rate than seen in some other countries. Multiple antimicrobial resistance was also noted especially among drugs readily available and commonly used. These data impact treatment choices in pneumococcal disease. Vaccine may play an important role in disease limitation. An effort to curtail the misuse of antibiotics, by prescription and otherwise, may prevent further increases in resistance rates.

Keywords: ***Pneumococcus, Antimicrobial resistance, Ghana, Less developed country, Developing world***

Résumé

Résistance à la Pénicilline streptocoque pneumonie soulève la question croissante dans le domaine de la pédiatrie, aux pays sous développés en particulier. A l'extérieur de l'Afrique du Sud, on a connue peu de choses en ce qui concerné S. pneumonie et ses susceptibilités on Afrique sous Sahara. L'objet de cette etude est de determiner la prevalence de la pneumocoque colonisation et la susceptibilité antimicrobien parmi les enfants dan la zone urbaine au Ghana.

Methodologie

La Nasopharyngele pneumocoque colonisation a été étudiée chez 311 enfants qui avaient reçu des soins à la polyclinique pour des enfants malades et dans la clinique pour l'immunisation a Kumasi, au Ghana. On avait fait le traitement de texte pour les isolés par rapport à la susceptibilité antibiotique à la pénicilline, tétracycline, érythromycine, chloramphenicole, cefuroxime, cefotaxime, ceftriaxone, et trimethopim-sulfamethoxazole.

Resultats

Plus de la moitic soit 51,4% des patients ont été colonisés avec S. pneumonie et 17% des isolés étaient résistants à la penicilline, avec des manifestations de résistance moyenne. Les entorse S. pneumonie étaient le plus souvent résistants par rapport au trimethoprim - sulfamethoxazole et tétracyline, et moins efficace par rapport aux chloramphénicol et cefuroxime et ils étaient presque uniformement sensitifs aux cefotaxime, ceftriaxonne et érythromycine.

Conclusion

Notre étude témoigne le taux élevé de la pneumocoque nasophargnite colonisation et le niveau touchant de la résistance de la pénicilline qu 'elle soit au taux moins inquiétant plus que l'on avait assisté dans d'autres pays. La résistance multiple antimicrobien a été également noté parmi les drogues facilement disponibles et ordinairement utilisées en particulier.

Ces données impact dex choix de traitement en ce qui concerne la maladie pneumocoque. La vaccine pourrait jouer un rôle prépondérant dans la limitation des maladies. Des efforts pour réduire l'abus des antibiotiques par des ordonnance, pourrait empêcher de plus des accroissements dans les taux de la résistance.

Introduction

Streptococcus pneumoniae continues to be a significant cause of morbidity and mortality in children. This is particularly true in less developed areas including sub-Saharan Africa where poverty often prohibits patients from receiving appropriate care Penicillin has provided efficacious and relatively inexpensive therapy for the treatment of pneumococcal disease for the past 50 years. However, increasing rates of penicillin resistance have been reported worldwide. Furthermore, those with high level resistance are frequently resistant to other classes of antibiotics. However, outside of South Africa, little is know about *S. pneumoniae* susceptibilities in sub-Saharan Africa. Our study was undertaken to determine the current prevalence of pneumococcal colonization and to assess the antimicrobial susceptibility of these organisms among children in an urban West African setting.

Methods

Study Population

Komfo Anokye Teaching Hospital (KATH) is a large inpatient and outpatient facility serving the population of Kumasi, Ghana (population approximately 600,000). Children aged 6- 12 months attending KATH Polyclinic for sick visits and attending KATH Immunization Clinic were selected for sampling. This age range was chosen because it reflects the majority of children seen for vaccinations in this setting. The study was approved by the Ethics Committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology in Kumasi. After informed consent was obtained from the parent or guardian, a short survey was administered in the vernacular language, Twi. Subjects were asked about their symptoms as well as use of antibiotics in the week prior to coming to clinic.

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Collection, isolation and susceptibility testing

Culture material was collected from the posterior nasopharyngeal wall with a wire mini-tip collection and transport system (Culturette, Becton Dickinson Microbiology Systems, Cockeysville, MD). Specimens were inoculated onto sheep blood agar plates within an hour of collection. Plates were incubated for 48 hours in a 5% carbon dioxide environment. *S. pneumoniae* isolates were identified based on colony morphology with confirmation using Optochin (ethyl hydrocuprein HCl) discs (BBL, Becton Dickinson Microbiology Systems).

Isolates were then screened for susceptibility to penicillin with a 1μg oxacillin disk (BBL, Becton Dickinson Microbiology Systems) using Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates with sheep blood. A zone of inhibition of less than 20mm indicated resistance. The strains were also tested for susceptibility using 30μg chloramphenicol, 15μg erythromycin, 30μg tetracycline and 30μg cefotaxime disks (Britannia, Buenos Aires, Argentina). The following National Committee for Clinical Laboratory Standards (NCCLS) were used to determine sensitivity in mm (resistant, intermediate, sensitive): oxacillin, ≤19, --, ≥20; chloramphenicol, ≤ 12, 13-17, ≥ 18; erythromycin, ≤13, 14-22, ≥23; tetracycline, ≤14, 15-18, ≥19; cefotaxime, ≤14, 15-22, ≥25. Oxacillin resistant strains were initially stored on glass beads at -30°C for later E-test minimum inhibitory concentration (MIC) testing, since the E-test strips were not available at the beginning of the study. After E-tests strips were obtained, all resistant isolates were tested immediately for the following antibiotics: ceftriaxone, penicillin, cefuroxime, chloramphenicol, erythromycin, and trimethoprim-sulfamethoxazole (TMP-SMX) by E-test method as previously described.[1] *S. pneumoniae* E-test MIC designations (susceptible, intermediately resistant, and resistant) were defined as follows (in microgram per milliliter): cefuroxime, 0.5 or less, 1,2 or more; erythromycin, 0.5 or less, 1, 2 or more; chloramphenicol, 4 or less, --, 8 or more; TMP - SM X, 0.5 or less, 1 to 2, 4 or more, ceftriaxone, 0.5 or less, 1,2 or more; and penicillin, less than 0.06, 0.06 to 1.0, 2 or more.

Statistical methods

Data were recorded on precoded case report forms and information entered onto a personal computer. Statistical analysis was performed by Chi square.

Results

During a 3-month period (June to August 1996), 311 specimens were collected. One hundred forty-seven (47.3%) were obtained from children attending immunization clinic with the remainder attending the polyclinic for sick children. There was an even distribution of males (158) and females (153).

Approximately half of the subjects had nasopharyngeal *S. pneumoniae* colonization (Table 1). Nine (6.0%) pneumococcal isolates did not survive prior to sensitivity testing. Of the remainder, over one-third tested oxacillin resistant. The carriage and oxacillin resistant rates did not differ significantly by sex or by type of clinic attended. Carriage rates were affected by antibiotic use in the week prior to the clinic visit. Those who had used antibiotics in the week prior but not the 24 hour period prior to attendance had the lowest pneumococcal carriage rate (25.0%) compared with those who had used antibiotics in the prior 24 hours (36.8%) and those who had not used antibiotics in the prior week at all (52.0%) ($p<0.5$). Oxacillin resistance rates, however, were not affected by antibiotic usage. Neither carriage rate nor sensitivity patterns were influenced by reported symptoms including respiratory illness. Thirty seven percent of pneumococcal isolates overall were oxacillin resistant by disk diffusion method.

Disk diffusion testing revealed that the *S. pneumoniae* strains were also frequently resistant to tetracycline, less so to chloramphenicol and were almost uniformly sensitive to cefotaxime and erythromycin (Table 2). While there was not a significant correlation between resistance to oxacillin and chloramphenicol there was between oxacillin and tetracycline ($p<0.05$).

Twenty-three (43%) of the oxacillin resistant strains were subsequently further subjected to MIC testing using the E-test method. Nine (39%) of these were confirmed penicillin resistant all with an intermediate degree of resistance. There was a high degree of resistance to trimethoprim-sulfamethoxazole (TIMP - SMX), less so to chloramphenicol and cefuroxime and 100% sensitivity to erythromycin and ceftriaxone (Table 3).

Discussion

Antimicrobial resistance in *S. pneumoniae* is becoming an alarming problem worldwide. There have been a number of studies addressing the issue in all the continents but generally less information has been reported from less developed countries especially in sub-Saharan African outside of South Africa.[2-6] Because of widespread unregulated antibiotic use one might expect problems, with antimicrobial resistance in such settings.

Before drawing conclusions, limitations of this study include: (1) Due to logistic difficulties in this developing country setting, only 43% of oxacillin resistant samples were available for further testing by the E-test method. However, these isolates should represent a random sample of the oxacillin screened strains as antimicrobial susceptibility should not impact the survival of the organism in storage. Despite this limitation, E-test analysis results were comparable to other similar studies.[7] (2) Our study population was limited to 6-12 month olds because the majority of children attending immunization clinic are in this age range and the same age group was studied in the polyclinic group. However, despite the

Table 1 Demographic/clinical characteristics and *S. pneumoniae* nasopharyngeal colonization/resistance patterns

	Total Study Subjects (n=311)	Pneumococcal colonization			*Oxacillin* resistance		
	%	No	%		No	%	
Total Study subjects	--	151	51.4	ns	53	37.3	ns
Male/female subjects	50.8/49.2	75/76	47.5/49.7	ns	27/26	37.5/37/1	ns
Immunization clinic/ polyclinic attendees	47.3/52.7	66/85	44.9/51/8	ns	26/28	44.1/33.7	ns
Antibiotic use							
<24 hours	12.2	14	36.8	8	61.5		
>24 hours, <1, week	6.4	5	25.0	p<.05	1	25.0	ns
none 78.5	127	52.0		43	35.8		
unsure	2.9	--	--	--	--	--	--
Respiratory symptoms in polyclinic attendees							
present	38.7	28	45.9		9	32.1	
absent	61.3	57	55.3	ns	19	33.9	ns

Table 2 Susceptibility to multiple antimicrobial agents by disk diffusion method

	Antimicrobial susceptibility or resistance (%)									
				Oxacillin resistance isolates (n=53)			Oxacillin susceptible isolates (n=89)			
Antimicrobial	**All isolates (n=142)**									
	S	I	R	S	I	R	S	I	R	
Oxacillin	62.7	--	37.3	--	--	--	--	--	--	
Tetracycline	34.5	2.1	63.4	22.6	0	77.4	41.6	3.4	55.1	p<.05
Chloramphenicol	86.6	0	13.3	86.8	0	13.2	86.5	0	13.5	ns
Erythromycin	99.3	0.7	0	100	0	0	98.9	1.1	0	ns
Cefotaxime	99.3	0	0.7	98.1	--	1.9	100	--	0	ns

Table 3 Susceptibility to multiple antimicrobial agents for oxacillin-resistant *S. pneumoniae* by E-test method

Antimicrobial susceptibility or resistance (%) (n=23)			
Antimicrobial	**S**	**IR**	**R**
Penicillin	61.9	39.1	0
Ceftriaxone	100	0	0
Chloramphenicol	91.3	--	8.7
Erythromycin	100	0	0
Trimethoprim Sulfamethoxazole	39.1	13.0	47.8
Cefuroxime	95.7	4.3	0

S, Susceptible; IR, intermediately resistant; R, resistant

possibility of higher carriage rates than in older children, [8,9] resistance rates beyond infancy would probably be similar.

Despite these limitations these data provide an idea of the pneumococcal carriage rate and susceptibility patterns in this West African setting. *S. pneumoniae* strains that colonize the nasopharynx may be associated with invasive disease.[10] Approximately half of our study sample had pneumococcal nasopharyngeal colonization which compared with other studies which have demonstrated approximately 25-50% colonization.[12-13.]

Of the 23 oxacillin resistant isolates available for further testing by E-test, 9(39%) were penicillin resistant, all demonstrating intermediate resistance. By extrapolating to all of the oxacillin resistant strains, the overall penicillin resistance rate as determined by E-test was 17%. Four to 70% resistance to penicillin has been reported from other areas of the world.[14-15] Forty percent of community acquired isolates from sick children in South Africa demonstrated penicillin resistance from 1989-1991.[6]

While specimens were frequently resistant to tetracycline (65% by disk diffusion) and TMP-SMX (61% by E-test) there was a lower rate of resistance to chloramphenicol (13% by disk diffusion and 9% by E-test) and cefuroxime (4% by E-test) and greater than 99% sensitivity to erythromycin (disk diffusion and E-test), ceftriaxone (disk diffusion and E-test) and cefotaxime (disk diffusion). This is consistent with the wide use of amoxicillin, tetracycline and sulfa drugs in Ghana on a prescription and non-prescription basis while erythromycin has been less commonly used. Cephalosporins have only recently been introduced into the country, are still not widely available and when obtainable are often prohibitively expensive. At the time of this study they were not on the national formulary. However, because penicillin resistance is mediated by penicillin binding proteins which can also cause resistance to cephalosporins, decreased susceptibility to the latter may develop as the degree of penicillin resistance increases. Compared to studies in Spain, Hungary and Pakistan, the tetracycline and TMP-SMX data compare to areas which have a high incidence of penicillin resistant *pneumococcus* with multiple drug resistance.[3,16,17] In South Africa, as in this study, resistance to chloramphenicol and erythromycin is not as common as in these other areas although in South Africa tetracycline resistance is also not as common as in our study and these other countries.[6]

While our study suggests a concerning level of penicillin resistance among pneumococcal strains it is not at the alarming level seen in other settings. The possible prevention of future increases in resistance is important. Frequent use of antimicrobials has been found to affect resistance rates[18-20] and carriage rates[21]. Widespread and indiscriminate use of antibiotics, both by prescription or otherwise, is the case in Ghana as in many other developing countries. The rational use of antibiotics must be encouraged among physicians, pharmacists and the public.

Pneumococcal vaccine may play an important role in limiting disease secondary to penicillin resistant pneumococcus, but more information regarding serotyping in this setting is needed.

These findings also raise the issue of management of infections caused by pneumococcus. Therapy is influenced by the availability of susceptibility testing, the severity of illness, clinical response, and availability of antimicrobial agents. Culture and sensitivity testing is available (depending on availability of reagents as well as power and water supply) at KATH, however, in most areas of Ghana it is not available. For mild to moderate pneumonia, amoxicillin or penicillin remains the mainstay of treatment, but in higher doses where pneumococcal resistance is likely.[22] Again, high dose penicillin can be used to treat bacteremia without meningitis due to organisms of intermediate susceptibility.[15] In most cases, serum and most tissue levels of penicillin will exceed the MIC of the organism. However, it is more difficult to reach high enough levels in the cerebrospinal fluid. It has been suggested that chloramphenicol may not provide adequate therapy for penicillin resistant pneumococcal meningitis as well.[2 -24] Most experts recommend empiric treatment with a third generation cephalosporin for meningitis in settings with penicillin resistant pneumococcus. However, in most areas of Ghana third generation cephalosporins are not available. In Kumasi, they can only be purchased in private pharmacies at a prohibitive cost because these drugs are not on the national formulary. While third generation cephalosporins would be useful in treating patients with otherwise resistant microbes, great care must be taken so that indiscriminate usage does not lead to reduced susceptibilities of these antimicrobials as well.

There is an alarming concern in other countries with multiple resistant pneumococci. The current policy for the empiric treatment of children with menigitis at KATH is high dose penicillin combined with chloramphenicol. Clinical trials would be useful to determine definitive recommendations for the treatment of CNS and non -CNS infection with penicillin resistant pneumococci. Continued surveillance to assess the prevalence of *S. pneumoniae* carriage, disease and resistance patterns is essential.

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