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ORIGINAL ARTICLE

Epidemiology of pneumococcal carriage in children under five years of age in Accra, Ghana

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Abstract

Background: Although the majority of pneumococcal infections occur in the developing world, pneumococcal epidemiology is poorly understood in these settings. The aim of the study was to investigate the epidemiology of pneumococcal carriage among children younger than 5 years at a paediatric healthcare centre in Ghana. *Method:* Four-hundred and twenty-three children were randomly sampled and nasopharyngeal specimens were collected from them. The specimens were cultured for *Streptococcus pneumoniae*, and the isolates were subjected to antibiotic susceptibility testing and serotyping by latex agglutination. Epidemiological data on demographic and clinical features of the study subjects were collected. *Results:* The prevalence of pneumococcal carriage was 48.9% (207/422), with age groups 43–48 months having the highest carriage prevalence. In the multivariate analysis, pneumococcal carriage was significantly associated with runny nose (odds ratio = 1.9, $p = 0.003$) and day-care attendance (odds ratio = 1.5, $p = 0.04$). No pneumococcal resistance was observed for ceftriaxone, while the prevalence of resistance to the other antibiotics tested was: cotrimoxazole 100%, ampicillin 88%, tetracycline 78%, penicillin 63% and erythromycin 24%. Fourteen different pneumococcal serogroups/serotypes were identified and serogroup 6 was the most prevalent (30%), followed by serotype 19 (20%). *Conclusions:* We conclude that pneumococcal carriage among the study children is high and the carried strains have a high level of resistance (> 50%) to several antibiotics. Ceftriaxone is a suitable antibiotic for treating pneumococcal infections in Ghana, and the use of this antibiotic coupled with the pneumococcal vaccination is expected to significantly reduce the burden of pneumococcal disease in the country.

Keywords: *Antibiotic resistance, Ghana, Pneumococcus, serotype, vaccine*

Introduction

Streptococcus pneumoniae, also referred to as the pneumococcus, is a Gram-positive, lancet-shaped bacterium usually occurring in pairs [1]. An important characteristic of the pneumococcus is the presence of a polysaccharide capsule, which defines over 90 capsular types [2–4]. *Streptococcus pneumoniae* is part of the normal bacterial flora of the upper respiratory tract of humans, and is mainly found in the nasopharynx [1]. Carriage of *S. pneumoniae* is higher in children than in adults, and the prevalence of colonization peaks around the age of 1–2 years [5]. Pneumococcal carriage rate is higher in developing than in developed countries owing to the wider range of distribution of risk factors such as overcrowding in the former [5].

Pneumococcus can sometimes be highly virulent, but more often is a member of the normal flora [6]. Globally, the annual incidence of pneumococcal infections is estimated to be one million, the majority of which occur in developing countries [7]. The burden of pneumococcal disease is exacerbated by the rising resistance of the organism to several antibiotics, and resistant strains have been reported worldwide [8]. Pneumococcal conjugate vaccines are being introduced in many developing countries [9]. However, it is unlikely that the vaccines will eliminate pneumococcal disease for several reasons, which include the limited serotype composition of the vaccine, capsular switching and serotype replacement [9,10]. Thus, pneumococcal antimicrobial therapy and issues related to its

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resistance remain important in the era of pneumococcal vaccination.

Although the majority of pneumococcal infections occur in the developing world [7], pneumococcal epidemiology in the developing world is poorly understood. In Ghana, the few pneumococcal studies that have been carried out focused on invasive disease [11–14]. Consequently, very little is known about the epidemiology of pneumococcal carriage in the country. Understanding the epidemiology of pneumococcal carriage is important, as carriage of the organism is a precursor for the development of pneumococcal disease, and is responsible for person-to-person transmission [15]. In this study, we present pneumococcal carriage data for children aged less than 5 years attending a paediatric hospital in Ghana, thereby providing information on carriage prevalence, risk factors of carriage, antibiotic resistance and serotype distribution.

Materials and methods

Study site

The study was conducted at the Princess Marie Louise Children's Hospital, located in Accra (the capital city of Ghana), in February 2011. Accra has a population of about two million people and there are 27 hospitals [16]. Princess Marie Louise Children's Hospital is one of the two hospitals in Accra that cater exclusively for children. This hospital is a primary healthcare facility, has a 62-bed capacity and receives over 70 000 clients annually. The hospital was established to care particularly for malnourished children; however, it currently sees children with all kinds of diseases. The common paediatric diseases reported to this hospital include diarrhoea, respiratory infections and malaria.

Sampling and data collection

This was a prospective cross-sectional study involving 423 children younger than 5 years who were randomly sampled through balloting of patients' folders at the Princess Marie Louise Children's Hospital. Nasopharyngeal specimens were collected from the study subjects according to the World Health Organization guidelines [17]. In brief, to obtain the specimen, the child's head was tipped slightly backwards and a nasopharyngeal swab passed directly backwards, parallel to the floor of the nasopharynx, until it reached the posterior pharynx. The swab was rotated at 180 degrees or left in place for 5 s to saturate the tip before it was slowly removed. Swabs were immediately immersed in 1 ml skim milk tryptone glucose–glycerin medium and transported on ice to the laboratory within 8 h. A structured questionnaire was used to collect data on risk factors of pneumococcal carriage from the study participants. The questionnaire covered four areas including demographic features, clinical features and household characteristics. Information on risk factors was obtained from guardians of the study children rather than from their hospital folders.

Laboratory analysis

Isolation and identification of Streptococcus pneumoniae. The nasopharyngeal specimens were inoculated immediately on to 5% sheep blood agar plates (containing 2.5 μg/ml of gentamicin). From the primary plate, presumptive *S. pneumoniae* colonies, based on colonial morphology and α-haemolysis, were picked and streaked out on a sheep blood agar plate. This was followed by application of an optochin disc and overnight incubation at 37°C in 5% carbon dioxide [17]. After incubation, the zone of inhibition around the optochin disc was measured; a measurement of at least 5 mm radius from the edge of the disc was considered susceptible and indicative of *S. pneumoniae* [18].

Serotyping of Streptococcus pneumoniae isolates

Serotyping of *S. pneumoniae* isolates was done by latex agglutination based on the method developed by Slotved et al. [19]. The latex agglutination test used is described in brief as follows. Ten microlitres of latex reagent was added to 10 μl of a broth (Todd–Hewitt) culture of *S. pneumoniae* on a card. The mixture was allowed to react for 5–8 s with slight agitation of the card. Agglutination observed within 5–10 s indicated a positive reaction, while agglutination after a period of more than 30 s was considered a false-positive reaction. After the tests, serotypes or serogroups were determined based on the chessboard system supplied by the Statens Serum Institut, Denmark [19].

Antibiotic susceptibility testing of Streptococcus pneumoniae isolates

Antibiotic susceptibility testing of *S. pneumoniae* isolates was done by the Kirby–Bauer method using *S. pneumoniae* NCTC 10319 as the control [20,21]. The antimicrobial drugs tested included ampicillin 10 μg, oxacillin 1 μg, tetracycline 30 μg, erythromycin 15 μg ceftriaxone 30 μg and cotrimoxazole 25 μg. In brief, the *S. pneumoniae* test isolate was emulsified in distilled water until the turbidity was similar to that of 0.5 McFarland's standard. A loopful of the bacterial suspension was inoculated on to Mueller–Hinton agar plates supplemented with 5% sheep blood. The

antibiotic discs were applied to the surface of the agar plate, and incubated at 37°C for 18–24 h. After incubation, the zones of inhibition around the antibiotic discs were measured and interpreted based on the breakpoint criteria of the Clinical and Laboratory Standards Institute on Antimicrobial Susceptibility Testing [22].

Data analysis

Data were entered into Microsoft Excel and imported into SPSS version 16 and Epi Info version 3.5.1 for all statistical analysis. Frequency tables were generated for the various variables and data described according to person, place and time. The prevalence of nasopharyngeal carriage of *S. pneumoniae* was presented as proportions of individuals in different age groups and gender. Antibiotic resistance rates were estimated and serotype distribution was determined. A logistic regression model was used to analyse exposures associated with carriage and the results were presented as odds ratios (ORs), p values and 95% confidence intervals.

Ethical approval

The study was approved by the Ethical Review Board of the Ghana Health Service and informed consent was obtained from guardians of the children who took part in the study.

Results

The 423 children recruited into the study comprised 226 males (53.4%) and 197 females (46.6%). Their age ranged from 3 to 59 months, with a median age of 18 months. In total, 179 (42.3%) of the participating children attended day care. About one-third of the children lived in different parts of Accra permanently and 292 (69%) of them lived in compound houses. None of the children recruited had received any pneumococcal vaccine. In assessing medical history as well as signs and symptoms of respiratory tract infection experienced by children in the previous month, 20 (4.7%) had asthma and nine (2.1%) had sickle cell disease as underlying diseases. Forty (9.5%) of the children had experienced episodes of otitis media. The signs and symptoms experienced by participants in the previous month included fever in 250 (59.1%), runny nose in 310 (73.3%), headache in 240 (57.0%), cough in 308 (72.8%), blocked nose in 190 (44.9%) and sneezing in 158 (27.4%).

The prevalence of *S. pneumoniae* carriage among the children was 48.9% (207/422). The prevalence of pneumococcal carriage was highest in the 43–48 month age group (Figure 1). In the multivariate analysis pneumococcal carriage was significantly associated with runny nose (OR = 1.9, $p = 0.003$) and day-care attendance (OR = 1.5, $p = 0.04$) (Table I). Of the 207 pneumococci isolates recovered from the study subjects, 85 were available for serotyping, 64 of which were from children with respiratory symptoms. As shown in Figure 2, 14 different serogroups/serotypes were identified and serogroup 6 was the most prevalent (30%). Other major serogroups/serotypes identified were serogroup 19 (20%), serogroup 23 (8%), serotype 14 (7%) and serotype 11 (6%). Overall, these five major serogroups/serotypes accounted for 71% of the serotyped isolates. The prevalence of nontypeable pneumococci (i.e. pneumococci showing negative results with type-specific antisera) was as high as 10%.

All the 207 pneumococci isolates recovered from the participants were subjected to antibiotic susceptibility testing. No resistance was observed for ceftriaxone, while the prevalence of resistance to the other tested antibiotics was: cotrimoxazole 100%, ampicillin 88%, tetracycline 78%, penicillin 63% and erythromycin 24%. The prevalence of multiple drug resistance (i.e. resistance to three or more antibiotics) was 87%.

Discussion

In this study, we investigated the epidemiology of pneumococcal carriage among children aged less than 5 years at a healthcare facility in Ghana, and reported an overall carriage rate of 48.9%. By comparison, a study on outpatients younger than 5 years in Kenya and Nigeria reported pneumococcal carriage rates of 57% [23]. In Ghana, our data concurs with a study conducted in Kumasi, in the middle belt of Ghana, which reported a carriage prevalence of 51.4% [24]. In contrast with our data, Donkor et al. [13] reported a pneumococcal carriage rate of 27% among children younger than 5 years attending a tertiary hospital in Accra (southern Ghana), while Dayie et al. [25] reported a carriage rate of 32% among nursery school children under 6 years in the same city. It is worth noting that, although the prevalence of colonization normally peaks around the age of 1–2 years, in the present study, the prevalence remains high up to 5 years of age and peaks only at 43–48 months (approximately 3.5–4 years) of age. This is interesting and further epidemiological studies are needed to elucidate this observation. The significant association of runny nose and day-care attendance with pneumococcal carriage has been previously reported in epidemiological studies carried out in Kenya [23]. Like our data, in the Kenyan study, the study subjects also experienced

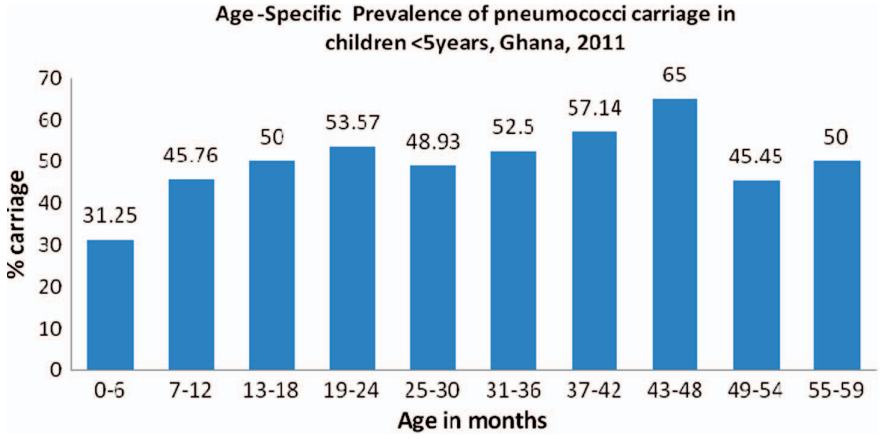

Figure 1. Age-specific pneumococci carriage in children under 5 years, Ghana, 2011.

coryza in the month prior to sampling [23]. Several risk factors of pneumococcal carriage, such as smoking [26], history of acute asthma [27] and nutritional status [26], reported by other investigators, were not observed in our study.

Much of the recent interest in the epidemiology of pneumococci involves tracing the spread of penicillin resistance. In this study, pneumococcal penicillin resistance was 62.8%, which is higher than rates reported in previous studies in Ghana [13,24,26]. While these differences may be due to the smaller number of isolates available for susceptibility testing in the previous Ghanaian studies [13,24], they may also represent the rapid pneumococcal evolution of antibiotic resistance occurring in many countries, including Ghana. Studies conducted in central and eastern Europe [28], as well as Korea [29], reported pneumococcal resistance to penicillin to be above 50%. The mechanism of resistance to penicillin and other beta-lactam antibiotics in pneumococci is attributed to variations in penicillin binding proteins and is disseminated among pneumococci through intraspecies or interspecies recombination [30].

We also observed a high prevalence of resistance for non-beta-lactam antibiotics, including tetracycline (67%) and cotrimoxazole (100%), which concurs with previous antibiotic resistance surveillance data in Ghana [13]. These drugs have been on the Ghanaian market for a long time and can be obtained over the counter without prescription. The high rate of usage of these antibiotics in Ghana may partly explain their resistance patterns, not only for pneumococcus but also for several other bacteria [31]. All the pneumococcal strains tested were susceptible to ceftriaxone, which is important information with regard to the treatment of pneumococcal infections in Ghana. Antibiotic treatment in Ghana is empirical in many cases, and evidence from this study suggests that ceftriaxone could be an appropriate antibiotic for this purpose.

Currently, pneumococcal vaccination using the 13-valent conjugate vaccine (PCV13) is underway in Ghana. One feature of the pneumococcal conjugate vaccine is its ability to eliminate pneumococcal carriage of serotypes included in the vaccine and therefore reduce pneumococcal disease [32]. However, potential problems are also anticipated with the vaccine, particularly serotype replacement, which has been observed in several countries where the pneu-

Table I. Multivariate analysis of pneumococcal carriage and risk factors among children under 5 years, Ghana, 2011.

Risk factor	OR	95% CI	*p*
Asthma	0.8471	0.3436–2.0883	0.7185
Blocked nose	0.8265	0.5631–1.2132	0.3305
Difficulty in breathing	0.6324	0.3991–1.0021	0.051
Cough	1.3517	0.8783–2.0801	0.1707
Day-care attendance[a]	1.4985	1.0169–2.2082	0.0409
Ear infection	1.9643	0.7129–5.4125	0.1917
Fever	0.9125	0.6192–1.3447	0.6434
Headache	1.0025	0.6821–1.4733	0.99
Children < 5 years in household	0.8462	0.5794–1.2359	0.3876
Number in household[b]	1.0936	0.9528–1.2553	0.203
Occupation[c]	0.9637	0.8493–1.0934	0.5657
Previous episodes of otitis media[d]	1.8407	0.9411–3.6	0.0746
Residential type	0.9522	0.6304–1.4383	0.816
Religion	1.1818	0.7415–1.8836	0.4824
Permanent resident in Accra	1.2107	0.6939–2.1125	0.5008
Runny nose	1.9236	1.2365–2.9928	0.0037
Sickle cell disease	0.5147	0.127–2.0856	0.3522
Sex	0.8785	0.5994–1.2878	0.5069
Smoking[e]	0.8579	0.5325–1.3822	0.5289
Sneezing	0.8062	0.5431–1.1967	0.2851
Sore throat	1.0457	0.426–2.5669	0.9223

OR, odds ratio; CI, confidence interval.
[a]Whether the child was attending nursery school.
[b]Total number of people living in a house.
[c]Five categories: artisans, professionals, traders, students and unemployed.
[d]Otitis media in the previous month.
[e]Whether a household member smokes indoors.

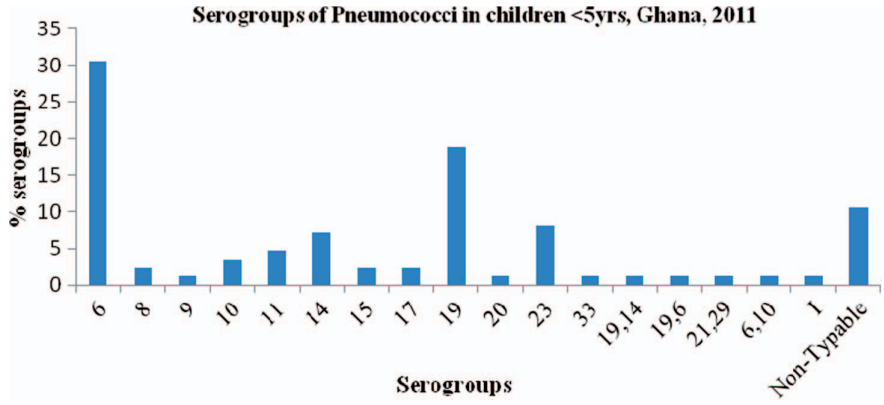

Figure 2. Distribution of pneumococci serogroups in children under 5 year, Ghana, 2011. I, serogroups (25, 38, 43, 44, 45, 40, 48); Non-typeable, 10% of isolates could not be typed using the Pneumotest-Latex kit.

mococcal vaccine has been in use for some time [9,10].

We conclude that pneumococcal strains carried by the study children have a high percentage resistance (>50%) to several antibiotics, including penicillin, which is commonly used in empirical treatment of pneumococcal infections in Ghana. Ceftriaxone is a suitable antibiotic for treating pneumococcal infections in Ghana, and the use of this antibiotic coupled with the pneumococcal vaccination is expected to significantly reduce the burden of pneumococcal disease in the country.

There are several limitations to this study. First, it would have been more accurate to determine pneumococcal carriage prevalence among healthy children than among sick children. Furthermore, we do not have information on the reasons why the study children had been assigned to the hospital. This information would have been very useful in the interpretation of the findings. Secondly, we did not have enough resources to carry out serotyping of all 207 isolates and in some cases we only determined serogroups. Thirdly, we did not collect information on antibiotic use or treatment among the study participants before sampling. It is possible that our sample is biased towards the more resistant strains as a result of antibiotic use.

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Declaration of interest: The authors report no conflicts of interest.

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