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Invasive disease and paediatric carriage of Streptococcus pneumoniae in Ghana

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Abstract

This study was carried out primarily to evaluate the public health burden related to Streptococcus pneumoniae in Ghana and to provide related preliminary molecular epidemiological data on the organism. Invasive and nasopharyngeal specimens were screened for S. pneumoniae, and isolates were subjected to serotyping, multilocus sequence typing (MLST) and antibiotic susceptibility testing. Overall, the prevalence of S. pneumoniae in cerebrospinal fluid (CSF) was 1.7%, in blood was 0.2%, and in nasopharyngeal specimens was 15.3%. The prevalence of multiple drug resistance among the isolates was 48.6%, while the percentage resistance to various drugs was in the range of 11.1–84.0%. Serotyping of the S. pneumoniae isolates showed 7 different serotypes (3, 6B, 9, 10, 14, 16 and 23F). The extent of coverage of serotypes by the 7-valent pneumococcal conjugate vaccine was 57.1%, for the 10-valent vaccine was 57.1%, and for the 13-valent vaccine was 71.4%. MLST of 7 housekeeping genes of the organism showed a high level of genetic diversity among the isolates. S. pneumoniae appears to be an important organism in invasive infections in Ghana, being the most prevalent organism in CSF in this study. The high multiple drug resistance of the organism observed heightens the public health burden, which may be controlled by pneumococcal conjugate vaccines to a large extent.

Introduction

Streptococcus pneumoniae is part of the normal bacterial flora of the upper respiratory tract, but is also associated with several invasive infections, including meningitis, pneumonia, and sepsis. Worldwide, the annual mortality of meningitis and pneumonia due to the organism is over 1.6 million [1,2]. Conditions including HIV infection, sickle cell anaemia and a variety of organ failures are associated with an increased risk of serious S. pneumoniae diseases. Though a global problem, the public health impact of S. pneumoniae is higher in the developing world, where children <5 years of age are most affected, and the organism is responsible for 10–20% of all deaths in this age group [1,3].

The public health burden related to S. pneumoniae is heightened by the increasing resistance of the organism to essential antimicrobial drugs, particularly penicillin, cephalosporins and macrolides. Resistant strains of the organism have been reported in both developing and developed countries, and contribute to the high mortality of its diseases. The enormous burden of S. pneumoniae, especially as a result of its increasing and widespread drug resistance, underscores the importance of control through vaccination. Quite recently, conjugate vaccines for S. pneumoniae have been developed to prevent infections in children, and the vaccines have been shown to be superior to the previous polyvalent polysaccharide vaccine of the organism [4,5].

In Ghana the isolation rate of S. pneumoniae is low, partly because of a lack of laboratory facilities and technical expertise required for the isolation of this organism. As a result there is inadequate information on its public health impact, though studies in some parts of the country have reported a high incidence of S. pneumoniae in meningitis [6,7]. Additionally, little is known epidemiologically about

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the organism, especially in relation to vaccination, which could be a major constraint to the effective control of the disease burden in Ghana. This research paper reports primarily on the public burden related to S. pneumoniae in terms of its invasive diseases and drug resistance, and also provides preliminary data on the molecular epidemiology of the organism in Ghana.

Materials and methods

Study sites and subjects

The study was carried out at 3 major hospitals in Ghana from July 2006 to December 2007. During this period, specimens of cerebrospinal fluid (CSF) were collected from meningitis patients, while blood specimens were taken from pneumonia and septicaemia patients. The routine at the study hospitals was to collect specimens from all patients who met the diagnosis inclusion criteria. Demographic characteristics of the patients, as well as conditions predisposing to increased risk of pneumococcal infection were also collected. A survey was also carried out to determine the nasopharyngeal carriage of S. pneumoniae among children aged <13 years. This was carried out at the Child Health Department of one of the study hospitals, namely Korle-Bu Teaching Hospital. This hospital is one of the largest in Ghana, and also a major referral centre. Because patients visit Korle-Bu Teaching Hospital from all over the country, the hospital was selected for the carriage study, in order to obtain a more representative sample. Based on 95% confidence limits with an allowable error of 10%, we randomly sampled at least 124 healthy children visiting Korle-Bu Teaching Hospital for a review; nasopharyngeal specimens were collected from the children for laboratory analysis.

Laboratory analysis of specimens

The specimens were cultured for S. pneumoniae on blood agar supplemented with 5% sheep blood agar, and suspected colonies of S. pneumoniae were confirmed by the optochin inhibition test [8]. CSF specimens were also screened for S. pneumoniae by the latex agglutination test [9]. The isolated S. pneumoniae strains were tested for susceptibility to a range of antimicrobial agents by the Kirby–Bauer method [10]. In addition, minimum inhibitory concentration (MIC) values were determined for the drugs using the Etest [9]. NCCLS breakpoints were used to determine the susceptibility of S. pneumoniae isolates. For penicillin, the most commonly used drug against S. pneumoniae, the MIC definition of resistance was a MIC value >1.0 mg/ml. S. pneumoniae isolates were serotyped using the latex agglutination reaction with specific antisera [9]. Multilocus sequence typing (MLST) was carried out on 7 housekeeping genes of the organism: *aroE*, *gdh*, *gki*, *recp*, *spi*, *xpt* and *ddl*. Standard protocols were followed for polymerase chain reaction (PCR) and direct sequencing of PCR products with the aid of an ABI Prism 310 genetic analysis system [11]. Allelic profiles were analysed with the aid of applications on the MLST homepage (http://spneumoniae.mlst.net). The ATCC 49619 strain of S. pneumoniae was used as a control in the laboratory tests.

Data analysis

The data collected were entered in MS-Excel and analysed to address the objectives of the study. The strategies taken to analyse the data involved descriptive statistics, including geometric means, frequencies, ranges and prevalence rates of the study variables. Significant differences, associations and interrelationships of the variables were also assessed at a level of $p < 0.05$. Specific analyses were carried out to: (1) determine prevalence rates of S. pneumoniae from specimens including blood, CSF and nasopharyngeal swabs; (2) compare prevalence rates of S. pneumoniae with other isolated organisms from blood and CSF; (3) determine prevalence rates of S. pneumoniae resistance to various antimicrobial drugs; (4) compare S. pneumoniae penicillin resistance in invasive disease and carriage; (5) estimate the burden of S. pneumoniae infections; (6) compare the burden of S. pneumoniae in different age groups; (7) assess the serotypes of S. pneumoniae isolated from study subjects; (8) evaluate the genetic diversity among the S. pneumoniae isolates; (9) assess the extent of coverage of S. pneumoniae serotypes by pneumococcal conjugate vaccines.

Results

Numbers of study subjects, clinical specimens and isolates

Over the study period, a total of 4566 patients at the study hospitals were clinically diagnosed with meningitis, pneumonia or sepsis. Their age ranged from 0.003 to 78 years, and a total of 1551 patients were aged <5 years. Clinical specimens collected from the patients for laboratory analyses included 3779 blood and 787 CSF specimens; 1220 blood and 331 CSF specimens were from patients aged <5 years, while 2559 blood and 456 CSF specimens were from patients aged ≥5 years. A total of 124 nasopharyngeal specimens were also collected for investigation. Overall, a total of 16 S. pneumoniae isolates were

obtained from invasive specimens (blood and CSF) and 19 from nasopharyngeal specimens.

Pathogens isolated from CSF

Out of the 787 CSF specimens cultured, 23 (2.9%) produced bacterial growth and a total of 11 different pathogenic organisms were isolated from the specimens. Table I shows the prevalence rates of the various organisms isolated from the CSF specimens. The most frequently isolated organism was S. pneumoniae with a prevalence rate of 1.1% among the study subjects. A total of 5 different Gram-negative organisms were isolated with a prevalence rate of 0.89%, comprising Escherichia coli (0.25%), Pseudomonas aeruginosa (0.25%), Salmonella spp. (0.13%), Proteus spp. (0.13%) and Flavobacterium meningosepticum (0.13%). Neisseria meningitidis was isolated with a prevalence rate of 0.38%, while Haemophilus influenzae, Staphylococcus aureus and viridans streptococci were each isolated with a prevalence rate of 0.13%. The only fungus isolated from the CSF was Cryptococcus neoformans with a prevalence rate of 0.13%. With the exception of S. pneumoniae, all the organisms isolated from the CSF specimens were isolated from patients aged ≥5 years. Out of the 9 isolates of S. pneumoniae from CSF, 4 were from children <5 years while 5 were from subjects aged ≥5 years; this represents prevalence rates of 1.2% (4/331) and 1.1% (5/456) in the 2 age groups, respectively, with no significant difference.

Pathogens isolated from blood

A total of 206 (5.5%) of the 3779 blood cultures produced growth. A wide range of organisms was isolated and the prominent ones are reported in Table II. The most frequently isolated organism was S. aureus with a prevalence rate of 1.32% among the study subjects. S. pneumoniae prevalence was 0.19%. A group of 5 Gram-negative organisms comprising Salmonella typhi (0.21%) and non-typhoidal salmonella (0.4%), Enterobacter (0.58%), Acinetobacter (0.56%), Klebsiella (0.45%), and E. coli (0.26%), were isolated from the blood specimens.

Table II also shows prevalence rates of the isolated organisms for study subjects aged <5 years and those aged ≥5 years. Overall, prevalence rates in subjects <5 years of age were 0.26–1.90% and in subjects aged ≥5 years were 0–0.18%. Significant differences in prevalence rates of the organisms between the 2 age groups were more commonly observed for the Gram-negative bacteria. For both age groups, S. aureus was the most prevalent organism.

Table I. Microbial pathogens isolated from cerebrospinal fluid specimens.

Organism	n[a]	%
Staphylococcus aureus	1	0.13
Streptococcus pneumoniae	9	1.1
Viridans streptococci	1	0.13
Escherichia coli	2	0.25
Pseudomonas aeruginosa	2	0.25
Salmonella spp.	1	0.13
Proteus spp.	1	0.13
Flavobacterium meningosepticum	1	0.13
Haemophilus influenzae	1	0.13
Neisseria meningitidis	3	0.38
Cryptococcus neoformans	1	0.13

[a]Number of isolates.

S. pneumoniae prevalence in invasive and non-invasive specimens

Based on positive cultures, the prevalence of S. pneumoniae in invasive specimens was 0.35% (16/4556), comprising 0.19% (7/3779) for blood and 1.1% (9/787) for CSF. Non-culture detection of S. pneumoniae in CSF specimens showed a prevalence rate of 18.2% (2/11). Thus the overall S. pneumoniae prevalence in CSF specimens based on culture and non-culture detection methods was 1.72% (11/798). The overall S. pneumoniae prevalence in invasive specimens in subjects <5 years was 0.58% (9/1220) and in those aged ≥5 years was 0.23% (7/3015). An overall S. pneumoniae prevalence rate of 15.3% (19/124) was observed for the nasopharyngeal specimens. The prevalence rates in subjects <5 years and those aged ≥5 years were 27.2% (9/33) and 10.3% (10/97), respectively.

Antimicrobial drug resistance of S. pneumoniae isolates

The prevalence of multiple drug resistance among S. pneumoniae isolated from the study subjects was 48.6% (17/35); the rates among invasive and non-invasive isolates were 50% (8/16) and 47.4% (9/19), respectively, while the rates among isolates from subjects <5 years and those aged ≥5 years were 56.3% (9/16) and 52.6% (10/19), respectively. Resistance to the various antimicrobial drugs tested were cefotaxime 11.1%, chloramphenicol 11.1%, ciprofloxacin 12.5%, cefuroxime 14%, penicillin 19.4%, erythromycin 28%, cloxacillin 43.6%, ampicillin 48%, tetracycline 62%, co-trimoxazole 84%, and gentamicin 84%. The MIC value ranges (mg/l) were as follows: chloramphenicol 0.75–8.0; cefotaxime 0.125–1.5; ampicillin 0.064–3.00; erythromycin <0.016–0.032; tetracycline 0.094–64.0; and penicillin 0.047–2.0.

Table II. Microbial pathogens isolated from blood specimens.

Organism	NT	%	NC	%	NA	%
Staphylococcus aureus	50	1.32	30	1.9	20	0.9
Streptococcus pneumoniae	7	0.19	4	0.26	3	0.13
Escherichia coli	10	0.26	6	0.39	4	0.18
Non-typhoidal salmonella	15	0.4	11	0.71	4	0.18
Salmonella typhi	8	0.21	5	0.32	3	0.13
Acinetobacter spp.	21	0.56	19	1.23	2	0.09
Enterobacter spp.	22	0.58	22	1.4	0	0
Klebsiella spp.	17	0.45	17	1.1	0	0

NT, total number of isolates from all subjects; NC, total number of isolates from subjects aged <5 y; NA, total number of isolates from subjects aged ≥5 years

Serotype distribution and genetic diversity of S. pneumoniae isolates

Thirteen S. pneumoniae isolates were serotyped; 61.5% (8) were typeable while 38.5% (5) were not. The typeable isolates were of 7 different serotypes: 3, 6B, 9, 10, 14, 16 and 23F.

Table III shows the MLST results of some S. pneumoniae isolates. Genetic analyses of the 7 housekeeping genes (*aroE*, *gdh*, *gki*, *recp*, *spi*, *xpt*, *ddl*) of the isolates showed wide variability as shown by the allele numbers corresponding to the various genes of the isolates; while the *spi* and *xpt* gene sequences were the same in some isolates, the other 5 housekeeping genes were entirely different. Ten (35.7%) of the 28 housekeeping genes sequenced showed novel sequences, and this included 1 gene each of *aroE* and *spi*, and 2 genes each of *gdh*, *gki*, *recp* and *ddl*.

Discussion

S. pneumoniae is an agent of immense public health importance and has attracted more attention recently, due to the development of pneumococcal conjugate vaccines. In this study, we evaluated the public health burden of S. pneumoniae and collected epidemiological data relevant for the utilization of pneumococcal conjugate vaccines in Ghana. Based on culture, the prevalence of S. pneumoniae in CSF was 1.1% and in blood was 0.2%. Recent studies based on blood cultures have reported prevalence rates of 4.9% in Tanzania, 2% in Kenya, 1.8% in Ethiopia, 1.4% in Uganda for CSF; and 1.4% in Kenya, 0.8% in Ethiopia, 0.2% in Uganda for blood [2]. In this study, the detection of S. pneumoniae antigens in CSF by latex agglutination showed an overall prevalence rate of 18.2%. Though this involved few specimens, it shows the prevalence of the organism is actually higher than that reported by culture (1.1%). Though not as reliable as culture, the latex agglutination test has been demonstrated to be quite reliable, yielding a specificity of 91% and sensitivity of 86.6% [12]. The relatively low rate of detection of S. pneumoniae and other organisms by culture is probably due to the treatment of patients with antimicrobial agents before samples were taken. This was confirmed in interviews and discussions with both patients and clinicians. It has been demonstrated that after the initiation of parenteral antibiotics, CSF sterilization may occur after 4 hfor S. pneumoniae, and pre-treatment of patients with antimicrobial agents before specimen collection could considerably reduce the isolation rate of bacteria in CSF, and to a higher extent in blood [13,14].

S. pneumoniae was the most prevalent organism in CSF and is probably the most important cause of meningitis in Ghana. In 2003, the organism was implicated in an outbreak of meningitis in Ghana [15]. In blood specimens, the prevalence of S. pneumoniae was relatively low for both age groups and S. aureus and Gram-negative organisms were more important. The prevalence of S. pneumoniae in

Table III. Multilocus sequence typing (MLST) of Streptococcus pneumoniae isolates.

Isolate	aroE	gdh	gki	recp	spi	xpt	ddl
G36	5	4	2	4	4	1	1
G18	51[a]	2[a]	12[a]	41[a]	55[a]	3	244[a]
G1222	1	1	8	18	6	3	8
G2508	12	184[a]	42[a]	107[a]	6	239	179[a]

aroE, gdh, gki, recp, spi, xpt, and *ddl* represent the housekeeping genes sequenced. [a]Indicates a novel allele and its sequence is very similar to that of the attached allele number as defined by the MLST database.

nasopharyngeal specimens was higher for children aged <5 years (27.2%) than those ≥5 years; prevalence rates of up to 87% have been reported in this age group [16]. S. pneumoniae occur as part of the normal flora in the nasopharynx. However, nasopharyngeal carriage of the organism is considered a risk factor for invasive disease, and this may partly account for the relatively high morbidity of S. pneumoniae in children aged <5 years [16,17], which was also observed in this study.

Multiple drug resistance, a major public health problem, occurred at a high prevalence rate of 48.6% among the S. pneumoniae isolates in our study, which is consistent with a recent report [18]. This discourages empirical antibiotic treatment of the organism and underscores the need for susceptibility testing. Disk diffusion test results were generally consistent with MIC values and showed high resistance to co-trimoxazole, gentamicin, tetracycline and ampicillin. Many microorganisms have been reported as having a high percentage resistance to most of these drugs for several years, and the rates of resistance have been rising over the years not only for clinical isolates but also for the normal flora of the healthy population [19,20]. Lower prevalence rates of resistance were observed for ciprofloxacin, chloramphenicol, cefotaxime and cefuroxime. With the exception of chloramphenicol, these drugs have been on the Ghanaian market for a relatively short period of time and therefore may not have been used or misused extensively. In addition, some of these drugs are expensive and are used only for serious infections, thus limiting their usage. The resistance rate against penicillin, which has been an important drug in the treatment of S. pneumoniae infections in Ghana, was 19.4%. A study in 2005 in Ghana reported a rate of 12% of intermediate penicillin resistance [21]. While S. pneumoniae is still susceptible to the drug, high resistance is predicted in the next couple of years due to poor enforcement of regulations restricting access of the general public to antimicrobial agents.

Serotyping of S. pneumoniae identified serotypes 3, 6B, 9, 10, 14, 16 and 23F. Most of these serotypes have been implicated in invasive infections worldwide. We did not encounter serotypes 1 and 5, which have been commonly reported in invasive infections in West Africa [21,22]. This may be due to the relatively small number of isolates serotyped following loss of viability of some of the S. pneumoniae isolates. The extent of coverage of serotypes by the 7-valent pneumococcal conjugate vaccine was 57.1%, for the 10-valent vaccine was 57.1%, and for the 13-valent vaccine was 71.4%.It is encouraging that about 57–70% of the serotypes are covered by conjugate vaccines and therefore this proportion of disease caused by S. pneumoniae may be preventable. Though a limited number of isolates was used in this evaluation, similar findings have been reported in other parts of Ghana and several other countries [11,21]. Owing to the limited number of isolates that were serotyped, it may be difficult to comment adequately on the relationship between serotype and drug resistance. However, the high multiple resistance among the S. pneumoniae isolates suggests significant resistance among the non-vaccine serotypes. Owing to serotype replacement, it may be necessary to monitor drug resistance of non-vaccine serotypes in this era of pneumococcal vaccination.

MLST is the definitive, internationally accepted method for monitoring the spread of clones through the S. pneumoniae population [23,24]. MLST of the 28 S. pneumoniae genes showed wide genetic variations, which is quite common with the organism [25]. The high proportion of novel sequences identified is interesting and shows that the population structure of the organism in Ghana may be quite different from what is known. A similar observation has also been made in S. pneumoniae isolates in the Gambia [26]. There is a need for further typing of S. pneumoniae from this region to provide more information on molecular clones particular to the region.

Our study showed that S. pneumoniae plays an important role in invasive infections in Ghanaian children, especially meningitis. The high multiple resistance of the organism, which limits treatment options, heightens the public health burden of the organism. This underscores the need for vaccination, and conjugate vaccines that were shown to have serotype coverage of up to 70% constitute a major intervention in reducing the public health burden of this organism in the country.

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Ethical approval: The protocol of the study was approved by the Ethical and Protocol Committee of the University of Ghana Medical School. Informed consent was obtained from study subjects before

enrolment, and in the case of children, consent was obtained from parents.

Declaration of interest: No conflict of interest to declare.

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