

Streptococcus pneumoniae serotypes/serogroups causing invasive disease in Riyadh, Saudi Arabia: extent of coverage by pneumococcal vaccines

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BACKGROUND: Serogroup distribution of sterile site pneumococcal isolates varies between developing and developed countries as well as between different geographical regions. The potential efficacy of any pneumococcal vaccine depends on the degree of representation of the prevalent serogroups in the vaccine. We conducted this study to determine the prevalent pneumococcal serogroups causing invasive infections in Riyadh, Saudi Arabia and to estimate the coverage by the various pneumococcal conjugate vaccines.

METHODS: *S. pneumoniae* isolated between February 2000 and November 2001 from sterile sites of patients of all age groups were collected from 8 major hospitals in Riyadh and serogrouped using the latex agglutination method.

RESULTS: Isolates from 78 patients, 72% of whom were children, were studied. Eighty-eight percent of the isolates belonged to only 10 serogroups/serotypes, namely 6 and 19, 1 and 15, 14 and 23, 7, 18 and 22, in descending order of frequency. Potential coverage of the 7-valent, 9-valent, and 11-valent conjugate vaccines were 54%, 65% and 73%, respectively. The rate of reduced penicillin susceptibility in the serogroups represented in the 7-valent conjugate vaccine was significantly higher than in the non-vaccine serogroups (62% vs. 25%; $P=0.0023$)

CONCLUSION: The currently available 7-valent pneumococcal conjugate vaccine provides sub-optimal coverage to serogroups causing invasive diseases in our community. However, this vaccine would be a useful adjunct to penicillin prophylaxis in at-risk patients in the community. The effectiveness of the vaccine would be greater if serotype 15 could be included.

Streptococcus pneumoniae (pneumococcus) is a leading cause of morbidity and mortality worldwide.¹ It causes a wide variety of diseases ranging from severe invasive infections such as bacteremia and meningitis to relatively benign infections such as otitis media and sinusitis. The pneumococcus is the commonest cause of community-acquired pneumonia and otitis media. It is estimated that pneumococcal infections lead to the death of over one million children below 5 years of age annually.² Efforts to prevent pneumococcal infections by immunization with the 23-valent polysaccharide vaccine have been of limited success due to its poor immunogenicity in children below 2 years of age.³ The recently produced protein-polysaccharide conjugate pneumococcal vaccines have good immunogenicity even in infants. The major drawback of this class of vaccines is that due to technical and logistic reasons, only a limited number of strains can be formulated in the vaccine. The prevalent pneumococcal serotypes responsible for invasive diseases vary between developing and developed countries.^{3,4} Thus, determination of the prevalent pneumococcal serotypes causing invasive disease in each region is crucial for the formulation of vaccines suitable for each region. The currently available 7-valent conjugated vaccine has proven efficacy in preventing invasive pneumococcal disease in infants;⁵ however, the serotypes included in the vaccine are those responsible for the majority of invasive infections in developed countries and therefore may not offer adequate coverage in most developing countries. In this study the pneumococcal serotypes prevalent in invasive disease in the city of Riyadh were determined and correlated with the efficacy of

coverage of the various conjugated vaccines and their suitability for use in this region.

Methods

The study was conducted between February 2000 and November 2001 in Riyadh, the capital of Saudi Arabia. Riyadh has a mixed population of nationals and expatriates of approximately five million. During this period, pneumococcal isolates obtained from normally sterile body sites in patients with invasive pneumococcal disease in eight major government hospitals in Riyadh were studied. The contributing hospitals were King Khalid University Hospital (KKUH) (700 beds), King Abdulaziz University Hospital (KAUH) (120 beds), Riyadh Medical Complex (RMC) (1000 beds), Yamamah Hospital, Sulaimania Children's Hospital (SCH), King Faisal Specialist Hospital (KFSH) (500 beds), Prince Salman Hospital and the Security Forces Hospital (SFH) (550 beds). These hospitals serve a population with a wide range of socioeconomic status and geographical location with different ethnic groups.

A patient was eligible if *S. pneumoniae* was isolated from blood, cerebrospinal fluid (CSF), peritoneal fluid, pleural effusion, synovial fluids or bone aspirate. Duplicate isolates from the same patient were excluded unless the second isolation was more than 30 days since the first isolation.

Laboratory procedure. Isolates from the various hospitals were subcultured on 5% sheep blood agar (BA) and incubated aerobically and anaerobically in 7% CO₂ at 37°C. α haemolytic colonies were identified first by optochin disc (5 μ g) (Oxoid) on BA medium, and all isolates sensitive to the optochin disc (≥ 12 mm) were further confirmed using the bile solubility test (10% Na desoxycholate). For serotyping each isolate was initially suspended in 10 mL of Todd-Hewitt's broth and incubated at 37°C for 24 hours after which a subculture was made on BA to check for purity. The broth culture was then centrifuged at 3000 rpm and a few drops of the centrifuged deposit were suspended in a vial with about 0.5-1 mL glycerol and placed in a deep freezer at -20°C for 24 hours before being stored at -70°C in liquid nitrogen. When ready for serotyping the vials were removed, left to thaw and inoculated again into Todd-Hewitt's broth and incubated at 37°C for 24 hours. A subculture was then made onto BA to be used for serotyping. The latex agglutination method was used as follows: each antiserum (Statens Seruminstitut, Copenhagen) was diluted 1 in 160 with sodium glycine buffer (pH 8.2). Equal volumes

of the diluted antiserum and latex suspension (polystyrene latex beads, Sigma USA) were mixed and incubated in water bath at 37°C for 2 hours. An equal volume of 0.1% albumin solution in sodium glycine buffer was then added as a preservative and stored at +4°C. The coated latex particles were brought to room temperature before use. Several colonies were added to a drop (50 μ L) of the sensitized particles on a black tile, admixed for 2 minutes and examined for agglutination with the naked eye. Suspensions remaining milky were considered negative.⁶ Isolates negative by all pooled sera as well as by omni serum were classified as non-typable.

Antibiotic susceptibility testing by disc (Oxoid) diffusion was done according to the guidelines of NCCLS.⁷ Resistance to penicillin was represented by a zone size of less than 19 mm to oxacillin 1 μ g. The minimal inhibitory concentration (MIC) to penicillin, ceftriaxone, and vancomycin was done using the Ettest (AB Biodisk, Sweden) on Isosensitest agar (Oxoid) containing lysed horse blood 5% at 37°C in 5-7% CO₂.

Estimation of vaccine coverage. For the vaccine coverage we calculated the percentage of invasive episodes caused by serotypes and serogroups represented in the respective vaccine formulation. We considered the disease-causing serogroup represented if any serotype of that group (e.g., 6B, 23F) was included in the vaccine formulation. The 23-valent polysaccharide vaccine includes serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. The 7-valent conjugate vaccine formulation includes the following serotypes (4, 6B, 9V, 14, 18C, 19F and 23F). The 9-valent conjugate vaccine formulation is 7-valent plus serotypes 1 and 5. The 11-valent formulation is 9-valent plus serotypes 3 and 7F.

All statistical analyses were performed with StatPac Gold statistical package. The χ^2 -test or Z-test were used for comparing proportions as appropriate. A *P* value of <0.05 was considered statistically significant.

Results

During the study period there were 78 patients with confirmed invasive pneumococcal infections in Riyadh. The contributions of the various hospitals to the 78 isolates were as follows: KKUH (22), KAUH (17), RMC (10), Yamamah Hospital (9), SCH (8), KFSH (7), Prince Salman Hospital (3) and SFH (2). The sources of the specimens were as follows: 63 from blood only, 4 from CSF only, and one each

from synovial fluid, bone and peritoneal fluid. In addition, there were 7 isolates from both blood and CSF and also one from both blood and synovial fluid; however, these were considered as one entity thus giving a total of 78 isolates (Table 1). Although the invasive infections were encountered all year round, 49 (63%) of 78 episodes occurred during the colder months of the year ($z=2.378$; $P=0.02$) from October to March. It is perhaps noteworthy that this period covered only 10 months of the 22-month study period as opposed to the other warmer months, which covered 12 months of the study period.

The age was recorded in 71 patients; in the remaining 7 patients we were unable to obtain their ages. Of these, 51 (72%) were children (<15 years old) and 20 (28%) were adults ($z=4.0906$; $P=0.0001$). Seventy-three (94%) of the 78 isolates were distributed among

only 17 different serotypes/serogroups (Table 1). Five (6%) of the isolates were nontypeable and one agglutinated only with pooled sera to group B. Of the 73 that were typeable, 64 (88%) belonged to only 10 serogroups/types and these were in descending order of frequency 6 and 19, 1 and 15, 14 and 23, 18 and 7, and 22 and 3. Serotypes 6 and 19 alone comprised 31% of the total number of isolates, each accounting for 15% of the total; serotypes 1 and 15 each accounted for 10% of the total and serotypes 14 and 23 accounted for 7% each. In general 4 serotypes (19, 6, 15 and 1) accounted for more than 50% of invasive disease.

The representatives of serogroups/serotypes causing invasive diseases in children differed from those causing disease in adults (Table 2). Serogroups/serotypes 23, 18 and 14 caused invasive disease in children only, with 23 and 14 causing 6 (22%) of 27 episodes of invasive disease in young children under 2 years of age. Apart from serogroup 19, which was isolated from 6 (30%) of 20 episodes in adults, no other serotype was dominant in adults. Serogroups/serotypes 3, 4, 6, 14 and 23 were most common in young children under 2 years old, and for older children and adults, the prevalent serogroups/serotypes were 1, 15, 18, 19 and 22. The uncommon serogroups/serotypes were 5, 9, 10, 11, 12 and 13. There were no isolates numbered higher than 23.

With respect to potential pneumococcal vaccine coverage, 91% (71/78) of the serogroups/serotypes from invasive infections in Riyadh are included in the 23-valent polysaccharide vaccine, as opposed to 54% (42/78) for the 7-valent conjugate vaccine, 65% (57/78) for the 9-valent conjugate vaccine and 73% (51/78) for the 11-valent conjugate vaccine ($P>0.05$, comparing the latter three vaccines) (Table 3). Sixty-three percent of serotypes causing invasive disease in young children are represented in the 7-valent vaccine and 45% in the older children and adults.

Antimicrobial resistance. Of the 78 pneumococcal isolates, 34 (44%) were of reduced susceptibility to penicillin (MIC=0.1-1.0 µg/mL). Of these, only one isolate had high level resistance (MIC ≥2.0 µg/mL, the actual MIC was 2.0 µg/mL). With the most current recommendations from the NCCLS,⁸ none of our isolates was resistant to ceftriaxone. The highest MIC for ceftriaxone was 1.0 µg/mL and the isolate was from a patient with bacteremia. The frequency of reduced susceptibility to penicillin differed among the different serogroups/serotypes. All the isolates of serogroup 23 were of reduced susceptibility to penicillin and the only isolate with high level resistance

Table 1. Frequency and site of isolation of different *S. pneumoniae* serogroups/serotypes isolated from 86 specimens during 78 episodes of invasive diseases in Riyadh, 2000-2001.

Serogroup/serotype	No. of Isolates (%)	Specimen			Total
		Blood	CSF	Others	
6	12 (15.4)	11	3	1 (bone)	15
19	12 (15.4)	12	0	0	12
1	8 (10.3)	8	0	0	8
15	8 (10.3)	8	1	1 (SYNF)	10
14	6 (7.7)	6	1	0	7
23	6 (7.7)	6	0	0	6
3	3 (3.8)	3	0	0	3
7	3 (3.8)	2	1	0	3
18	3 (3.8)	3	0	0	3
22	3 (3.8)	2	1	1 (PF)	4
4	2 (2.6)	1	1	1 (SYNF)	3
5	1 (1.3)	1	0	0	1
9	1 (1.3)	1	0	0	1
10	1 (1.3)	0	1	0	1
11	1 (1.3)	1	0	0	1
12	1 (1.3)	0	1	0	1
13	1 (1.3)	1	0	0	1
Group B	1 (1.3)	0	1	0	1
Non typeable	5 (6.4)	5	0	0	5
Total	78	71	11	4	86

SYNF: Synovial fluid, PF: Peritoneal fluid

was also in this serogroup. In contrast, all serotype 1 isolates were sensitive. Fifty percent or more of the isolates from each of the serogroups/serotypes 6, 15, 19 and 23 were of reduced susceptibility. Twenty-six of 42 (62%) isolates that are incorporated in the 7-valent vaccine were resistant to penicillin, while only 9 of 36 (25%) non-vaccine isolates were penicillin resistant ($\chi^2=9.2329$; $P=0.0023$).

Discussion

Numerous epidemiological surveillance reports published in North America, Europe, Asia and Africa refer to the distribution of the capsular type of *S. pneumoniae* isolated from invasive infections and the resistance of these isolates to penicillin.⁹⁻¹⁵ In Saudi Arabia, there is a paucity of such studies. Memish et al recently reported the serotypes of 50 sterile site isolates from the Kingdom.¹⁶ This study was, however, mainly in adults and older children, with only 6 isolates coming from children under 10 years of age.

The most frequent isolates belonged to only 10 serogroups/serotypes (namely, 6 and 19, 1 and 15, 14 and 23, and 3, 7, 18 and 22, in descending order). Although serotype 1 was not isolated from any of our children less than 2 years of age, it was responsible for 33% of the cases in Bedouin children less than 2 years old and 15% of cases among Jewish children in Southern Israel.¹⁰ Additionally although serotype 5 is dominant among isolates from developing countries,¹⁷ it was only rarely encountered (1.3%) in our study or by Memish et al,¹⁶ just as in reports from the U.S.A. and Spain.^{17,18} Our other serogroups/serotypes (19, 6, 1, 14, 23 and 7) are found in developing countries as often as in developed countries. Serotype 14 was more frequently isolated from children less than 2 years old than older children and adults whilst serotype 1 was isolated exclusively in older children, findings that are similar to those of a study in Argentina.¹⁹ Finally, although serotype 15 is encountered in both developing and developed countries, it appears at the low end of the order of frequency in both groups¹⁹ whilst in our study it is second highest in the order of frequency. Obviously, for an effective conjugate vaccine for use in Saudi Arabia, serotype 15 must be included.

Our 6 most common isolates constitute 52 of the total and are distributed in serogroups/serotypes 6, 19, 1, 15, 14 and 23. Of these, 36 (69%) belong to serogroups/serotypes 6, 19, 14, and 23. These four serogroups/serotypes are generally considered less invasive.²⁰ Of those that cause severe disease, only serotype 1 was among the top six serogroups/serotypes in our study and contributed only 11.5% of infections by

Table 2. Serogroup/serotype distribution of the most frequent pneumococci causing invasive diseases in children vs. adult patients in Riyadh.*

Serogroup/serotype	No. children (<15 yrs)	No. adults (≥15 yrs)	Not known	Total
19	4	6	2	12
6	10	1	1	12
15	5	3	—	8
1	4	4	—	8
23	6	0	—	6
14	5	0	1	6
3	2	1	—	3
18	3	0	—	3
7	2	0	1	3
4	2	0	—	2
22	0	2	1	3

* $\chi^2=21.336$; P value = 0.0189 for the difference between children and adults.

Table 3. Coverage by different pneumococcal vaccines of serotypes isolated from invasive infections in Riyadh, 2000-2001.

Category	Total no. of isolates	Number of strains covered (%)			
		23-valent polysacch.	7-valent conjugate	9-valent conjugate	11-valent conjugate
Overall	78	71 (91)	42 (54)	51 (65)	57 (73)
Pediatric (All)	51	48 (94)	30 (59)	35 (69)	39 (76)
Pediatric (<2 Yrs)	27	27 (100)	17 (63)	18 (67)	21 (78)
Adults	20	17 (85)	8 (40)	12 (60)	13 (65)

these six serogroups/serotypes. In general, our isolates in descending order of frequency do not follow either that of developed or developing countries.

Of special public health concern in both developed and developing countries has been the emergence and rapid rise in the proportion of pneumococcal strains exhibiting intermediate or high levels of resistance.^{12,21} Consequently, treatment of invasive pneumococcal disease is becoming increasingly difficult. One of the options left is the prevention of infection, especially in those at high risk of developing invasive disease, with vaccination.

In planning a strategy for developing a pneumococcal vaccine it must be recognized that there are geographic and age-related differences in the incidence of certain serogroups. Consequently, it has been proposed that each serogroup be considered a separate pathogen from an epidemiological standpoint.²² The currently available vaccines are composed of polysaccharides separately derived from the outer capsule of each of 23 strains. These vaccines have been shown to be clinically effective against bacteraemic disease in adults and also to be highly cost-effective when provided as a routine immunization in the elderly. However, these vaccines are insufficiently immunogenic in infants and young children. To improve on these vaccines has led to the development of protein-conjugate vaccines. Because of the complexity of the process, conjugate vaccines currently available contain only 7 to 11 serotypes.³

Although the overall coverage of the 23-polyvalent polysaccharide vaccine was 91% in our study, its use would be of limited value in the community since 72% of our patients with invasive pneumococcal disease are young children under 2 years of age. However, the conjugate vaccines that are recognized as effective in this age group have a rather low coverage for this group in our study. The coverage is only 63%, 67% and 78% for the 7-valent, 9-valent and 11-valent conjugate vaccines, respectively. This finding, however, is not surprising since only 4 of the commonly isolated serotypes in our study are represented in the 7-valent vaccine as against 6 of the 7 most common serotypes in North America.³

However, the small number of isolates limits our estimate of vaccine coverage in this age group. Another limitation in this study is our inability to identify all the isolates to the specific serotypes, but only to the serogroup level. Consequently, this could result in an over-estimation of vaccine coverage. However, in view of the fact that there is some cross-reactivity among serotypes in some serogroups,^{23,24} we believe that our estimates may not be overly optimistic.

Although the formulation of the vaccines may not be optimum in our community, they may still offer satisfactory protection because immunization in children may prevent acquisition of vaccine-type pneumococci,²⁵ and thus help reduce the spread of resistant strains. It is noteworthy that in our study 44% of the strains are of reduced susceptibility to penicillin with 63% of 4 of our most common serotypes (6,15,19 and 23) being resistant to penicillin. Furthermore, the rate of reduced susceptibility of our serotypes represented in the 7-valent vaccine is significantly higher than in the non-vaccine serogroups (P value=0.0023). In light of this high level of penicillin insusceptibility in our strains, particularly the vaccine strains, and based on our estimate of satisfactory protection from the conjugate vaccines (albeit not optimum), we still recommend vaccination to patients at risk in our community (e.g., sickle cell disease patients along with penicillin prophylaxis) until a more effective vaccine becomes available. Further studies of a larger number of patients from all over the country are desperately needed before any pneumococcal conjugate vaccine is introduced in the routine vaccination schedule in this country.

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