Penicillin resistance in serogroups/ serotypes of *Streptococcus pneumoniae* causing invasive infections in Central Saudi Arabia

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ABSTRACT

Objective: To determine the minimum inhibitory concentrations (MICs) of penicillin, ceftriaxone and vancomycin of serogroups/serotypes of *Streptococcus pneumoniae* (*S. pneumoniae*) from invasive diseases in all age groups from major hospitals in Riyadh, Kingdom of Saudi Arabia (KSA).

Methods: All isolates of *S. pneumoniae* from patients with invasive pneumococcal infections between February 2000 and November 2001 were prospectively collected from 8 major hospitals in Riyadh, KSA. The isolates were confirmed as *S. pneumoniae* at the King Khalid University Hospitals, Riyadh, KSA and then serogrouped/serotyped using the agglutination method. The MICs for penicillin, ceftriaxone and vancomycin were carried out using the E-test.

Results: Forty-three percent of the isolates were resistant to penicillin mostly of the intermediate type (97%). The resistant strains were mainly confined to serogroups/ serotypes 6, 23, 19 and 15 and the 7-valent conjugate vaccine covers 76% of the penicillin-resistant strains. Only one isolate was resistant to ceftriaxone.

Conclusion: In view of the rather insignificant level of highly resistant-penicillin strains and the virtual absence of resistance to ceftriaxone we would like to suggest using ceftriaxone for treating invasive pneumococcal infections outside the central nervous system. We recommend that the conjugate vaccine would be a useful adjunct to penicillin prophylaxis in patients at risk in our community.

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Streptococcus pneumoniae (*S. pneumoniae)* is a leading cause of morbidity and mortality worldwide.[1] It is estimated that pneumococcal infections lead to the death of one million children <5 years annually.[2] Before 1967, *S. pneumoniae* strains were considered universally susceptible to penicillin, which was also considered the antibiotic of choice for the treatment of most pneumococcal infections. Although in 1945 Eriksen[3] had demonstrated the ability of *S. pneumoniae* to become penicillin-resistant by exposing strains to increasing concentrations to penicillin, strains with reduced susceptibility to penicillin were not documented until 1965.[4] Since then drug resistant pneumococci have been reported in

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Australia and have emerged as a worldwide problem, most severe in Spain, Hungary and South Africa where as many as half of all pneumococcal isolates have reduced susceptibility to penicillin.[5] Parallel with the spread of penicillin resistance has been the increase in the resistance to other antibiotics often used as second, third, or fourth line of treatment of pneumococcal infection.[6] The more recent identification of cephalosporin-resistant strains is a cause for concern.[5,7] The pattern of antimicrobial susceptibilities in serogroups/serotypes of *S. pneumoniae* in invasive disease in Riyadh, Kingdom of Saudi Arabia (KSA) and for that matter, all over the KSA has not been well documented. Most of the published results from KSA so far have dealt with infections from both sterile and non-sterile sites.[8-12] Only one report from a tertiary center with special patient population, dealt with infections from sterile sites.[13] Since there is such a wide geographical variation in the prevalence of antibiotic resistance in strains of *S. pneumoniae*, careful selection of an effective antibiotic for the initial empirical treatment requires an awareness of patterns of antimicrobial susceptibility in the patient's geographical area. This study conducted over a 22-month period was to document the susceptibility patterns of invasive *S. pneumoniae* isolates to penicillin and ceftriaxone. The isolates were obtained from major hospitals in Riyadh.

Methods. The study was conducted between February 2000 and November 2001 in Riyadh, the capital of KSA, with a population of approximately 5 million of mixed nationals and expatriates. During this period, pneumococcal isolates obtained from normally sterile body sites in patients with invasive pneumococcal disease in 8 major government hospitals in Riyadh were prospectively collected and transported to King Khalid University Hospital (KKUH), laboratory for further study. The contributing hospitals were KKUH with 700 beds, King Abdul-Aziz University Hospital (KAUH) with 120 beds, Riyadh Medical Complex (RMC) with 1,000 beds, Al-Yamama Hospital, Sulaimania Children's Hospital (SCH), King Faisal Specialist Hospital and Research Centre (KFSH&RC) with 500 beds, Prince Salman Hospital and Security Forces Hospital (SFH) with 550 beds. These hospitals serve a population with a wide range of socio-economic 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 same patient were excluded unless the second isolation was >30 days since the first episode.

Statistical analysis was carried out using Stat Pac Gold statistical package. The x^2 test was used for comparing proportions. A $p<0.05$ was considered significant.

Laboratory procedure. Isolates from the various hospitals were subcultured on 5% sheep blood agar (BA) and incubated aerobically and anaerobically in 7% CO_2 at 37°C. α–hemolytic colonies were identified first by Optochin disc (5μg) on BA medium, and all isolates sensitive to the Optochin disc (>12 mm) were further confirmed using the bile solubility test (10% Na desoxycholate). Strains were serotyped using the agglutination method, with standard antiserum (Statens Seruminstitut, Copenhagen, Denmark). An Antibiotic susceptibility test by disc diffusion was carried out according to the guidelines of National Committee on Clinical Laboratory Standards.[14] Resistance to penicillin was represented by a zone size of <19 mm to Oxacillin 1 μg. The minimal inhibitory concentration (MIC) to penicillin, ceftriaxone and vancomycin was carried out using the E-test (AB Biodisk, Sweden) on Isosensitest agar (Oxoid) containing lysed horse blood 5% at 37ºC in 5-7% CO_2.

Definitions. Penicillin resistance was classified as intermediate if MIC>0.1 to 1.0 μg/ml or high if MIC >2 μg/ml and for ceftriaxone resistance was classified as intermediate resistance if MIC >1.0 μg/ml and high resistance if MIC >2.0 μg/ml. Vancomycin was considered susceptible if MIC <1.0 μg/ml.[15]

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 (for example 6B, 23F and so forth) is included in the vaccine formulation. The 23-valent polysaccharide vaccine includes the following 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 (7-V) conjugate vaccine formulation includes the following serotypes (4, 6B, 9V, 14, 18C, 19F and 23F). The 9-valent (9-V) conjugate vaccine formulation is 7-V plus serotypes 1 and 5. The 11-valent (11-V) formulation is 9-V plus serotypes 3 and 7F.

Results. During the study period, there were 78 patients (72% children) with invasive pneumococcal infections in Riyadh. The sources of the isolates were as follows: 63 from blood only, 4 from cerebrospinal fluid (CSF) only, one each from synovial fluid, bone, and peritoneal fluid. In addition there were 7 isolated 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. The contributions of various hospitals to the isolates were as follows: KKUH (22), KAUH (17), RMC (10), Al-Yamama Hospital (9), SCH (8), KFSH (7), Prince Salman Hospital (3) and SFH (2). Thirty-four of the 78 (43%) pneumococcal isolates

were penicillin-resistant (MIC has ranged between 0.125-2.0 μg/ml). The rate of penicillin resistance among the most frequently isolated serogroups/serotypes is shown in **Table 1**. The most noticeable observation of the results is that resistant strains were mainly confined to 4 of the 10 serogroups/serotypes in which resistant isolates were obtained. The 4 serogroups/serotypes were 6, 23, 19 and 15 in descending order of frequency. These 4 serogroups/serotypes contributed to 26 of the 34 typable resistance pneumococcal isolates (76%) with serogroup 6 being the most frequent resistant (26%) isolate. It is also noteworthy that almost all the typable penicillin-resistant strains were of the intermediate level of resistance 33/34 (97%) and only one was highly resistant with a MIC of 2.0 μg/ml. Of further interest also is the fact that all the 6 isolates of serogroup 23 in the study were resistant to penicillin and these included the highly resistant strain as well as the only ceftriaxone-resistant strain. Also of interest is the absence of resistance in serotype 1 (the common serotypes causing invasive disease in our study). There was no resistance to vancomycin. The resistance rate in both children and adults was approximately in the same proportion as in the number of cases. Of the 34 isolates with penicillin resistance, 26 (76%) were from serogroups/serotypes contributing to the 7-V vaccine (p=0.0024) (**Table 2**). Serotype 15 contributed to 5 of the remaining 9 resistant isolates. This table also shows that there will be no significant extra vaccine coverage given by either the 9-V of the 11-V in the penicillin non-susceptible isolates.

Table 1 - Rate of reduced penicillin susceptibility among the most frequent pneumococcal Serogroups/Serotypes causing invasive diseases in Riyadh, Kingdom of Saudi Arabia (2000-2001)*.

Serogroup/ Serotype	Total N of Isolates	Reduced susceptibility n	(%)
6	12	9	(75)
19	12	6	(50)
1	8	0	(0)
15	8	5	(63)
14	6	2	(33)
23	6	6	(100)†
3	3	1	(33)
7	3	0	(0)
18	3	1	(33)
22	3	1	(33)
4	2	1	(50)
9	1	1	(100)
10	1	1	(100)
Total	**68**	**34**	**(50)**

*x^2 = 21.8228, p=0.0053
†one isolate has a high-level resistance

Table 2 - Rate of reduced Penicillin-susceptible strains among *Streptococcus pneumoniae* serotypes included in the currently available conjugate vaccines versus non-vaccines.

Serogroups	7-valent vaccine	9-valent vaccine	11- valent vaccine
Vaccine serogroups			
N isolated	42	51	57
N penicillin-non-susceptible (%)	26 (62)	26 (51)	27 (47)
Non-vaccine serogroups			
N isolated	36	27	21
N penicillin-non-susceptible (%)	9 (25)	9 (33)	8 (38)
x^2 (p-value)	9.23 (0.0024)	1.57 (0.2107)	0.22 (0.6357)

Discussion. Over the past 2 decades, strains of *S. pneumoniae* have become increasingly resistant to penicillin which for a long time was the antibiotic of choice for all pneumococcal infections. For an informed decision on the empiric use of penicillin in such clinical settings, there must be a documentation of, not only the prevalence of penicillin-resistant S. pneumoniae, but also the degree of such resistance, in all areas since the patterns of resistance differ in different geographical areas. In this study, 43% of our invasive pneumococcal strains were penicillin-resistant and this puts Riyadh in the same top bracket of penicillin-resistant pneumococcal strains as isolates from Spain, South Africa and Hungary.[5] This high rate in the study compares favorably with the equally high rate of 51% in an earlier study in Riyadh published in 2002[13] but contrasts sharply with report from other centers in KSA between 1986 to 1999 when penicillin-resistant pneumococci ranged from 0%[8,9] to between 14.6-24.6%.[10-12] As regards ceftriaxone, only one study[13] has reported a moderate level of resistance of 7%; however, the isolates from that report were from one hospital, which dealt with special patient population at high risk for infection with resistant isolates. Out of the 34 isolates that were non-susceptible to penicillin in our study, only one

(2.9%) was highly resistant. In a study in Sao Paulo (Brazil) Berezin et al[16] only one of 10 penicillin non-susceptible strains was highly resistant to penicillin. The overall prevalence rate of the study in Sao Paulo however, was only 9.8% as compared to our study (43%). The interesting finding in our study was that although we had a high prevalence of penicillin-non-susceptible strains similar to countries like Spain and South Africa, only one isolate was resistant to ceftriaxone. In Forward's[6] review of the epidemiology of penicillin resistance in *S. pneumoniae*,[6] he noted that the serotypes most frequently associated with penicillin resistance in the United States of America were 6A, 6B, 19F and 23F constituting 87% of the resistant serotypes. In Canada 19F, 23F, 16A, 6A, 6B and 9V were the prevalent serotypes. In our study the penicillin resistant serogroups/serotypes were mainly confined to 4 viz 6, 23, 19 and 15 and these contributed 79% of the resistance isolates. Of special interest was the fact that all the isolates of serogroup 23 were resistant to penicillin including the only ceftriaxone-resistant strain.

What are the clinical implications of our study? Apparently, the impact of invasive infections by penicillin non-susceptible strains in Riyadh may not be severe. In view of the rather insignificant levels of highly resistant-penicillin strains and the virtual absence of resistance to third generation cephosphorins, specifically ceftriaxone, we would like to suggest the use of high doses of ceftriaxone for invasive pneumococcal infections outside the central nervous system. Our suggestion is based on the fact that beta-lactam antibiotics reach serum levels high enough to overcome such intermediate resistance.[17] We concede that our study size was small and was not designed to enable us to make such a forthright suggestion; however, we were encouraged by the conclusions of Silverstein et al,[17] as well as those of Palleres et al[18] that in settings where the penicillin resistance is rare, clinicians should feel comfortable using conventional beta-lactam therapy without the addition of vancomycin for presumed non-meningitic pneumococcal infections. However, we would also like to recommend a continuous monitoring of susceptibility of pneumococci to ceftriaxone so that a timely decision could be made on whether the addition of vancomycin would ever be necessary. The finding that 26 (76%) of the penicillin non-susceptible isolates in this study are covered by 7-V conjugate vaccine might suggest that using this vaccine in Riyadh could help reduce the risk of invasive infections with penicillin-resistant pneumococci. Additionally, the reduction in colonization with serogroups/serotypes in the vaccine will minimize the spread of these resistant strains. However, the vaccine would have had a greater impact on both the prevalence of penicillin-resistant pneumococcal disease and its spread in Riyadh if the formulation had included serotype 15 since this serotype has a high rate of reduced penicillin-susceptibility (63%) as well as being one of the more common causes of invasive infections in Riyadh.

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