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Implications of *Streptococcus pneumoniae* Penicillin Resistance and Serotype Distribution in Kuwait for Disease Treatment and Prevention[▽]

Eiman M. Mokaddas,* Vincent O. Rotimi, and M. John Albert

Department of Microbiology, Faculty of Medicine, Kuwait University, Jabriya, Kuwait

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Streptococcus pneumoniae **causes serious infections. Treatment is difficult because of the emergence of penicillin resistance in *S. pneumoniae*. Pneumococcal vaccines offer the promise of control and prevention of pneumococcal infections. Serotype prevalence and penicillin susceptibility data for a country will predict the usefulness of the vaccines in that country. In Kuwait, the 23-valent polysaccharide and the 7-valent conjugate vaccines are being used without knowledge of the prevalent serotypes in the country. To obtain the necessary background information, data on penicillin susceptibility and serogroups were obtained from 397 consecutive clinical isolates collected during 2004 and 2005. Two hundred fifty-three isolates (64%) were penicillin resistant, and resistance was significantly higher in patients ≤15 years old and among the upper respiratory tract and eye isolates. The most common serotypes were 23F, 19F, 6A, 6B, 14, and 19A. Among the penicillin-resistant strains, the most common serotypes were 23F, 19F, 6B, 14, and 9A. Among the invasive strains, the most common serotypes were 14, 23F, 19A, and 9V. The polysaccharide vaccine gave 82% coverage against invasive infections in all age groups >2 years. The coverage of the 7-valent conjugate vaccine against invasive serotypes in children ≤2 years old was 55%. This moderate coverage by the conjugate vaccine against invasive infections in children necessitates a revised strategy on the use of the present conjugate vaccine and shows the need for formulation of an improved vaccine for superior coverage for Kuwait and possibly other countries of the Arabian Gulf.**

Successful treatment of *Streptococcus pneumoniae* infections remains a challenge. *S. pneumoniae* is a major cause of community-acquired pneumonia, meningitis, and otitis media in adults and children in developed countries (6, 10, 23). Surveillance data continue to reveal increasing resistance of *S. pneumoniae* to a variety of antimicrobial agents, including penicillins, cephalosporins, macrolides, and quinolones. Currently, in some areas of the world, up to 40% of clinical infections are caused by a pneumococcal strain that is resistant to at least one drug, and 15% are due to strains resistant to three or more drugs (27a). The situation in Kuwait is not different, as indicated by a study in 2001, which revealed that the prevalence of penicillin-resistant *S. pneumoniae* (PRSP) is 63%; almost half of these strains were multidrug resistant (20). Preventive strategies for pneumococcal infection include targeted use of the 23-valent polysaccharide pneumococcal vaccine in individuals older than 2 years of age and routine immunization of infants and children under 2 years of age with the 7-valent polysaccharide-protein conjugate pneumococcal vaccine. The conjugate vaccine is also recommended for children between 2 and 5 years of age who have not already received the vaccine and are also at high risk of severe pneumococcal disease (2, 3; Centers for Disease Control, unpublished data). Prevention of infection also is a key means of combating the spread of antibiotic-resistant strains. A previous study conducted a decade ago, involving a small number of isolates, has given an indication of the prevalent *S. pneumoniae* serotypes in Kuwait (1). Since Kuwait is a country with a large expatriate population and movement of people, there are bound to be temporal changes in the prevalent serotypes. The 23-valent vaccine was introduced in Kuwait in the late 1970s for high-risk individuals more than 2 years old, including those with sickle cell disease, heart disease, bronchopulmonary disease, hepatic disease, diabetes mellitus, and malignancies, and for healthy adults over 65 years old. The 7-valent conjugate vaccine for routine immunization of infants was introduced in Kuwait in August 2006. These vaccines are being used without knowledge of the serotypes circulating in this country. Thus, the usefulness of the pneumococcal vaccination program in Kuwait is not clear. The current study was therefore undertaken to determine the prevalent serotypes and the usefulness of the polysaccharide and conjugate pneumococcal vaccines in Kuwait. The penicillin susceptibilities of isolates were also determined. To attain the status of universal vaccines, the pneumococcal vaccines should be effective in all countries of the world. Therefore, country-specific data on coverage of disease-causing serotypes by vaccines from different parts of the world are required. Data from the Arabian Gulf are sparse.

MATERIALS AND METHODS

Kuwait. Kuwait is a relatively small country of approximately 17,820 km^2 situated in the Arabian Gulf region. It has a population of approximately 3 million people, of which the native population is under 1 million. The remainder are expatriates from different parts of the world working in the oil-rich economy. The population is served by 11 secondary and tertiary care hospitals and 30 primary care clinics.

* Corresponding author. Mailing address: Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait. Phone and fax: (965) 533 2719. E-mail: e.mokaddas@hsc.edu.kw.

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Bacterial strains. A total of 404 *S. pneumoniae* isolates from various infections, such as pneumonia, otitis media, sinusitis, conjunctivitis, meningitis, and septicemia, from patients attending the above-described treatment centers during a period of 2 years, between January 2004 and December 2005, were available for the study. The isolates from blood and cerebrospinal fluid (CSF) were considered invasive, and isolates from upper respiratory tract specimens (antral wash and middle ear fluid), lower respiratory tract specimens (sputum and endotracheal aspirates), and eye were considered noninvasive. There were a large number of isolates from conjunctivitis cases referred to the laboratories. We could not ascertain a reason for this. All isolates were sent in skim milk transport medium to the *S. pneumoniae* Reference Laboratory, Microbiology Department, Faculty of Medicine, Kuwait University, Kuwait, for study. The demographic data for all patients, such as age, sex, and whether inpatient or outpatient, and the sources of the isolates were carefully recorded. In the reference laboratory, the isolates were confirmed as *S. pneumoniae* by positive tests for alpha-hemolysis on blood agar, optochin susceptibility, and bile solubility.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing of isolates against penicillin was done using the Etest method (AB Biodisk, Solana, Sweden) on Mueller-Hinton agar (BBL Microbiology Systems, Becton Dickinson, and Cockeysville, MD) supplemented with 5% defibrinated sheep blood. Inocula were prepared in Trypticase soy broth (Oxoid, Basingstoke, Hampshire, United Kingdom) by suspending pneumococcal colonies grown overnight on sheep blood agar to a density that matched a 0.5 McFarland opacity. Clinical and Laboratory Standards Institute (CLSI) guidelines (5) for broth microdilution were used to determine quality control ranges and interpretation criteria for resistance as recommended by the manufacturer of Etest strips. Control strains *S. pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, *Haemophilus influenzae* ATCC 49247, and *Enterococcus faecalis* ATCC 29212 were tested in parallel with each run as recommended by CLSI (5).

Definition of susceptibility. A strain was considered penicillin susceptible if the MIC was <0.06 μg/ml, intermediately resistant if the MIC was 0.12 to 1.0 μg/ml, and fully resistant if the MIC was ≥ 2 μg/ml.

Serogrouping and serotyping. Serogrouping and serotyping of the isolates were done by the Quellung reaction with a complete set of specific rabbit pneumococcal antisera in the Danish checkerboard typing system (Statens Serum Institute, Copenhagen, Denmark) as previously described by Skov Sorensen (25).

Statistical analysis. Differences in prevalence of resistance between two groups were assessed by the chi square test. A P value of ≤ 0.05 was considered significant.

RESULTS

Out of the 404 isolates, 397 (98.3%) were available for this study. The demographic data for patients and the origins of isolates and their penicillin resistance are shown in Table 1. The male-to-female ratio was 1.5:1, and the majority of the patients were children <5 years old (30%) and elderly persons ≥ 65 years old (28%). The isolates from the ≤ 5-year age group had a higher prevalence of penicillin resistance than those from the 16- to 64-year age group ($P < 0.001$). Similarly, the isolates from the 6- to 15-year age group had a higher prevalence of penicillin resistance than those from the 16- to 64-year age group ($P \leq 0.05$). The differences between the other age groups were not significant. The prevalence of penicillin resistance among the upper respiratory tract isolates was significantly higher than that among the lower respiratory tract and blood isolates ($P < 0.025$ for both comparisons). The eye isolates had a significantly higher prevalence of penicillin resistance than those from the lower respiratory tract ($P \leq 0.01$). The differences between the other sites were not significant. Forty-one percent and 43% of the invasive isolates were in the pediatric age group of <15 years and in the elderly age group of >65 years, respectively.

The distribution of the serotypes of 397 invasive and noninvasive isolates and their penicillin susceptibilities according to different age groups are shown in Table 2. Among these isolates, 329 (82.9%) could be serotyped and the remainder were either nontypeable or autoagglutinating. The distributions of the predominant serotypes among the four age strata were similar, and the serotypes were 23F, 19F, 6A, 6B, 14, and 19A. For all these serotypes, the numbers of invasive isolates were smaller than those of noninvasive isolates. For the total number of patients, the most common serotypes among the invasive isolates, in descending order, were 14, 23F, 19A, and 9V, whereas those among the noninvasive isolates were 23F, 19F, 6A, 6B, 19A, 14, 9A, 9V, and 11A. Among the penicillin-resistant strains, the most common serotypes were 23F, 19F, 6B, 14, and 9A.

TABLE 1. Penicillin resistance of *S. pneumoniae* isolates by age, sex, and site of infection

Patient characteristic	No. (%) of isolates:			
	Total	Resistant to penicillin[a]	Invasive	Noninvasive
Age (yr)				
≤5	120	85 (71)	14 (12)	106 (88)
6–15	73	50 (68)	4 (5)	69 (95)
16–64	92	48 (52)	7 (8)	85 (92)
≥65	112	70 (63)	19 (17)	93 (83)
Sex				
Male	238	154 (65)	27 (11)	211 (89)
Female	159	99 (62)	17 (11)	142 (89)
Site of infection				
Upper respiratory tract	146	95 (65)		
Lower respiratory tract	97	48 (61)		
Blood	42	19 (45)		
CSF	2	2 (100)		
Eye	115	81 (70)		
Other	13	8 (62)		

[a] Differences in penicillin resistance between the ≤5-year and 16- to 64-year age groups ($P \leq 0.001$), between the 6- to 15-year and 16- to 64-year age groups ($P \leq 0.05$), between the upper respiratory and lower respiratory tract isolates ($P \leq 0.025$), between the upper respiratory tract and blood isolates ($P \leq 0.025$), and between the eye and lower respiratory tract isolates ($P \leq 0.01$) are significant. Other comparisons are not significant.

The various scenarios regarding the coverage of *S. pneumoniae* serotypes in different infections and age groups are shown in Table 3. The coverage of serotypes from invasive infections for ages above 2 with the 23-valent polysaccharide vaccine was 82%, while the coverage of invasive infections in children below 2 years of age and in children up to 5 years of age with the 7-valent conjugate vaccine was 55% and 62%, respectively.

DISCUSSION

In our study, the prevalence of PRSP isolates exceeded 60% in the pediatric and elderly populations. Similar results have been reported from Saudi Arabia (19) and the United States (8, 9). Similar to the results from the surveillance study in the United States (8, 9), PRSP was seen less in blood and other sterile body fluids, and the prevalence of resistant strains was highest in isolates from the upper respiratory tract. This is different from a Saudi Arabian study in 2004, where more resistant isolates were seen in invasive infections (19). In stud-

TABLE 2. Distribution of invasive and noninvasive *S. pneumoniae* serotypes and their penicillin susceptibility status according to different patient age strata

Age (yr)	Total no. of isolates	Serogroup	No. of isolates (no. invasive, no. noninvasive)		
			Susceptible	Intermediate	Fully resistant
<2	8	6A	2 (0, 2)	6 (1, 5)	0
	6	6B	0	5 (1, 4)	1 (0, 1)
	4	9A	1 (0, 1)	3 (1, 2)	0
	6	14	1 (0, 1)	5 (2, 3)	0
	5	19A	3 (1, 2)	2 (1, 1)	0
	23	19F	1 (0, 1)	21 (1, 20)	1 (0, 1)
	20	23F	1 (1, 0)	17 (2, 15)	2 (0, 2)
	4	Nontypeable	1 (0, 1)	3 (0, 3)	0
	3	Autoagglutinating	2 (0, 2)	1 (0, 1)	0
2–5	3	6A	3 (1, 2)	0	0
	4	6B	1 (0, 1)	3 (0, 3)	0
	2	9A	2 (0, 2)	0	0
	3	14	0	3 (1, 2)	0
	5	19A	3 (1, 2)	2 (0, 2)	0
	8	19F	0	6 (0, 6)	2 (0, 2)
	8	23F	0	6 (0, 6)	2 (0, 2)
	4	Nontypeable	2 (0, 2)	2 (0, 2)	0
	4	Not agglutinable	0	4 (0, 4)	0
6–15	9	6B	0	9 (0, 9)	0
	4	9A	1 (0, 1)	3 (0, 3)	0
	6	14	0	6 (2, 6)	0
	11	19A	6 (0, 6)	5 (1, 4)	0
	12	19F	0	10 (0, 10)	2 (0, 2)
	24	23F	0	22 (1, 21)	2 (1, 1)
	4	Nontypeable	1 (0, 1)	3 (0, 3)	0
	3	Autoagglutinating	1 (0, 1)	2 (0, 2)	0
>15	7	1	7 (3, 4)	0	0
	7	3	7 (1, 6)	0	0
	3	4	3 (2, 1)	0	0
	5	5	5 (2, 3)	0	0
	23	6A	15 (1, 14)	8 (0, 8)	0
	9	6B	0	7 (1, 6)	2 (0, 2)
	1	7B	0	1 (0, 1)	0
	2	7F	2 (0, 2)	0	0
	3	8	3 (0, 3)	0	0
	6	9A	0	6 (0, 6)	0
	3	9L	2 (0, 2)	1 (0, 1)	0
	1	9N	1 (0, 1)	0	0
	12	9V	0	10 (2, 8)	2 (2, 0)
	4	10A	3 (1, 2)	1 (1, 0)	0
	11	11A	7 (0, 7)	4 (0, 4)	0
	1	11C	1 (1, 0)	0	0
	2	13	2 (0, 2)	0	0
	7	14	0	5 (0, 5)	2 (2, 0)
	4	15A	0	4 (3, 1)	0
	2	15B	0	2 (0, 2)	0
	7	15C	0	7 (0, 7)	0
	1	16F	1 (0, 1)	0	0
	3	17F	3 (1, 2)	0	0
	3	18C	3 (0, 3)	0	0
	2	19B	1 (0, 1)	1 (0, 1)	0
	4	19F	0	4 (1, 3)	0
	3	20	3 (1, 2)	0	0
	1	22F	1 (0, 1)	0	0
	5	23A	3 (0, 3)	2 (0, 2)	0
	3	23F	1 (0, 1)	0	2 (0, 2)
	2	28A	2 (0, 2)	0	0
	1	29	0	1 (0, 1)	0
	1	31	1 (0, 1)	0	0
	1	33F	1 (0, 1)	0	0
	5	34	5 (0, 5)	0	0
	1	35	1 (0, 1)	0	0
	1	37	1 (0, 1)	0	0
	1	47	1 (0, 1)	0	0
	20	Nontypeable	11 (0, 11)	7 (0, 7)	2 (0, 2)
	26	Autoagglutinating	15 (0, 15)	11 (0, 11)	0

ies carried out in Austria (22) and Uruguay (15), the prevalence of PRSP among invasive isolates was low. In another study carried out in Uruguay and Argentina (7), where invasive pneumococcal disease in hospitalized children 5 years old and younger was surveyed, 36% had PRSP, of which more than half had high-level resistance. Although the number is very small, both the CSF isolates in the current study were penicillin resistant. In a previous study from Kuwait (1), 3 of 7 CSF isolates (43%) were penicillin resistant.

TABLE 3. Coverage of *S. pneumoniae* serotypes in Kuwait by pneumococcal vaccines

Age (yr) and vaccine	*S. pneumoniae* serotypes	% coverage by vaccine[a]
≥2, 23-valent[b]	All	64
	Invasive	82
	Penicillin resistant	76
<2, 7-valent[c]	All	72
	Invasive	55
	Penicillin resistant	82
<5, 7-valent	All	69
	Invasive	62
	Penicillin resistant	80

[a] Coverage was calculated with autoagglutinating strains excluded.
[b] Polysaccharide vaccine.
[c] Conjugate vaccine.

Our study also showed that invasive diseases were seen mainly at the extremes of age. This is similar to the finding of a study in Riyadh, Saudi Arabia (27), where 72% of invasive *S. pneumoniae* isolates were from children.

The predominant serotypes in Kuwait reported 7 years ago (1) and in the present study are essentially similar (23F, 19F, 6A, 19A, and 14), indicating no change in the major serotype distribution in the intervening period in Kuwait. This serotype distribution is similar to the distribution reported by Twum-Danaso et al. (27) in Riyadh, Saudi Arabia, for the period 2000 and 2001. However, another study covering three major provinces of Saudi Arabia, including the central province where Riyadh is located, during 2000 reported a different distribution of serotypes (19). In that study, serotypes 19F, 9A, 4, and 14 were the most frequently isolated serotypes among the invasive strains, and serotypes 4, 6A, and 9V were the most frequently isolated serotypes among the noninvasive strains. The number

of isolates was too small to identify geographical predominance of serotypes. That study showed that the distribution of serotypes might vary for different regions of a large country like Saudi Arabia. Another report that is concordant with ours is from Turkey (29), where the predominant serotypes were 19, 23, 9, and 14. A study in England and Wales (26) found a slightly different order of predominance of the serotypes, i.e., 14, 9, 1, 19, 6, 4, 23, 3, 8, 12, and 18, in invasive pneumococcal disease in all age groups.

In our study, the serotype distribution of PRSP was essentially similar to the distribution among the susceptible strains. However, a few serotypes, i.e., 15A, 15B, and 15C, were found mostly in the PRSP group. Additionally, serotype 23F comprised 36% of all the fully resistant *S. pneumoniae* isolates, similar to the results of the previous study from Kuwait (1). On the other hand, in the study from Riyadh, Saudi Arabia (27), serogroup 6 was the predominant serotype among the resistant isolates. Most of the typeable strains in the Saudi Arabian study had intermediate resistance to penicillin, as was the case in the present study; the only fully resistant isolate was in serogroup 23F. In that Saudi Arabian study, serogroup 1 was associated with a significant number of invasive diseases, but no serogroup 1 isolate was penicillin resistant. Our finding is, however, slightly different from reports of other studies from Saudi Arabia (11, 19), which showed that serotype 23F was not common among the PRSP isolates. However, the predominant serotypes in both of these Saudi Arabian studies (11, 19) were 9V, 6A, 15B, and 19F. A study from Turkey in 2003 (29) reported the predominance of serotype 9. A study from South Africa (17) found that serotypes 6, 19, and 14 comprised 92% of PRSP isolates from blood and CSF, a finding very similar to ours.

Ideally, the pneumococcal serotypes included in the conjugate vaccines should be selected to cover the strains that cause most invasive pneumococcal diseases in children throughout the world (12, 13). In Kuwait, the 7-valent conjugate vaccine, which includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, was introduced recently. However, the 23-valent polysaccharide vaccine is currently available in Kuwait for high-risk groups of patients only. The data from our study have demonstrated that there is a good coverage (76 to 82%) of strains, including the resistant and invasive isolates from ages above 2 years, by the polysaccharide vaccine. Superior coverage rates of 96.1% (17) and 97.4% (15) were demonstrated for the 23-valent vaccine in South Africa and Uruguay, respectively. Furthermore, our data showed coverage rates of 82% in children below 2 years of age and 80% in children up to 5 years of age by the 7-valent conjugate vaccine against PRSP. This appeared to be similar to the data from Turkey, where the 7-valent vaccine showed very good coverage against PRSP (29). Similarly, a Saudi Arabian study (27) reported a coverage rate of 76% for penicillin-resistant isolates.

On the other hand, our data showed a relatively low coverage rate of 55% in children under 2 years of age and a moderate coverage rate of 62% in children up to 5 years of age by the 7-valent conjugate vaccine against invasive infections. In a study by Fouda et al. (11) from Saudi Arabia, a similar coverage rate of 55.5% was reported for the 7-valent conjugate vaccine against invasive infections. However, other studies (16, 18, 24, 26, 27) have reported variable coverage rates, ranging from low (26%) to high (95%), for invasive pneumococcal isolates.

As children less than 2 years of age do not develop immunity following vaccination with the 23-valent polysaccharide vaccine, they are the real targets for the more effective conjugate vaccine in preventing invasive pneumococcal disease. The implication of the moderate coverage of the conjugate vaccine in Kuwait is that many children below the age of 2 years will become infected despite vaccination. Therefore, the current policy of introducing the 7-valent conjugate vaccine as part of the national immunization program in Kuwait needs reevaluation in the light of our findings.

It is obvious that the currently licensed conjugate pneumococcal vaccine offers various degrees of coverage against invasive pneumococcal infections in different parts of the world. This is worrying. It appears that we need country-specific conjugate vaccines to offer better coverage against the circulating serotypes. This is not possible from an economic point of view, since for vaccine manufacturers, making small doses of country-specific vaccines is not profitable. An alternative would be to have region-specific vaccines to cover the serotypes across many countries of the region to cater to larger populations, thus making the vaccine-making enterprise economically worthwhile. Comparison of serotypes in Saudi Arabia and Kuwait shows the prevalence of similar serotypes in these neighboring countries. Similar serotype prevalence surveys need to be extended to other countries of Gulf Co-operation Council (which includes Kuwait, Saudi Arabia, Bahrain, Qatar, Oman, and the United Arab Emirates) to ascertain whether the serotypes are similar enough to warrant the production of a conjugate vaccine specific for these countries. Such surveys are under way in these countries, and even though preliminary data from Qatar and Oman are available, the numbers of isolates are too small to make meaningful comparisons (14).

While the percent coverage of serotypes causing invasive disease can contribute importantly to country-specific estimates of the direct impact of vaccine, it is not reliable when used as the sole indicator of anticipated conjugate vaccine impact. For example, even though the serotypes represented in the 7-valent conjugate vaccine are less for invasive diseases in indigenous populations than in the general populations in the United States and Australia, the impact of reducing the incidence of invasive diseases was greater in the indigenous populations than in the general populations (3, 21, 28). This would indicate an emphasis on vaccine-attributable reduction in disease after vaccination rather than on coverage of serotypes in the vaccine against serotypes causing disease. Nevertheless, a formulation with a serotype composition more suited to local needs would be expected to provide even greater benefit (4).

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