

The Prevalence of Antimicrobial Resistance and Serotypes of *Streptococcus pneumoniae* in the Kingdom of Bahrain

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Abstract

Streptococcus pneumoniae (*S. pneumoniae*), also known as pneumococcus, is found in the upper respiratory tract of 5-70% of healthy people. It causes otitis media, pneumonia and invasive pneumococcal diseases (IPD). The group with the highest risk for pneumococcal diseases are children, elderly people and people with chronic systemic illnesses. The identification of these serotypes is crucial to monitor the prevalent serotypes, to track resistance patterns, and to evaluate the efficacy of treatment and vaccination. Our aim was to determine antibiotic resistance in *S. pneumoniae*, identify the most common serotypes, determine the association between highly resistant strains and most common serotypes, and select an effective vaccine against the predominant serotypes in the Kingdom of Bahrain. One hundred isolates were included in the study. Antimicrobial susceptibility was determined by agar dilution and serotypes were determined by the Quellung reaction. Antimicrobial resistance rates were highest for penicillin in children \leq 5 years old (34%) and elderly patients (29%). For the serotypes distribution of *S. pneumoniae*, the predominant serotypes were 19, 6, 23, 3 and 14 in the different age groups. The predominant serotypes that showed resistance to three antimicrobial agents were 19, 6, 23 and 14. The increasing pneumococcal resistance to penicillin, erythromycin, and tetracycline indicates the need for preventive measures, antibiotics use monitoring and stewarding. In light of the expanding prevalence of *S. pneumoniae* resistant to multiple antimicrobial agents, the need for an effective pneumococcal vaccine has become very important.

Keywords: Antimicrobial resistance, Pneumococcal vaccine, Serotypes, *Streptococcus pneumoniae*

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INTRODUCTION

Streptococcus pneumoniae also known as pneumococcus, is found in the upper respiratory tract of 5-70% of healthy people. It causes otitis media, pneumonia, sinusitis and also invasive pneumococcal diseases (IPD)^{1,2}.

The serotypes identification is crucial to monitor the prevalent serotypes, resistance patterns, and to evaluate the efficacy of treatment and vaccination³. Serotype distribution in different countries is shown in Table 1.

Table 1. Serotype distribution in different countries⁴

Serotype	Canada (n = 15)	U.K. (n = 9)	Spain (n = 61)	Sweden (n = 223)	U.S. (n = 46)	Total (n = 354)
1		22	2	4	2	3
3		11	16	6	15	9
4				7	11	6
5			2	<1	9	2
6A				3		2
6B			2	4	4	3
7F	13		5	10	2	8
8	7		5	1	2	2
9N				3		2
9V	7	11	3	13	9	11
11A			3	1	2	2
12F			5	<1	2	1
14	53	22	11	16	7	15
18C	7	11	8	1	2	3
19A	7		7	5	2	5
19F		11	7	3	2	3
22F			2	4	7	3
23F			8	10	11	9
Other	7	11	15	9	11	10

Note: Blank cells indicate that <5 isolate were found.

Although no data on serotypes are available, yet the use of pneumococcal vaccines is common in the Kingdom of Bahrain. This present study was aimed to determine the serotypes and antibiotic susceptibility to penicillin, erythromycin, and tetracycline used for the treatment of pneumococcal infection.

MATERIALS AND METHODS

Bacterial isolates

A total of 100 *S. pneumoniae* isolates from patients were evaluated after getting an ethical approval from Arabian Gulf University. There were sixty-two isolates from Salmaniya Medical Complex (SMC) and thirty-eight isolates from Bahrain Defence Force Hospital (BDF), that were isolated from inpatients and outpatients with various infections, such as pneumonia, otitis media, sinusitis, conjunctivitis, meningitis, and

septicemia. Isolates from blood or cerebrospinal fluid (CSF) were considered invasive, and isolates from upper respiratory tract samples (e.g., ear swab), lower respiratory specimens (e.g., sputum, and deep tracheal aspirates), eye swab, pus, anal swab or wound fluids were considered noninvasive. The demographic data for all patients such as sex, age, and nationality, as well as clinical diagnosis and the sources of the isolates were recorded. All collected strains were taken as subculture isolates from hospital lab on sheep blood agar plates and incubated at 37°C for 18-24 hours in the presence of 5% CO₂. Several colonies of the bacterial culture were then picked, placed in skimmed milk, and stored at -80°C until further testing. The isolates were confirmed as *S. pneumoniae* by positive tests for alpha-hemolysis on blood agar, optochin susceptibility, and bile solubility.

Study design

It was a cross sectional study. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used.

Antibiotic sensitivity testing

The dilution of antimicrobial agents was made in δH_2O . Two ml of the previous antimicrobial concentration was poured into the Petri dishes. Then 18 ml of Mueller Hinton agar (MHA) with sheep blood was poured into plates. After that, plates to be used were left at room temperature overnight to dry. The colonies were emulsified in Mueller Hinton broth where the suspension optical density was adjusted to 0.5 of McFarland standards. Then 2 μ l of the colony suspension was inoculated as a spot onto the surface of each agar plate. The *S. pneumoniae* ATCC 49619 strain was used as quality control for testing. The plates were incubated at 37°C in a CO₂ incubator for 18-24 hours. The strains and sensitivity testing (MIC) were performed according to Clinical and Laboratory Standards Institute guidelines (CLSI), see Table 2⁵.

Serotyping

Pneumococcal serotypes were determined using the Quellung reaction with the Pneumotest kit which contains 12 pneumococcal

Table 2. Antimicrobial breakpoints for *S. pneumoniae*

Antimicrobial	Breakpoints		
	Sensitive (S)	Intermediate (I)	Resistance (R)
Penicillin	MIC ≤ 0.06	MIC= 0.122-1	MIC ≥ 2
Erythromycin	MIC ≤ 0.25	MIC= 0.5	MIC ≥ 1
Tetracycline	MIC ≤ 2	MIC= 4	MIC ≥ 8

*Antimicrobial breakpoints (minimum inhibitory concentration MIC (μg/ml)) for *S. pneumoniae* based on the guidelines of the Clinical and Laboratory Standard Institute⁶.

pool antisera produced by the Statens Serum Institut, Copenhagen, Denmark⁷.

Statistical analysis

The chi-square test was used to identify differences in prevalence of antimicrobial resistance. A p-value of < 0.05 was considered significant.

RESULTS

The demographic data for patients is shown in Table 3. The male-to-female ratio was 1.4:1. Many of the patients were children ≤ 5 years old (44%), the next biggest group was of patients 16–64 years of age (34%). There was no statically significant difference between the age groups in this study.

Table 3. Antimicrobial resistance of *S. pneumonia* isolates by age, sex, and site of infection

Patient characteristics	No. (%) of isolates				Invasive	Noninvasive
	Total	Resistant to penicillin	Resistant to erythromycin	Resistant to tetracycline		
Age (yr)						
≤ 5	44	15 (34)	17 (39)	14 (32)	10 (34)	34 (77)
6 – 15	5	1 (20)	3 (60)	3 (60)	1 (20)	4 (80)
16 – 64	34	9 (26)	11 (32)	12 (35)	7 (21)	27 (79)
≥ 65	17	5 (29)	6 (35)	5 (17)	5 (17)	12 (71)
Sex						
Male	58	17 (29)	21 (36)	19 (33)	14 (24)	44 (76)
Female	42	13 (40)	16 (38)	15 (36)	9 (21)	33 (79)
Site of infection						
Eye	28	7 (25)	8 (29)	7 (25)		
Lower respiratory tract	24	8 (33)	9 (38)	9 (38)		
Blood	21	4 (19)	9 (43)	9 (43)		
Upper respiratory tract	16	8 (50)	8 (50)	6 (38)		
Other	10	2 (20)	2 (20)	2 (20)		
CSF	1	1 (100)	1 (100)	1 (100)		

The predominant serotypes were 19, 6, 23, 3, and 14

Table 4. Distribution of serotypes (3, 6, 14, 19, 23) of invasive and noninvasive *S. pneumoniae* and their antimicrobial resistance according to different age groups. *, †, ‡, §, ¶, **, ††, †††

Age (year)	Total no. of isolates	Serotypes	No. of isolates (no. invasive, no. noninvasive)									
			*PS	†PI	‡PR	§ES	EI	¶ER	**TS	††TI	†††TR	
≤5	2	3	2 (1,1)	0	0	2 (1,1)	0	0	2 (1,1)	0	0	
	2	23	2 (0,2)	0	0	2 (0,2)	0	0	2 (0,2)	0	0	
	3	Non-typeable	1 (0,1)	0	2 (0,2)	3 (0,3)	0	0	3 (0,3)	0	0	
6-15	4	14	1 (0,1)	1 (1,0)	2 (0,2)	2 (0,2)	0	2 (1,1)	2 (0,2)	0	2 (1,1)	
	7	6	2 (1,1)	2 (2,0)	3 (1,2)	1 (1,0)	0	6 (3,3)	3 (2,1)	0	4 (2,2)	
	11	19	3 (0,3)	3 (1,2)	5 (1,4)	7 (1,6)	0	4 (1,3)	7 (1,6)	0	4 (1,3)	
	1	3	1 (0,1)	0	0	1 (0,1)	0	0	1 (0,1)	0	0	
	1	14	0	1 (0,1)	0	0	0	1 (0,1)	0	0	1 (0,1)	
16-64	1	19	0	0	1 (0,1)	0	0	1 (0,1)	0	0	1 (0,1)	
	2	Non-typeable	1 (0,1)	1 (0,1)	0	2 (0,2)	0	0	2 (0,2)	0	0	
	3	14	1 (0,1)	1 (0,1)	1 (0,1)	1 (0,1)	0	2 (0,2)	1 (0,1)	0	2 (0,2)	
	4	6	2 (0,2)	2 (2,0)	0	3 (1,2)	0	1 (1,0)	3 (2,1)	0	1 (1,0)	
	5	3	5 (1,4)	0	0	5 (1,4)	0	0	5 (1,4)	0	0	
≥65	5	19	1 (1,0)	2 (0,2)	2 (1,1)	3 (1,2)	0	2 (1,1)	2 (1,1)	0	3 (1,2)	
	5	23	2 (0,2)	0	3 (0,3)	2 (0,2)	0	3 (0,3)	2 (0,2)	0	3 (0,3)	
	1	14	1 (0,1)	0	0	1 (0,1)	0	0	1 (0,1)	0	0	
	2	3	2 (0,2)	0	0	2 (0,2)	0	0	2 (0,2)	0	0	
	4	23	0	1 (0,1)	3 (1,2)	0	0	4 (1,3)	2 (0,2)	0	2 (1,1)	
	5	6	2 (1,1)	2 (1,1)	1 (0,1)	3 (2,1)	0	2 (0,2)	3 (2,1)	0	2 (0,2)	

*PS: Penicillin-sensitive §ES: Erythromycin-sensitive ** TS: Tetracycline-sensitive
 †PI: Penicillin-intermediate ||EI: Erythromycin-intermediate †† TI: Tetracycline-intermediate
 ‡PR: Penicillin-resistant ¶ER: Erythromycin-resistant †††TR: Tetracycline-resistant

Penicillin resistance was higher among upper respiratory tract than among blood isolates. The 100 pneumococcal isolates were first tested for antimicrobial susceptibility. The sources of the isolates, and their antimicrobial resistance pattern are shown in Table 3. The prevalence of penicillin-resistant isolates in this study was high in children ≤ 5 years old (34%) and elderly patients (29%). For erythromycin resistance, high rates were found in patients between 6–15 years old (60%) with only five isolates which is a very small group and in children ≤ 5 years old (39%). For tetracycline resistance, high prevalence rate was found in the patients between 6-15 years old (60%) again with only five isolates which is a very small group and in patients between 16–64 years old (35%). There was no significant difference in resistance for any of the antibiotics between the age groups. The prevalence of penicillin resistance among the upper respiratory tract isolates (8 of 16) was significantly higher than that among the blood isolates (4 of 21) (p=0.046). Among the 100 isolates, 39 were sensitive to all the three antibiotics and 26 isolates showed resistance to more than one antibiotic. No differences were found in antibiotics resistance between upper and

lower respiratory tract infections. The antibiotics resistance between invasive and noninvasive isolates was also not significantly different.

Of the isolates, 95 (95%) were serotyped and 5 (5%) were unidentifiable. The predominant serotypes among all patients were the serotypes 19 (n=17), 6 (n=16), 23 (n=11), 3 (n=10), and 14 (n=8) is shown in Table 4. The distribution of these serotypes were not different between the age groups. The strains with serotypes 19, 23, 6, and 14 were most often resistant to antibiotics, while of the five most common strains the one with serotype 3 was never found to be resistant to antibiotics.

All serotypes of invasive infections in children are included in the vaccines

The prevalence of *S. pneumoniae* serotypes in different infections as per the age group in children is shown in Table 5. The PPV23 vaccine given to individuals of ages >2 and <65 years in high risk groups includes the 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 serotypes of the isolates of invasive infections observed in children > 2 years old were covered by the PPV23 vaccine. Also, in the groups of at

Table 5. *S. pneumoniae* serotypes identified in children and their coverage by pneumococcal vaccines

Age (yr) and vaccines	<i>S. pneumoniae</i> isolates (n=number of strains)	coverage by vaccine	non-coverage by vaccine (non covered serotypes)
≥ 2, PPV23	All (n=100)	91.4 %	8.6 % ((16, 36, 37), (21, 39), (24, 31, 40) and 3 strains were non-typeable)
	Invasive (n=23)	100 %	-
	Penicillin-resistant (n=30)	95.2 %	4.8 % (one strain was non-typeable)
	Erythromycin-resistant (n=37)	100 %	-
	Tetracycline-resistant (n=34)	100 %	-
< 2, PCV13	All	66.7 %	33.3 % (10, 11, two strains were 15, 17, 20, 22, (24, 31, 40), and two strains were non-typeable)
	Invasive	100 %	-
	Penicillin-resistant	77.8 %	22.2 % (11 and one strain was non-typeable)
	Erythromycin-resistant	88.9 %	11.1 % (20)
	Tetracycline-resistant	85.7 %	14.3 % (20)
≥ 2 and < 5, PCV13	All	69 %	31 % (10, 11, three strains were 15, 17, 20, 22, (21, 39), (24, 31, 40) and three strains were non-typeable)
	Invasive	100 %	-
	Penicillin-resistant	78.6 %	21.4 % (11 and two strains were non-typeable)
	Erythromycin-resistant	87.5 %	12.5 % (15 and 20)
	Tetracycline-resistant	84.6 %	15.4 % (15 and 20)

risk children below 2, and healthy children up to 5 years old, the vaccine PCV13, that includes the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F covered all of the serotypes causing invasive infections in these children. PPV23 covered also the serotypes that were antibiotic resistant. The vaccine coverage of nontypeable strains are not known.

Table 6. Common serotypes in all age groups

Serotypes	Total number of isolates (n)
14	9
3	10
23	11
6	16
19	17

DISCUSSION

This is the first study of the kind in the Kingdom of Bahrain to determine *S. pneumoniae* serotypes. The most common serotypes in all age groups were 19, 6, 23, 3 and 14 which is shown in Table 6.

Similar results were reported in a Saudi Arabian study in the period between 2000 to 2001⁸, in Kuwait⁹, Oman¹⁰, and Turkey¹¹. In contrast, different predominant serotypes were found in some European countries such as Denmark like 1, 4, 14, 6A + 6B, 7F, 9V, 3, 12F, and 8¹² and Germany like 19F, 23F, and 6B¹³.

This study is different from ones conducted in the Kingdom of Saudi Arabia¹⁴ and Kuwait⁹, where the most common serotypes were 6, 19, 1 and 3. Similar results to the current study were obtained in Burkina Faso¹⁵ as well as in England and Wales¹⁶ for the serotypes 6, 19, 1 and 3. The same study in England and Wales gave a different result for the serotypes (14, 9, 4, 23, 3, 12 and 18). Serotypes 19, 23, 3, 6 and 14 were the predominant serotypes among the noninvasive isolates in all age groups in our study. Similar results were found for some serotypes (3 and 6A) in the Kingdom of Saudi Arabia¹⁴, Kuwait (23F, 19F, 6A, 6B and 14)⁹ and a study done in Thailand (2007) reported similar serotypes (19, 6 and 23)¹⁷.

The prevalence of penicillin-resistant isolates in this study was high in children ≤ 5 years old (34%) and in elderly patients (29%). Similar results were found in studies from Kuwait⁹, US¹⁸, and Caledonia¹⁹. For erythromycin resistance, the highest rates were found in patients between

6–15 years old (60%) and in children ≤ 5 years old (39%). This is similar to the result from US²⁰ but different from results reported in Thailand (2005), where more resistant isolates were seen in the age group < 6 years (37.9%) and between 15–60 years old (27.5%)²¹. For tetracycline resistance, the highest prevalence rates were found in the patients between 6–15 years old (60%) and in patients between 16–64 years old (35%). In Spain, a similar finding was reported for the adults²².

According to the site of infection, in this study, the highest prevalence rate for penicillin- and erythromycin-resistant strains were found in upper respiratory tract isolates, similar result for penicillin were reported in Kuwait⁹ and US¹⁸. Another study from Saudi Arabia in 2004¹⁴, Iran²³, and Taiwan²⁴ showed highest resistance rates in blood and CSF isolates. But those studies were different from the current one because highest prevalence was found in blood and CSF isolates. For tetracycline resistance, the highest prevalence was found in blood, which is different from UAE²⁵ where the highest rate was seen in lower respiratory tract isolates. This study also found that invasive infections were found mainly in the group of children ≤ 5 years old (34%). Similar findings were reported in the Kingdom of Saudi Arabia⁸, Kuwait⁹, and Spain²².

When looking at antibiotic resistant strains, resistance was most commonly found for the three antibiotics in the serotypes 19, 6, 23 and 14. In Kuwait, a study was conducted in which similar results were obtained for penicillin-resistant serotypes (23F, 19F, 6B and 14)⁹. On the other hand, in the Kingdom of Saudi Arabia, a study showed that the main serotypes among the multidrug-resistant isolates were 19F, 23F and 6A¹⁴. In Turkey¹¹ and South Africa²⁶, also comparable researches were performed. The most frequently encountered serotypes were 6, 19, 1, 23, and 14 in Turkey and 6, 19, 14, and 23 in South Africa.

In the Kingdom of Bahrain, the 7-valent conjugate vaccine, which includes serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, was used to cover the strains that cause most invasive pneumococcal diseases in children. Recently, 13-valent conjugate vaccine, which includes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, was introduced to cover more pneumococcal serotypes. Also, the 23-valent

polysaccharide vaccine (PPV23) is available in the Kingdom of Bahrain for high-risk groups such as HIV positive individuals, sickle cell disease patients and elderly above 65 years old. This study demonstrated that PPV23 had an excellent coverage (91.4%) for all strains, with even 100% coverage for invasive, erythromycin resistant, and tetracycline resistant isolates and high coverage (95.2%) for penicillin-resistant isolates for children ≥ 2 years old. Our results demonstrated higher coverage rates by PPV23 for invasive and penicillin-resistant strains than in Kuwait (76 to 82%)⁷. Also, high coverage was reported in South Africa (97.4%)²⁶ and Uruguay (96.1%)²⁷.

For PCV13 coverage for children < 2 years old, the vaccine showed good coverage rates for all serotypes (66.7%), penicillin-resistant (77.8%), erythromycin-resistant (88.9%), tetracycline-resistant (85.7%) and excellent coverage rate for invasive isolates (100%). High coverage rate demonstrated in the study from Chile which showed coverage rate of (80%) for all serotypes²⁸. On the other hand, this present study reported higher coverage rate for invasive isolates than another study in Spain (77.9%)²⁹ and Thailand (85.7%)³⁰. For children < 5 years old, this study showed coverage rates of (69%) for all serotypes, (100%) for invasive isolates and (78.6%), (87.5%), (84.6%) for penicillin-, erythromycin-, and tetracycline-resistant isolates, respectively. On the other hand, high coverage rate was demonstrated in a study from China (87.8%) for all serotypes³¹. In Thailand³⁰, lower coverage rates was reported for IPD, it was (87.8%).

In conclusion, in light of the increasing prevalence of *S. pneumoniae* resistant to multiple antimicrobial agents, the local policies and the immunization should consider these changes and adapt accordingly.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

HAM and KMB designed the experiments. HAM performed the experiments. HAM and KMB analyzed the data and HAM wrote the manuscript. KMB supervised and reviewed the manuscript. HAM and KMB approved the manuscript.

DATA AVAILABILITY

All datasets generated or analyzed in this research are included in the manuscript.

ETHICS STATEMENT

Informal consent was taken before the study from each patient. Study approval was issued and maintained by the ethical committee from Arabian Gulf University, Manama, Bahrain before starting the project.

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