

# Serotype Prevalence and Penicillin-susceptibility of *Streptococcus pneumoniae* in Oman

Mubarak M. Al-Yaqoubi,<sup>1</sup> Kamal M. Elhag<sup>2</sup>

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## Abstract

**Objectives:** to determine the prevalent serotypes of *Streptococcus pneumoniae* and the rate of penicillin-nonsusceptibility among pneumococci in Oman.

**Methods:** *Pneumococcal* isolates encountered during the period of September 2002 to December 2007 in the Royal Hospital were serotyped. Clinical information as well as the penicillin susceptibility reports were retrieved from the hospital information system and medical records.

**Results:** 120 strains of *Streptococcus pneumoniae* were isolated of which 85 strains were serotyped. 20 different serotypes were identified; the most common serotypes were 9A, 6B, 19F, 14 and 23F. 56% of the strains were not susceptible to penicillin, while 99% of these were susceptible to ceftriaxone. 74.3% and 46.1% of the serotypes are covered by the pneumococcal polysaccharide vaccine and the 7-valent pneumococcal conjugate vaccine respectively.

**Conclusion:** Certain few pneumococcal serotypes such as 9A, 6B and 19F are more prevalent in the Omani community than others. More than half of *S. pneumoniae* are not susceptible to penicillin while the great majority of the strains are susceptible to ceftriaxone.

**Keywords:** Pneumococcal Serotypes Penicillin susceptibility in pneumococci.

## Introduction

Infections caused by *Streptococcus pneumoniae* are common worldwide and the mortality associated with invasive pneumococcal disease (IPD) such as meningitis and bacteraemia remains high at 5-35% depending on site of infection, age and comorbidity.<sup>1</sup> The polysaccharide capsule is the major virulence factor and its structure divides pneumococci to more than 90 serotypes.<sup>2</sup> Studies that looked into the relation between pneumococcal serotypes with invasiveness and the course of pneumococcal infections are few and the results vary.<sup>3</sup>

The introduction of the 7-valent pneumococcal conjugate vaccine in the United States and Europe has led to significant decreases in the incidence of pediatric invasive pneumococcal disease.<sup>4</sup> There are some data relating to the burden of pneumococcal disease as well as the incidence of penicillin-nonsusceptibility in pneumococci in this part of the world.<sup>5-7</sup> Previous studies from Oman looked into the issue of penicillin resistance.<sup>8</sup>

It is of interest to monitor the pneumococcal serotypes prevalent in the Omani community especially since the 7-valent pneumococcal conjugate vaccine has been introduced in the country recently (2008). The Objective of this study is to determine the prevalent serotypes of *Streptococcus pneumoniae* as well as the rate of penicillin-nonsusceptibility among pneumococci.

## Methods

Pneumococcal isolates from clinical specimens (Blood, CSF, Respiratory and other) encountered during the period between September 2002 and December 2007 in the Royal Hospital, a 600 bedded-tertiary care hospital serving Muscat area as well as being a referral health care centre for patients from all over Oman, were identified by conventional methods using Gram stain, alpha-haemolysis and optochin-susceptibility, saved in skimmed milk at -80 degrees centigrade for serotyping. Isolates were sent to WHO (EMRO) laboratory in Cairo for serotyping.

The hospital information systems (Medicom and Al-Shifa) were used to retrieve the reported susceptibility testing. Susceptibility to penicillin and ceftriaxone was done using CLSI (previously NCCLS) guidelines. Penicillin susceptibility was determined by oxacillin 1gm disc (disc diffusion method).

Strains with an oxacillin inhibition zone of <20 mm were considered non-susceptible and were further tested by E-test (AB Biodisk, Sweden). E-test was also used to test pneumococcal strains for susceptibility to ceftriaxone. The isolate was considered susceptible to penicillin if the MIC was  $\leq 0.06$   $\mu\text{g/ml}$ , intermediately-susceptible if the MIC was 0.1-1.0 and resistant if the MIC was  $\geq 2.0$   $\mu\text{g/ml}$ . Strains were considered susceptible to ceftriaxone if the MIC was  $\leq 1$   $\mu\text{g/ml}$ , intermediately-susceptible if the MIC is 2  $\mu\text{g/ml}$  and resistant if the MIC was  $\geq 4$   $\mu\text{g/ml}$ .

Mubarak M. Al-Yaqoubi ✉  
Department of Microbiology, Khoula Hospital, Muscat, Sultanate of Oman.  
E-mail: mubarak18@gmail.com

Kamal M. Elhag  
Department of Microbiology, Royal Hospital, Muscat, Sultanate of Oman.

For each pneumococcal isolate, the patient characteristics, co-morbidities and risk factors for pneumococcal disease, diagnosis, treatment and outcome were retrieved from the Hospital Information System or paper medical records of patient files.

Co-morbidity was defined as the presence of splenectomy, diabetes, chronic liver/renal disease or malignancy. The study was approved by the ethics committee at the Royal Hospital

## Results

A total of 120 pneumococcal isolates from various clinical specimens including blood (50), respiratory specimens (49), CSF (4) and other specimens (17) were collected. The clinical characteristics of the patients are shown in Table 1.

Different age groups were represented in the study. 69.2% of patients had co-morbid conditions or risk factors for pneumococcal disease. Pneumonia was the most common diagnosis (53.3%) followed by meningitis (13.3%) and septicaemia with unknown focus (7.8%). Other infections represented 25.6% of total number of patients for whom the diagnosis could be retrieved from medical records system. The outcome could be traced for 80 patients. Of these, 15 (18.7%) died, while 65 (81.3%) survived.

23 different serotypes (Table 2) could be identified among the 85 strains that were serotyped. Serotypes 6B, 19F and 23F were the most prevalent (12% each), followed by serotypes: 9A and 14 (7.1% each). Serotypes 1 and 11A were less common with 5 (6%) and 4 (4.7%) strains respectively.

Each of the serotypes: 3, 5, 10A, 19A and 23A were identified in 3 (3.5%) of the 85 strains. Serotypes 4, 16F and 7F were identified each in 2 (2.3%) strains, while only 1 strain was found to belong to each of serotypes 6A, 12F, 15A, 18C, 33A, 33F, 34 and 35B.

The susceptibility to Penicillin and ceftriaxone could be retrieved from the hospital laboratory information system for 77 and 85 strains respectively. Only 34 (44%) strains were susceptible to Penicillin, while 43 (56%) were not susceptible including 16 strains that were fully resistant. 81 (99%) strains were susceptible to ceftriaxone and only 1 (1%) strain was found to be intermediately susceptible.

32 of the 43 strains that were not susceptible to penicillin were serotyped. Of these, eight different serotypes were identified (Table 3). Six strains belonged to each of serotypes 6B and 23F while five strains were found to be serotype 19F.

Information concerning the outcome of patients could be retrieved for 81 patients. 15 of them died, 10 (66.7%) of whom had positive blood cultures with *Streptococcus pneumoniae*. Isolates from 10 of these patients were serotyped. Two strains were identified as serotype 19F, while 1 strain was found to belong to each of serotypes: 1, 3, 7F, 9A, 14, 19A and 33F and one strain was non-typable.

Penicillin susceptibility was available for 11 strains out of the 15-death-associated stains. Eight (72.7%) strains were susceptible

while 3 (27.3%) were not susceptible to penicillin. The status of co-morbidity was known for 13 of those who died. 10 (76.9%) of them had co-morbid conditions while 3 (23.1%) did not have any co-morbidity.

**Table 1: Clinical Characteristics of Patients**

Patients' variables	Number (%)
<b>Age</b>	
≤2	27 (28.1)
2-5	7 (7.3)
6-20	15 (15.6)
21-59	31 (32.3)
≥60	16 (16.7)
*All	96 (100)
<b>Sex</b>	
Male	54 (56.3)
Female	42 (43.7)
*All	96 (100)
<b>Co-morbidity/Risk Factors</b>	
With	63 (69.2)
Without	28 (30.8)
*All	91 (100)
<b>Diagnosis</b>	
Pneumonia	48 (53.3)
Septicemia	7 (7.8)
Meningitis	12 (13.3)
Other	23 (25.6)
*All	90 (100)
<b>Outcome</b>	
Death	15 (18.7)
Survival	65 (81.3)
*All	80 (100)

All: Number of patients for whom information could be retrieved\*

**Table 2:** Serotypes of *Streptococcus pneumoniae* strains isolated

Serotype	Total Number (%)	Blood	CSF	Respiratory Specimens	Other Specimens
1	5 (5.9)	5			
3	3 (3.5)			2	1
4	2 (2.4)	2			
5	3 (3.5)	2			1
6A	1 (1.2)	1			
6B	10 (11.8)	4		3	3
7F	2 (2.4)	1		1	
9A	6 (7.1)	1		3	2
10A	3 (3.5)	2		1	
11A	4 (4.7)	1		2	1
12F	1 (1.2)	1			
14	6 (7.1)	4		1	1
15A	1 (1.2)				1
16F	2 (2.4)			2	
18C	1 (1.2)			1	
19A	3 (3.5)	2		1	
19F	10 (11.8)	3	1	6	
23A	3 (3.5)			3	
23F	10 (11.8)	2	1	7	
33A	1 (1.2)			1	
33F	1 (1.2)			1	
34	1 (1.2)			1	
35B	1 (1.2)			1	
Non-Typable	5 (5.9)	1		4	
Total		32	2	41	10

**Table 3:** Serotypes of Penicillin-nonsusceptible pneumococcal (PNSP) strains isolated

Serotype	No(% total number of PNSP)	% from total number of each serotype	Blood	CSF	Respiratory Specimens	Other Specimens
6A.	1 (3.1)	100	1			
6B	6 (18.8)	60	2		2	2
9A	3 (9.4)	50	1		1	1
11A	2 (2.2)	50	1		1	
14	3 (9.4)	50	2		1	
19F	5 (15.6)	50		1	4	
23F	6 (18.8)	60	1	1	4	
33A	1 (3.1)	100			1	
Non-Typable	5 (15.6)	100	1		4	
Total	32		9	2	18	3

## Discussion

Blood and CSF specimens represented 54 out of 120 specimens reflecting the fact that Royal hospital is a tertiary care hospital receiving severe cases mainly. Consequently, it is not surprising that 69.2% of the patients had associated co-morbid conditions which are known to be associated with serious pneumococcal infections.<sup>9,10</sup> The five most prevalent serotypes (6B, 19F, 23F, 9A, 14) represent almost half (49.6%) of the encountered pneumococcal isolates in this study, and they comprise more than two thirds (71.9%) of PNSP. Similar findings were shown in a study from Kuwait.<sup>5,11</sup> The 3 most prevalent serotypes (6B, 19F, 23F) were represented by 10 (11.8%) strains each and this has been demonstrated in other studies.<sup>5,11,12</sup> These serotypes are fortunately represented in the new 7-valent conjugate pneumococcal vaccine which has been recently introduced in the Sultanate of Oman. This 7-valent conjugate vaccine containing the serotypes 4, 6B, 9V, 14, 18C, 19F and 23F covers 46.1% of the strains encountered in this study which agrees with findings by other workers from the region.<sup>5,6</sup> The 23-valent pneumococcal polysaccharide vaccine provides coverage of 74.3% of the strains encountered in this study. Looking into the invasive isolates encountered in this study (ie CSF and blood culture isolates), 51.5% and 100% coverage is provided by the 7-valent conjugate vaccine and the 23-valent polysaccharide vaccine respectively. Although the 23-valent polysaccharide vaccine has excellent coverage in terms of prevalence of pneumococcal serotypes, the fact that it is less effective in immunosuppressed patients and ineffective in children below 2 years of age limits its usefulness.<sup>13</sup> Because it is effective in immunosuppressed individuals and children below 2 years of age, the introduction of the 7-valent pneumococcal conjugate vaccine to childhood immunization program in Oman is a very welcomed step forward to reduce the burden of pneumococcal infections in the community. However, the coverage of the newly introduced 7-valent pneumococcal conjugate vaccine can be improved. In this, we agree with other researchers from the region that the prevalence of pneumococcal serotypes in this region should be taken into consideration to formulate a vaccine which provides better coverage.<sup>5</sup>

Penicillin non-susceptibility was demonstrated in 56% of pneumococci in this study, this goes along with findings in other countries in the region.<sup>5,7,14</sup> Previous study from Oman showed that 54.6% of *S. pneumoniae* colonizing nasopharynx of children were penicillin non-susceptible strains.<sup>8</sup> The most prevalent strains (6B, 19F, 23F) represent more than half (53.2%) of the resistant pneumococcal strains; an additional argument in favor of the 7-valent vaccine. The percentage of PNSP covered by the 7-valent conjugate vaccine is 62.6% while the 23-valent polysaccharide vaccine provides a coverage of 72% of the PNSP encountered in this study. The introduction of the new PCV-7 has reduced the burden of pneumococcal disease in other parts of the world<sup>15</sup> although the impact of penicillin resistance among pneumococci causing invasive pneumococcal disease has varied in different studies.<sup>16,17</sup>

Continuous monitoring of circulating serotypes of pneumococci in the community is necessary to measure the impact of the newly introduced vaccine on the incidence of pneumococcal disease as well as the impact on penicillin resistance among pneumococci.

## Conclusion

Our study provides insight on the prevalent pneumococcal serotypes in the Omani community during the period from 2002 to 2007 in a national referral hospital and is the first study from Oman in this regard. It also addressed the issue of penicillin resistance among pneumococci and provides recent Data. It is however limited by the small sample number. Further studies with a larger sample involving all regions of Oman to explore the prevalent pneumococcal serotypes and antimicrobial susceptibilities of *S. pneumoniae* are warranted.

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