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Serotype distribution and antibiotic resistance among invasive *Streptococcus pneumoniae* from Oman post 13-valent vaccine introduction

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ABSTRACT

Objective: This study was undertaken to determine the serotype distribution and drug susceptibility patterns in pneumococcal isolates recovered from patients with invasive pneumococcal disease (IPD).
Methods: All invasive pneumococcal isolates received between June 2014 and June 2016 were included in the study as part of a national laboratory-based IPD surveillance program. Isolates recovered from clinical specimens of normally sterile body sites were included.
Results: A total of 41 different serotypes were identified among the 132 pneumococcal isolates included in this study. The most prevalent serotypes/serogroups were 12 (8.3%), 15 (8.3%), 19 F (7.6%), 3 (6.1%), and 19A (6.1%);. It was observed that overall vaccine coverage rates for pneumococcal conjugate vaccines (PCV), PCV7, PCV10 and PCV13 were 15.9%, 24.2% and 37.1% respectively. 56.8% (n = 75) of the isolates were non-susceptible to at least one antibiotic and 40.9% (n = 54) of the isolates were resistant to PEN (M). 18.9% (n = 25) of the isolates were multi-drug resistant (MDR).The case fatality rate was 15.9%.
Conclusion: Our study results call for broader vaccine coverage, emphasizes the need to introduce the conjugate pneumococcal vaccine for the high risk adult population and stress the importance of continuous surveillance of serotypes and antimicrobial resistance to guide vaccine development and antimicrobial stewardship activities.

Introduction

Streptococcus pneumoniae is a leading cause of serious illness, including bacteremia, meningitis and pneumonia among children and adults worldwide (Hackel et al., 2013; Nuorti et al., 2010; Thigpen et al., 2011). The World Health Organization (WHO) estimated that approximately 541,000 children under 5 died in 2008 of pneumococcal disease and most pediatric pneumococcal deaths occur in developing countries (WHO, 2008; O'Brien et al., 2009). In 2006, the incidence of IPD among Omani children under 2 was 26.1 per 100,000, which was found to be higher than the IPD incidence in the neighboring countries (Al Awaidy et al., 2012). *S. pneumoniae* was found to be the most common cause of laboratory confirmed meningitis in Oman although this trend is declining (Padmann et al., 2018).

There are over 94 pneumococcal serotypes but only few account for the majority of IPD worldwide (Hausdorff et al., 2000). Based on that, 3 multivalent pneumococcal conjugate vaccines (PCV) have been licensed in past 15 years; PCV7, PCV10 and PCV13. A large reduction in IPD and pneumonia has been seen in countries that have introduced PCV (Whitney et al., 2003; Lexau et al., 2005; Simonsen et al., 2014). With the introduction of PCVs, there are reports of serotype repalcement, where non-vaccine serotypes fillthe niche created by decrease in vaccine serotypes (Weinberger et al., 2011; Wyres et al., 2013; Spratt and Greenwood, 2000). Emerging *S. pneumoniae* strains resistant to the commonly used antimicrobial drugs such as beta-lactams and macrolides have been frequently described worldwide (Woodford and Livermore, 2009; Henriques-Normark and Tuomanen, 2013).

In Oman, the first pneumococcal conjugate vaccine that was introduced to the extended program of immunization was PCV7 in 2008. In November 2010, PCV7 was replaced by PCV10 which was later replaced by PCV13 in February 2012 given at 2, 4, 6 months of age to provide more serotype coverage. The national decision to

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switch from PCV7 to PCV13 was mainly driven by international reports of emerging multidrug resistant 19A serotype but no national surveillance program was available to monitor the trend locally. The coverage rate for pneumococcal vaccine is 99% according to latest reports from the extended immunization program in Oman.In adults,the 23 valent poly saccharide vaccine is offered for high risk groups.There are few published studies on pneumococcal disease in Oman (Al Awaidy et al., 2012; Al-Yaqoubi and Elhag, 2011) but all the studies which were carried out on isolates prior to the introduction of PCV7 relied on facility-based data and did not involve other centers and therefore did not appropriately reflect the national situation. A national lab-based invasive pneumococcal surveillance program was established in 2014, included all the health establishments in Oman and provided information about antimicrobial susceptibility of the isolates in addition to serotypes. Our study analyzes this multicentric national IPD surveillance database with the objective to fill the existing epidemiological and clinical gaps and direct future decisions about immunization.

Materials and methods

Study design

This study, part of the national surveillance program, was conducted to aid in the decision-making process for Oman's national immunization program. From June 2014 to June 2016, 14 regional and tertiary care hospitals form the 11th governorates participated in this laboratory-based surveillance program for IPD. At each center, isolates and epidemiological data were collected.

Invasive pneumococcal isolates were recovered from clinical specimens of normally sterile body sites such as blood and cerebrospinal fluid. The laboratory criteria for IPD was adopted from the Center of Diseases Control and Prevention manual (CDC, 2010). One isolate per patient was included. For each patient, relevant demographic data including age, sex, clinical presentation and outcome of the infection were collected and analyzed. Patients were stratified into 3 age groups; $\leq$5 years, 6 to 50 years and $\geq$51 years for purpose of analysis. Outcome was defined as complications, recovery or death. Case fatality rate was defined as the proportion of patients who died within 30 days of IPD diagnosis.

Identification of pneumococcal isolates

S. pneumoniae isolates were received at Central Public Health Laboratories (CPHL) from various hospitals on 5% blood agar plates. The isolates were preserved at −80°C in cryovials until analysis.

The *S. pneumoniae* isolates were re-cultivated at CPHL using 5% sheep blood agar and incubated at 37 °C in the presence of 5% CO_2 overnight (18–24 h). Isolates were identified by phenotypic characteristics and with Vitek® 2 compact microbial analysis system as per manufacturer's instructions (bioMérieux, Craponne, France). Real time PCR method targeting lytA gene (found on all the pneumococcal isolates irrespective of serotypes) was used to confirm pneumococcal identity as previously described (CDC, 2010).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done on all strains for 12 different antibiotics (penicillin, ceftriaxone, cefotaxime, meropenem, amoxicillin, oxacillin, erythromycin, clindamycin, chloramphenicol, trimethoprim/sulfamethoxazole, vancomycin and levofloxacin). MIC values were determined by E-test (bioMérieux, Craponne, France) for penicillin, ceftriaxone and cefotaxime. Manufacturer's guidelines were followed to perform the E-testing. Disk diffusion testing on Mueller Hinton Agar with 5% sheep blood was done on all other antibiotics. The latest Clinical and Laboratory Standards Institute (CLSI) M100S document was used for the interpretation of results. Isolates that were resistant to at least 1 drug in at least 3 different classes of antibiotics were defined as multidrug resistant (MDR) (CLSI, 2014; Magiorakos et al., 2012). *S. pneumoniae* ATCC® 49619 and *Staphylococcus aureus* ATCC® 25923 were used as quality control isolates.

Serotyping

Capsular typing was performed by a combination of the latex agglutination reaction method using type specific antisera and molecular methods (Real time PCR and conventional PCR).

Latex agglutination method

ImmuLex™ Pool Antisera (SSI Diagnostica, Hillerød, Denmark) wereused for initial typing. The method and interpretation was performed as per the manufacturer's instructions.

Serotyping by PCR

For genomic DNA extraction, fresh overnight grown bacterial cells were suspended in 1 ml of phosphate buffered saline to achieve 0.5 McFarland's turbidity. Bacterial suspension was centrifuged at 8000 rpm for 5 min. Bacterial pellet were processed further using the QIAamp® DNA Mini kit (Qiagen Cat No. 51304, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Purity and quantity of eluted genomic DNA was documented using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). DNA was stored at −20 °C for later analysis.

A multiplex real time PCR scheme targeting 21 different serotypes or serogroups using primers/probes previously described (available at http://www.cdc.gov/streplab/pcr.html) was used. Seven different sets of multiplex PCR were used to determine major pneumococcal serotypes/serogroups as earlier defined by Pimenta et al. (2013).

A conventional PCR method was used to determine serotypes not covered by latex and real time PCR methods. The primers for conventional PCR were previously defined (available at https://www.cdc.gov/streplab/pcr.html/).

Ethical approval

This study is part of the national surveillance system. The bacterial isolates were analyzed anonymously, thus ethical approval was not required and patient confidentiality was not an issue.

Statistical analysis

IBM SPSS version 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The Chi-square test was used for the comparison between serotypes and resistance to penicillin. As a rule of thumb, a 2-tailed cut-off of $p < 0.05$ was considered to be statistically significant.

Results

Demographics, clinical spectrum and outcome

A total of 132 *S. pneumoniae* isolates from sterile sites of patients with invasive pneumococcal disease were collected at CPHL in Muscat as part of ongoing national IPD surveillance study. During the study period from 2014 to 2016, almost equal proportions of

isolates were collected each year; 35.6%, 34.8% and 29.8% respectively. Invasive pneumococcal isolates were collected from all major government hospitals ($n = 14$) across all regions of Oman. Of the total IPD cases, 47.7% were from Muscat followed by the Dhofar (22.0%) and Dakhliya (12.1%) regions. The Muscat region contributed to approximately 50% of the IPD cases because it is the most populated city in Oman, and it has 4 major tertiary care hospitals where seriously and acutely sick patients are admitted.

Of the 132 isolates, blood was the predominant specimen ($n = 122$, 92.4%), followed by cerebrospinal fluid (CSF) ($n = 8$, 6.1%) and other body fluids ($n = 2$, 1.6%). The pneumococcal infection rate was significantly higher ($p = 0.005$) among male patients ($n = 82$, 62.1%) than female patients ($n = 50$, 37.9%) with male to female ratio of 1.64:1. The mean age of patients was 38.7 (range 15 days to 95 years). Most of the patients were ≥51 years ($n = 60$, 45.5%) followed by 6–50 years ($n = 37$, 28.0%) and ≤5 years ($n = 35$, 26.5%). Among those ≤5 years, the majority of cases were in infants ≤1 year (65%).

More than half of the total isolates were recovered from patients who had been diagnosed and were getting treated for pneumonia (52.3%, $n = 69$). Meningitis (17.4%, $n = 23$), septicemia (17.4%, $n = 23$), and skin and soft tissue infection (5.3%, $n = 7$) were other major clinical presentations.

Out of 132 patients, 102 (77.3%) were successfully treated and discharged after clinical improvement while 21 patients (15.9%) died during treatment. Outcome data for 9 patients (6.8%) were not available and 1 patient (0.8%) was reported to have permanent hearing loss. The case fatality rate was higher among the first (≤5years) and third (≥51 years) age groups, 14.2% and 21.7% respectively, than the second (6–50) age group (8.1%). Though death rates were higher among high risk groups, these variations were found to be statistically insignificant ($p = 0.384$). Outcome was also independent of serotype ($p = 0.770$), diagnosis ($p = 0.138$) and patient gender ($p = 0.581$). Age- and gender-specific frequencies along with rates of deaths and MDR status among the isolates are shown in Table 1.

Serotype distribution

A total of 41 different serotypes were identified among 132 pneumococcal isolates (it is important to note that 6 isolates remained non-typeable). Capsular agglutination and multiplex real time PCR results were compared and found to be 100% concordant. Many sub-serotypes were identified by careful interpretation of latex agglutination and real time PCR results. Six isolates (4.5%) could not be identified by either method and were marked as non-typeable. The most common serotypes/serogroups were 12 (8.3%), 15 (8.3%), 19F (7.6%), 3(6.1%), 19A (6.1%) and 22 (5.3%) among all age groups. These serotypes represent 41.7% of the total serotypes. Moderate prevalence was observed for serotypes 1(3.8%), 7F (3.8%), 17F (3.0%), 18C (3.0%) and 9N/L (3.0%). Serotype/serogroup distribution data is shown in Table 2.

Analysis of serotype distribution showed age-dependent variation. Serotype variations among different age groups were statistically significant ($p = 0.023$), though the number of isolates for many serotypes was low. The predominant serotypes among children ≤5 were 12 (11.4%), 19F (11.4%), 23A (8.6) and 16F (8.6%). These serotypes accounted for 40% of the total serotypes in this group. Serotype distribution among the other high risk group (≥51 years) was remarkably different and predominant serotypes/serogroups were 3 (13.3%), 15 (8.3%), 19A (8.3%), 19F (6.7%), 22 (6.7%), 12 (5.0%) and 11 (5.0%). Though, these leading serotypes contribute to 53.3% of the total serotypes among ≥51 age group; this group had more diversity in serotype distribution.

Serotypes/serogroups 1 (13.5%), 12 (10.8%), 15 (10.8%) and 7F (8.1%) were the most common serotypes among the 6- to 50-year-old age group and comprise 43.2% of total serotypes.

Some serotypes were reported only among one age group and not in others. Serotype 16F ($n = 3$) and 23A ($n = 3$) were reported only among children ≤5 years. Serotype 3 ($n = 8$, one of the predominant serotypes) and serotype 11F/B/C ($n = 3$) were found only among patients ≥51 years, while serotype 1 ($n = 5$) was found only among those 6–50. Major variations of serotype distribution are shown in Figure 1.

Table 1
Age-wise distribution of gender, death rates and multidrug resistance rates.

Age group (in years)	Age group frequency	Gender		Mortality	MDR
		Male	Female		
≤5	26.5 % ($n = 35$)	51.4 % ($n = 18$)	48.5 % ($n = 17$)	14.2 % ($n = 5$)	20 % ($n = 7$)
6–50	28.8 % ($n = 37$)	72.9 % ($n = 27$)	27.0 % ($n = 17$)	8.1 % ($n = 3$)	13.5 % ($n = 5$)
≥51	45.5 % ($n = 60$)	61.6 % ($n = 37$)	38.3 % ($n = 23$)	21.7 % ($n = 13$)	21.7 % ($n = 13$)

Table 2
Detailed serotype/serogroup distribution among all age groups.

Serotype/serogroup	Frequency		Contribution (%)
	No.	%	
12, 15	11	8.3	16.6
19F	10	7.6	7.6
3, 19A	8	6.1	12.2
22	7	5.3	5.3
1, 7F	5	3.8	7.6
17F, 18C, 9N/L	4	3	9
11F/B/C, 11A/D, 16F, 23A, 35B, 6B, 9A	3	2.3	16.1
8, 13, 10B, 18A/B, 23F	2	1.5	7.5
4, 5, 6D, 10F/C, 14, 19, 24, 28, 29, 34, 37, 39, 10A, 23B, 6A, 6C, 7B/C, 7F/A	1	0.8	14.4
Non-vaccine group (NVG)	6	4.5	4.5

Vaccine coverage

The overall vaccine coverage (for the circulating serotypes over the 2 year period) for all the conjugate vaccines was low. We observed that overall vaccine coverage rates for PCV7, PCV10 and PCV13 were 15.9%, 24.2% and 37.1% respectively among all age groups in our study. The distributions of vaccine serotypes were variable among different age groups although the differences were statistically non-significant. PCV13 vaccine coverage rates and vaccine serotype distribution against first, second and third age groups were 26.5%, 34.2% and 43.3% respectively.

Vaccine coverage rates for PCV7, PCV10 and PCV 13 along with the serotypes are shown in Figure 2.

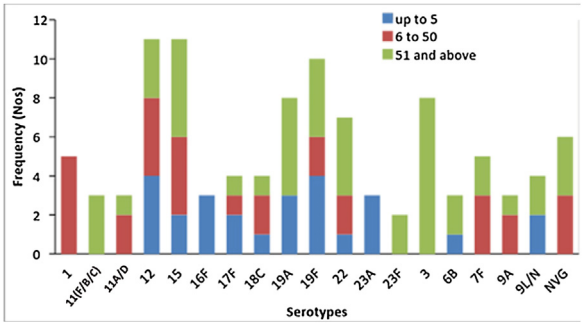

Figure 1. Serotype/serogroup distribution among different age groups (only predominant serotypes were included).

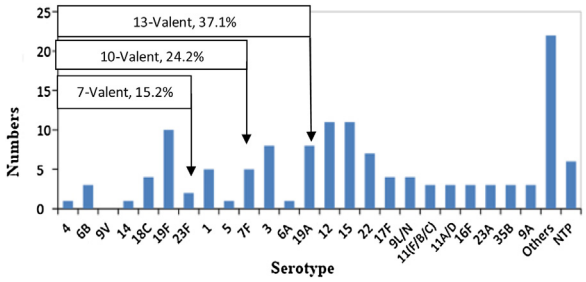

Figure 2. Serotype/serogroup distribution and PCV coverage among the pneumococcal isolates of all age groups.

Antimicrobial susceptibility testing

Non-susceptibility rates for PEN were very high with 40.9% of the isolates being non-susceptible per the meningitis breakpoints (M) while only 1.5% were non-susceptible per non-meningitis breakpoints (NM).

Ceftriaxone (CRO) and cefotaxime (CTX) non-susceptibility rates were 3.8% and 3.1% respectively (as per meningitis criteria) and 0.8% each (as per non-meningitis criteria). Rates of non-susceptibility to erythromycin (ERY), clindamycin (CLI), and trimethoprim-sulfamethoxazole (SXT) were; 28.1% (25.8% R, 2.3% I), 16.7% (16.7% R, 0.0% I) and 32.6% (26.5%R, 6.1% I) respectively. Lower non-susceptibility rates were detected for meropenem (MEM), amoxicillin (AMX), chloramphenicol (CHL) and levofloxacin (LUV); 1.5%, 2.3%, 1.5% and 0.8% respectively. All the isolates were susceptible to vancomycin (VAN). Antimicrobial susceptibility data is shown in Table 3.

Antimicrobial susceptibility rates for all the antimicrobial agents were non-significantly variable among different age groups except for PEN (M) where non-susceptibility rates were significantly higher in high risk age groups. PEN (M) non-susceptibility rates among age groups of those ≤5 years, 6–50 years and ≥51 years were 64.7%, 18.4% and 41.7% respectively ($p = <0.005$).

The results of non-susceptibility rates among different age groups and non-susceptibility rate comparison between serogroups 12 and 15 and other serotypes are shown in Table 3.

Out of 132 isolates, 56.8% ($n = 75$) of the isolates were non-susceptible to at least one antibiotic. We found 18.9% ($n = 25$) to be multidrug resistant (MDR). All the MDR isolates were resistant to erythromycin. PEN-M, clindamycin and SXT were the antimicrobial agents commonly found resistant among these MDR isolates. Out of 25 MDR isolates, more than half (56%, $n = 14$) were of serotype 19 F ($n = 8$) and serogroup 15 ($n = 6$). 19F serotype appeared as major concern because 80% ($n = 8$) of total 19F isolates ($n = 10$) were MDR.

Serogroups 15 and 12 were the most predominant serotypes among invasive pneumococcal isolates in our study. Non-susceptibility rates were significantly higher among serogroups 12 and 15 than among other serotypes to ERY, CLI and CHL ($p = <0.001$, <0.001 and 0.001 respectively). The differences of non-susceptibility rates to all other antibiotics were not significant (Table 4).

Table 3
Antimicrobial susceptibility of the 132 invasive pneumococcal isolates to 11 common antibiotics.

Antibiotic	Site	Susceptibility (%)			MIC (μg/ml)		
		R	I	S	MIC50	MIC90	MIC range
PEN	Meningitis	40.9	0.0	59.1	0.032	0.50	0.016–4
PEN	Non-meningitis	0.0	1.5	98.5	0.032	0.50	0.016–4
CRO	Meningitis	0.8	3.0	96.2	0.016	0.25	0.004–2
CRO	Non-meningitis	0.0	0.8	99.2	0.016	0.25	0.004–2
CTX	Meningitis	0.8	2.3	97.0	0.023	0.25	0.002–2
CTX	Non-meningitis	0.0	0.8	99.2	0.023	0.25	0.002–2
MEM		1.5	0.0	98.5	0.008	0.13	0.002–2
AMX		0.8	1.5	97.7	0.023	0.50	0.003–8
ERY		25.8	2.3	72.0			
CLI		16.7	0.0	83.3			
CHL		1.5	0.0	98.5			
SXT		26.5	6.1	67.4			
VAN		0.0	0.0	100.0			
LVX		0.8	0.0	99.2			

Antibiotic abbreviations: PEN (penicillin), CRO (ceftriaxone), CTX (cefotaxime), MEM (Meropenem), AMX (amoxicillin), ERY (erythromycin), CLI (clindamycin), CHL (chloramphenicol), SXT (trimethoprim-sulfamethoxazole), VAN (vancomycin), LUV (levofloxacin).

Discussion

This is the largest multicenter prospective study to date in Oman to evaluate IPD serotypes, clinical outcomes and antimicrobial susceptibility patterns as part of a national laboratory-based surveillance program.

In this study, most cases of IPD occurred in those >51 years of age and this can be explained by the fact that these patients more commonly have risk factors for IPD more common than the other age groups and that they are not covered by the national vaccine program. This age group was also found to have the highest mortality rate (21.6%) compared to the other 2 groups. This finding

Table 4
Non-susceptibility rate comparison among 12 and 15 serogroups and other serotypes.

Antimicrobial agents	Overall non-suscptibility rates (%)	Non-susceptibility rates among age groups (%)				Non-susceptibility rates among serotypes (%)		
		≤5 years	6–50 years	≥51 years	p-Value	12&15 group	Others	p-Value
PEN_NM	1.5	0.0	0.0	3.3	0.296	0	1.8	0.524
PEN_M	40.9	62.9	18.4	41.7	<**0.001**	31.8	42.7	0.342
CRO_NM	0.8	0.0	0.0	1.7	0.546	0.0	0.9	0.653
CRO_M	3.8	2.9	0.0	8.3	0.136	0.0	5.5	0.262
CTX_NM	0.8	0.0	2.6	1.7	0.654	0.0	1.8	0.524
CTX_M	3.1	0.0	0.0	6.7	0.084	0.0	3.6	0.364
MEM	1.5	0.0	0.0	3.3	0.296	0.0	1.8	0.524
AMX	2.3	0.0	0.0	5.0	0.159	0.0	2.7	0.433
ERY	28.1	32.4	28.9	25	0.739	63.6	20.9	<**0.001**
CLI	16.7	20.6	15.8	16.7	0.847	59.1	9.1	<**0.001**
CHL	1.5	0.0	0.0	3.3	0.296	9.1	0.0	**0.001**
SXT	32.6	26.5	34.2	35.0	0.669	40.9	30.9	0.361
VAN	0.0	0.0	0.0	0.0	NA	0.0	0.0	NA
LVX	0.8	2.6	0.0	0.8	0.288	0.0	0.9	0.653

Antibiotic abbreviations: PEN (penicillin), CRO (ceftriaxone), CTX (cefotaxime), MEM (Meropenem), AMX (amoxicillin), ERY (erythromycin), CLI (clindamycin), CHL (chloramphenicol), SXT (trimethoprim-sulfamethoxazole), VAN (vancomycin), LUV (levofloxacin).

has been reported in previous studies (Inverarity et al., 2011) with old age reported as a risk factor for mortality in IPD cases (Navarro-Torné et al., 2015).

Although several studies and reports of IPD distribution by age have illustrated a U-shaped curve with the highest incidence in children <5 and adults >65 (Melegaro et al., 2006; Robinson et al., 2001), our study showed that the number of cases among children ≤5 years were the lowest; which might be due to the implementation of the pneumococcal conjugate vaccine in childhood. The universal vaccination program has been in place since 2008.

The high male to female ratio found in our study was previously reported by others in previous studies (Chaïbou et al., 2014).

The overall case fatality rate was 15.9% with the highest rates found among those ≥51 years of age (21.6%). Our fatality rate was lower than previously reported in Japan (24.1%) (Hanada et al., 2016), but higher than that reported in Europe (9%) (Navarro-Torné et al., 2015). Although statistically not significant ($p = 0.138$), the case fatality rate among those diagnosed with pneumonia of 21.7% was higher than those diagnosed with meningitis (12.5%) and other clinical presentations. This finding is different from previous studies which report a higher mortality rate of meningitis (Navarro-Torné et al., 2015), probably due to the fact that more than half of the patients in our study with pneumonia (50.7%) were ≥51 years old.

In our study, the most prevalent pneumococcal serotypes/serogroup were 12, 15, 19F, 3, 19A and 22. The predominant serotypes among children ≤ 5 years of age were 12 (11.4%), 19F (11.4%), 23A (8.6) and 16F (8.6%). These serotypes accounted for 40% of the total serotypes. Serotype distribution among adults ≥51 years was remarkably different and predominant serotypes were 3 (13.3%), 15 (8.3%), 19A (8.3%), 19F (6.7%), 22 (6.7%), 12 (5.0%) and 11 (5.0%). Interestingly this is quite different from recent studies published in the Gulf Cooperation Council (GCC) countries regarding the prevalent serotypes. For example, a recent laboratory-based surveillance study showed the prevalence of 23F, 6B, 19F, 4, 14 and 19A among pneumococcal strains from children under 15 years of age, collected between 2009–2012 in Saudi Arabia (Al-Sheikh et al., 2014). In this study the serotype coverage of PCV 7, PCV10 and PCV13 were 77%, 81% and 90% respectively. This serotype distribution was also similar to other previous studies from Saudi Arabia (Shibl et al., 2012). However, these studies were mainly of child populations, as there are limited data on prevalence of pneumococcal serotypes in the adult population from the GCC countries (Feldman et al., 2013).

This difference of serotype distribution and vaccine coverage was also observed in a recent study on IPD burden and serotype distribution in Kuwait where the most prevalent serotype among children under the age of 5 were 19F, 19A, 6A, 8 and 15B, while the serotype prevalent among adults >50 was 14, 3, 1, 19F and 8 (Mokaddas and Albert, 2012).

In comparison to one study done in Oman in the pre-pneumococcal vaccine period (Al-Yaqoubi and Elhag, 2011), it is clear that a shift in pneumococcal serotypes occurred as a result of the vaccine introduction. The vaccine serotypes decreased from 82.4% among the IPD isolates collected before the introduction of the PCV7 vaccine to 37.9% collected after the introduction of the PCV13 vaccine. On the other hand, non-vaccine serotypes increased from 17.6% before PCV7's introduction to 62.1% after the introduction of PCV13. This finding confirms the findings of several studies reporting serotype replacement causing IPD after the introduction of PCVs (Feikin et al., 2013; Weinberger et al., 2011). The distribution of the vaccine serotypes versus non-vaccine serotypes was more prominent among those ≤5 years (26.5% versus 73,5%).

We found that outcome was independent of serotype ($p = 0.770$). This supports previous studies showing no difference in severity of disease or mortality when comparing different serotypes. (Chaïbou et al., 2014; Choi et al., 2008; Zurawska et al., 2017). Outcome was also independent of patient gender ($p = 0.581$).

Antibiotic non-susceptibility in the most prevalent serotypes 12 and 15 which are not covered by the PCV13 vaccine was significantly higher for erythromycin, chloramphenicol and clindamycin. Similar findings were observed in a study from Hong Kong where strains not covered by PCV13 such as serogroup 15 were found to have high erythromycin non-susceptibility of 79.3% (Chan et al., 2016). Another study in Korea showed similar higher rates of resistance among serogroups not covered by PCV13, with resistance rates to penicillin, erythromycin and MDR reaching86%, 90.5% and 81.5% respectively (Choe et al., 2016).

Out of the 25 MDR isolates, more than half of them (56%, $n = 14$) were of serotypes 19F ($n = 8$) and 15 ($n = 6$). The 19F serotype appeared as major concern because 80% ($n = 8$) of the total 19F isolates ($n = 10$) were MDR. This is different from previous studies carried out in the post PCV 7 vaccination showing MDR cases mostly encountered among the 19A serotype (Choi et al., 2008; Robinson et al., 2001). Serotype 19F is covered by PCV13 vaccine and among the 10 cases found, only 4 cases were in the first age group and only 2 cases had been vaccinated.

Our findings support the National Antimicrobial Guidelines where recommended empirical therapy for invasive pneumococcal infection is ceftriaxone and vancomycin until antimicrobial susceptibility testing is available and ceftriaxone sensitivity is confirmed.

This study also emphasizes the role of molecular method in serotyping the pneumococcal strains as this method came in concordance with the conventional agglutination methods and in some instances could differentiate between serotypes detected only as a group by conventional method.

This study emphasizes the previous findings that the clinical and economic burden of IPD remain a challenge especially in adult populations which calls for serious consideration to introduce the pneumococcal vaccine in the national adult vaccination program and the need to sustain IPD surveillance (Ludwig et al., 2012).

The study has several limitations, first, there is a possibility of under reporting from regional hospitals. Second, not all suspected meningitis cases had a CSF sample obtained due to parents/patient refusal (Padmann et al., 2018; MoH Oman, 2017). Third, we did not include patients' immunization status and previous history of antibiotics. These limitations may underrepresent the true prevalence of serotype, associated outcome and resistance prevalence. In addition some strains and serogroups were not further characterized to serotype level; this is may be due to limitations of the molecular and agglutination method used or presence of a novel serotype not detected by these methods. Further studies using reference methods will be undertaking to characterize these strains in future.

Conclusion

Invasive pneumococcal disease remains a concern with a high fatality rate even in countries like Oman where PCV has been part of the childhood immunization program since 2008. The change in serotypes prevalence and antimicrobial resistance pattern continue to be a challenge that needs continuous monitoring by the national IPD surveillance program. The prevalence and mortality rates within the adult population in this study calls for serious consideration of introducing the vaccine as part of the adult immunization program.

Conflict of interest

The authors declare no conflict of interest.

Use of trade names is for identification only and does not imply endorsement by the Central Public Health Laboratories or by the Directorate General of Communicable Disease and Surveillance.

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