# RESEARCH NOTE

## Distribution of serotypes and antibiotic resistance of invasive pneumococcal disease isolates among children aged 5 years and under in Saudi Arabia (2000–2004)

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### ABSTRACT

To determine vaccine coverage of invasive pneumococcal disease (IPD) in Saudi Arabian children aged 5 years and under, 350 IPD isolates were tested for antibiotic susceptibility. Of these 46%, 42% and 12% were penicillin-sensitive, pencillin-intermediate, and penicillin-resistant, respectively. Rates of resistance to erythromycin and cefotaxime were 26% and 6%, respectively. The potential serotype coverage of the PCV7 vaccine in Saudi Arabia among children <5 years of age is 62%, and vaccine coverage significantly improved in children <2 years of age, to reach 83% against IPD isolates. PCV7 is expected to have a substantial impact on the burden of invasive and antibiotic-resistant pneumococcal disease in Saudi Arabia.

**Keywords** Invasive pneumococcal disease, Saudi Arabia, serotype

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*Streptococcus pneumoniae* is one of the most common bacterial causes of morbidity and mortality worldwide, causing life-threatening infections such as meningitis, pneumonia and febrile bacteraemia [1,2]. The WHO reports that one million children, mostly young and living in developing countries, die from pneumococcal disease annually [3]. The severity and frequency of *S. pneumoniae* infection, as well as the emergence of drug-resistant isolates, has led to renewed emphasis on the prevention of pneumococcal disease.

A seven-valent conjugate vaccine (PCV7) (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F), which was first introduced in the USA [4–6], is now licensed throughout the world. Before introduction of PCV7 into the national immunization programme, invasive pneumococcal disease (IPD) had resulted in 175 000 hospitalizations and up to 12 500 deaths annually in the USA [7]. The vaccine now offers protection against seven of the more than 90 pneumococcal capsular serotypes, and includes those serotypes responsible for most infections in industrialized countries [5]. Because of geographical variations in serotype distribution, the vaccine may not be universally optimal, and knowledge of regional pneumococcal serotype distribution is therefore needed to evaluate the potential impact of vaccination in a population being immunized. The present study was undertaken to determine the serotype distribution of invasive *S. pneumoniae* isolated from children across Saudi Arabia and to correlate this distribution with susceptibility to penicillin, erythromycin and cefotaxime.

In total, 350 invasive *S. pneumoniae* isolates were collected between 2000 and 2004 by 25 microbiology laboratories throughout Saudi Arabia and was sent to King Saud University for characterization. Participating laboratories were non-randomly selected, to be as representative as possible of a wide range of patient demographics and diverse geographical locations, from both hospitals and community-based laboratories throughout Saudi Arabia. It was estimated that these hospitals provided inpatient services to approximately half the population.

All isolates were confirmed as *S. pneumoniae e* by colony morphology and haemolysis on blood agar plates, and by optochin sensitivity and bile solubility testing. The incidence rate of IPD was not calculated, because the isolates represented only a sample of all the invasive isolates during the study period.

Serotyping was performed by the standard capsular reaction test with the chessboard system and specific sera (Statens Serum Institute, Copenhagen, Denmark) [8]. All isolates were tested for antibiotic sensitivity against penicillin, erythromycin and cefotaxime using Etest strips (AB

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Biodisk, Solna, Sweden) according to the manufacturer's instructions and Mueller–Hinton agar supplemented with 5% sheep blood. The breakpoints recommended by the CLSI were used to interpret MIC values [9]. MIC value categories used for penicillin were as follows: sensitive, 0.01–0.1 mg/L; intermediate, 0.1–1.0 mg/L; and resistant, ≥2 mg/L [9]. Quality control strains (*S. pneumoniae e* ATCC 49619) were included with each run. Multidrug resistance was defined as resistance to the three antibiotics tested.

During the study period, 82% of the invasive isolates were recovered from blood and 18% from cerebrospinal fluid and other normally sterile fluids. Approximately 45% were isolated from children aged <2 years.

All invasive isolates were serotyped, with *c*. 15% (51, 14.6%) of them being non-typeable (omni-serum-positive, but pool-specific-negative and factor-specific sera-negative).

Table 1 shows the most common serotypes found among the invasive isolates from children aged <5 years. In children <2 years of age, serotypes 14, 23F, 6B and 19F were the most prevalent. Overall, 62% of isolates from all age groups and 83% of isolates from children aged <2 years were represented in PCV7. Serotypes of invasive *S. pneumoniae e* in relation to PCV7 serotypes are shown in Table 1.

**Table 1.** Frequency of different serotypes among the 350 invasive isolates collected from 25 Saudi hospitals between 2000 and 2004 from children aged 5 years and under

| Serotype | Children aged <2 years, no. (%) | Children aged between 2 and 5 years, no. (%) | Children aged 5 years, no. (%) | All children, no. (%) |
|---|---|---|---|---|
| 14 | 35 | 18 | 2 | 55 |
| 23F | 31 | 13 | 2 | 46 |
| 6B | 24 | 8 | 4 | 36 |
| 19F | 19 | 8 | 4 | 31 |
| 18C | 11 | 6 | 3 | 20 |
| 9V | 7 | 9 | 2 | 18 |
| 4 | 5 | 4 | 2 | 11 |
| VST | 132 (83.0) | 66 (62.3) | 19 (22.4) | 217 (62) |
| 19A | 3 | 1 | 2 | 6 |
| 3 | 2 | 1 | 4 | 7 |
| 24 | 2 | 3 | 2 | 7 |
| 15 | 2 | – | 4 | 6 |
| 6A | 1 | 1 | 4 | 6 |
| 23A | 1 | 2 | 3 | 6 |
| 7 | 1 | 2 | 3 | 6 |
| 11 | 1 | 4 | 1 | 6 |
| 23B | 1 | 1 | 3 | 5 |
| 5 | 1 | 2 | 2 | 5 |
| 22 | 1 | 3 | – | 4 |
| 12 | – | 1 | 6 | 7 |
| 8 | – | 1 | 5 | 6 |
| 1 | – | 3 | 2 | 5 |
| NVST | 16 (10.1) | 25 (23.6) | 41 (48.2) | 82 (23.4) |
| Non-typeable | 11 (6.9) | 15 (14.1) | 25 (29.4) | 51 (14.6) |
| Total | 159 | 106 | 85 | 350 |

VST, vaccine serotype; NVST, non-vaccine serotype.

Most invasive isolates collected from children <5 years of age had decreased susceptibility to penicillin, with 54% of the isolates being resistant to penicillin; 42% had intermediate resistance, and 12% were fully resistant. The frequency of resistance among invasive serotypes included in PCV7 is shown in Table 2.

Resistance to a third-generation cephalosporin (cefotaxime) was low (6%). Approximately 18% of the penicillin-resistant isolates were also resistant to erythromycin. Approximately 60% of the isolates were resistant to at least one of the three antibiotics tested. Four serotypes (6B, 19F, 23F and 14) accounted for 80% of the invasive isolates with resistance to one or more of the antibiotics tested (penicillin, erythromycin and cefotaxime). Five isolates (all serotype 19F) with penicillin MICs >8 mg/L were encountered.

Recent local studies in Saudi Arabia have shown that almost 60% of *S. pneumoniae e* isolates are non-susceptible to penicillin (46% penicillin-intermediate and 18% penicillin-resistant) [10]. This may be partially due to the misuse of antibiotics within the country, as antibiotics can be purchased without a prescription. Rates of resistance to macrolides, chloramphenicol and trimethoprim–sulphamethoxasole are 29%, 14% and 62%, respectively [10]. Local data further reveal that Saudi Arabia has four predominant clones, namely of serotypes 19F, 6B, 18C and 23F, which have been associated previously with decreased susceptibility to penicillin [11].

Previously published studies [12, 13] have shown that penicillin resistance in pneumococci isolated from children who are carriers is *c*. 18% in Saudi Arabia.

The potential serotype coverage of PCV7 in Saudi Arabia among children <5 years of age is 62%, and vaccine coverage significantly improved in children aged <2 years of age to reach 83% against IPD isolates. A recent study on serotypes from IPD patients in Riyadh, based on 75 isolates, has been reported [14]. Although it was found that serotypes 14, 23F and 4 were predominant, only a few isolates were from children <5 years of age [14].

Most penicillin-resistant isolates belonged to serogroups 9, 14, 19 and 23 and were also multiresistant. In contrast, intermediate resistance was found in other serotypes [15]. The finding that PCV7 included serotypes that accounted for the majority of the least penicillin-susceptible, as

**Table 2.** Distribution among 350 invasive pneumococcal isolates (vaccine serotype and non-vaccine serotype), according to antibiotic resistance

| | Penicillin, no. (%) | | | | Erythromycin[a], no. (%) | Cefotaxime[a], no. (%) |
|---|---|---|---|---|---|---|
| | S | I | R | Total | R | R |
| 14 | 20 | 31 | 4 | 55 | 13 | 4 |
| 23F | 19 | 21 | 6 | 46 | 19 | 3 |
| 6B | 15 | 14 | 7 | 36 | 16 | 4 |
| 19F | 4 | 19 | 8 | 31 | 11 | 3 |
| 18C | 7 | 12 | 1 | 20 | 5 | – |
| 9V | 6 | 9 | 3 | 18 | 3 | – |
| 4 | 6 | 4 | 1 | 11 | 3 | 2 |
| VST | 77 (35.5) | 110 (50.7) | 30 (13.8) | 217 (62.0) | 70 (32.3) | 16 (7.4) |
| 19A | 3 | 1 | 2 | 6 | 2 | – |
| 3 | 5 | – | 2 | 7 | 2 | – |
| 24 | 5 | 2 | – | 7 | 2 | – |
| 15 | 3 | 2 | 1 | 6 | 3 | – |
| 6A | 1 | 3 | 2 | 6 | 3 | 2 |
| 23A | 4 | 1 | 1 | 6 | – | 1 |
| 7 | 6 | – | – | 6 | – | – |
| 11 | 6 | – | – | 6 | – | – |
| 23B | 2 | 2 | 1 | 5 | 1 | – |
| 5 | 4 | – | 1 | 5 | 1 | 1 |
| 22 | 4 | – | – | 4 | – | – |
| 12 | 7 | – | – | 7 | – | – |
| 8 | 6 | – | – | 6 | – | – |
| 1 | 3 | 1 | 1 | 5 | 2 | 1 |
| Non-typeable | 25 | 25 | 1 | 51 | 5 | 0 |
| NVST | 84 (63.2) | 37 (27.8) | 12 (9) | 133 (38) | 21 (15.8) | 5 (3.8) |
| Total (VST + NVST) | 161 (46) | 147 (42) | 42 (12) | 350 (100) | 91 (26) | 21 (6) |

VST, vaccine serotype; NVST, non-vaccine serotype. [a]Erythromycin and cefotaxime resistance was based on the CLSI (2003) recommended breakpoints for MIC interpretation.

well as the multidrug-resistant, pneumococci is encouraging. Therefore, the vaccine could reduce the impact of penicillin-resistant strains on invasive disease and potentially control the rapidly emerging drug-resistant pneumococci in this part of the world.

In conclusion, this surveillance over a period of 4 years of invasive isolates has documented the serotype distribution and antibiotic resistance rates in invasive isolates among Saudi children. These data serve to substantiate the potential role for PCV7 in Saudi Arabia. Continued surveillance that monitors changes in serotype distribution over time will be important, particularly to observe changes that may result from the introduction of targeted or widespread vaccination programmes.

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## TRANSPARENCY DECLARATION

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# RESEARCH NOTE

## Carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* isolates in the United Arab Emirates

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### ABSTRACT

Five carbapenem-resistant *Acinetobacter baumannii* isolates, collected from the United Arab Emirates in 2006, were investigated to identify the mechanism(s) responsible for carbapenem resistance. Genotyping was performed by pulsed-field gel electrophoresis, and the location of the $bla_{OXA-23}$ gene was determined by using the endonuclease I *Ceu*I technique and mating-out assays. The four isolates in which the $bla_{OXA-23}$ gene was located on the chromosome within a Tn*2006* composite transposon were clonally related. The single non-clonally related isolate harboured the $bla_{OXA-23}$ gene on a 70-kb transferable plasmid. This study reports on the dissemination of OXA-23-producing *A. baumannii* isolates in the Middle East.

**Keywords** *Acinetobacter baumannii*, carbapenem resistance, OXA-23, transposon, United Arab Emirates

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Carbapenem resistance in *Acinetobacter baumannii* is increasingly being observed worldwide. Several studies have reported metallo-β-lactamases (MBLs) [1], but the most common mechanism is related to carbapenem-hydrolyzing class D β-lactamases (CHDLs) [2]. Three main acquired CHDL gene clusters have been described in *A. baumannii*, containing $bla_{OXA-23}$-like, $bla_{OXA-40}$-like or $bla_{OXA-58}$-like genes. The $bla_{OXA-23}$ gene is either plasmid-borne or chromosome-borne [3,4], and *A. radioresistens* was recently identified as the progenitor of $bla_{OXA-23}$-like genes [5].

From July 2006 to November 2006, five carbapenem-resistant *A. baumannii* isolates causing fatal infections in five patients were collected from the adult intensive-care unit of Sheikh Khalifa Medical City. Whereas two isolates were considered to be colonizing agents, three were involved in infections, two of which caused septicaemia. The age of the patients ranged from 30 to 60 years, and all of them had been treated with colistin and tobramycin, except patient 3, who was treated with colistin and tigecycline (Table 1).

The isolates were identified after 16S rRNA gene sequencing [6]. MICs of imipenem and meropenem were determined using antibiotic disks from Sanofi Diagnostics Pasteur (Marne-La-Coquette, France) [7] and were found to be 32 mg/L (Table 2). The isolates were resistant to almost all antibiotics, including aminoglycosides, fluoroquinolones and β-lactams (Table 2).

The production of MBLs was evaluated using the MBL Etest, combining imipenem and EDTA as recommended by the manufacturer (AB Biodisk,

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