

Emerging resistant serotypes of invasive *Streptococcus pneumoniae*

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Background: *Streptococcus pneumoniae* is the leading cause of meningitis and sepsis. The aim of the study was to analyze the distribution, vaccine serotype coverage, and antibiotic resistance of *S. pneumoniae* serotypes isolated from patients with invasive diseases, after the introduction of pneumococcal 7-valent conjugated vaccine (PCV-7).

Methods: A total of 134 isolates were collected from blood and cerebrospinal fluid specimens at Hamad Hospital during the period from 2005 to 2009. Isolate serotyping was done using the Quellung reaction. The prevaccination period was considered before 2005.

Results: The most common serotypes for all age groups were 3 (12.70%), 14 (11.90%), 1 (11.90%), 19A (9.00%), 9V (5.20%), 23F (5.20%), and 19F (4.50%). Coverage rates for infant <2 years for PCV-7, the 10-valent conjugated vaccine (PCV-10), and the 13-valent conjugated vaccine (PCV-13) were 34.78%, 52.17%, and 78.26%, respectively. Coverage rates of these vaccines were 50%, 67.86%, and 75% for the 2–5 years age group; 27.12%, 40.68%, and 64.41% for the age group 6–64 years; and 25%, 33.33%, and 66.67% for the ≥65 years age group, respectively. The percentage of nonsusceptible isolates to penicillin, cefotaxime, and erythromycin were 43.86%, 16.66%, and 22.81%, respectively. Thirty-seven isolates (32.46%) were multidrug resistant (MDR) and belonged to serotypes 14, 19A, 19F, 23F, 1, 9V, 12F, 4, 6B, 3, and 15A. Compared to previous results before the introduction of PCV-7, there was a significant reduction in penicillin-nonsusceptible *S. pneumoniae* from 66.67% to 43.86%, and a slight insignificant reduction in erythromycin nonsusceptible strains from 27.60% to 22.8%, while there was a significant increase in cefotaxime nonsusceptible strains from 3.55% to 16.66%.

Conclusion: Invasive pneumococcal strains and the emergence of MDR serotypes is a global burden that must be addressed through multiple strategies, including vaccination, antibiotic stewardship, and continuous surveillance.

Keywords: *Streptococcus pneumoniae*, Qatar, vaccine, serotype distribution, antibiotic resistance

Introduction

Streptococcus pneumoniae is one of the most common human pathogens, and prior upper respiratory tract colonization of the nasopharynx by *S. pneumoniae* is a prerequisite for invasive pneumococcal diseases (IPD).¹ It is the most common cause of community-acquired pneumonia, bacteremia, meningitis, otitis media, and other invasive diseases worldwide and is responsible for significant morbidity and mortality, particularly in young children.²

The World Health Organization (WHO) in 2005 estimated that pneumococcal disease is responsible for 1.6 million deaths annually, >800,000 of which are in children ≤5

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years of age mostly from developing countries.^{3–6} The spread of human immunodeficiency virus, which increases the risk of pneumococcal diseases up to 40-fold, has exacerbated the situation in some of these countries.⁷

There are 94 pneumococcal serotypes on the bases of their reactions with serum antibodies,^{8,9} and these vary according to age, race, and geography. Globally, 20 serotypes were associated with over 80% of IPD in different age groups, with 13 most common serotypes causing 70%–75% of invasive diseases in children. As a result, the WHO recommended the inclusion of pneumococcal conjugate vaccines in national immunization programs.⁶ At present, there are three pneumococcal conjugated vaccines, pneumococcal 7-valent conjugated vaccine (PCV-7), which includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, responsible for much pediatric IPD in many Western countries,¹⁰ 10-valent conjugated vaccine (PVC-10) (PCV-7 plus serotypes 1, 5, and 7F), and 13-valent conjugated vaccine (PCV-13) (PCV-10 plus serotypes 3, 6A, and 19A). PCV-7 was licensed in Qatar in August 2005, and PCV-13, in November 2010.

Initial treatment of pneumococcal disease was with optichin, a drug related to quinine that, although effective, had to be abandoned for clinical use because of ocular toxicity;¹¹ currently, it is used as a diagnostic tool for the identification of *S. pneumoniae* in the laboratory. Thereafter and for many years, penicillin provided very effective treatment for *S. pneumoniae* infections. The emergence of *S. pneumoniae* strains with resistance not only to penicillin, but also to other antibiotics has been reported in the last two decades.^{12,13} This has contributed to treatment failure, at least in pneumococcal meningitis.¹⁴ In the most recent Spanish cohort study, nonsusceptibility to penicillin was detected in 4.2% of IPD patients.¹⁵

Penicillin-resistant *S. pneumoniae* (PRSP) was first reported in 1967;¹⁶ subsequently, *S. pneumoniae* isolates with high level resistance to penicillin and multidrug resistance were reported in South Africa in the late 1970s.¹⁷ Higher prevalence rates of PRSP were reported in Europe and USA in 1990.^{18,19}

The resistance pattern of *S. pneumoniae* continues to evolve under the selective pressure of multiple factors, such as the indiscriminate use of antimicrobials,²⁰ clonal spread,²¹ and vaccination.^{22–24} More recently, certain emerging serotypes exhibit decreased susceptibility to several agents including ceftriaxone,²⁵ with the expansion of the multidrug resistant (MDR) population.²⁶

The aim of this study was to analyze serotype distribution of 134 invasive *S. pneumoniae* isolates in Qatar over a 4-year period, in the 7-, 10-, and 13-valent vaccines in different age groups and assess their antimicrobial susceptibility

pattern compared to that before the introduction of the PCV-7 vaccine.

Materials and methods

Patients and specimen collection

The isolates used in this study were collected from patients attending Hamad Hospital and emergency clinics at Hamad Medical Corporation, Qatar, during the period between January 2005 and March 2009. IPD was defined according to the Centers for Disease Control definition as isolation of *S. pneumoniae* from a normally sterile body site (eg, blood and cerebrospinal fluid [CSF]). Isolates of this study were isolated from blood and CSF by culturing on 5% sheep blood agar and incubating at 37°C in the presence of 5% CO₂ for 24 hours. Alpha-hemolytic colonies were subcultured and further identified as *S. pneumoniae* by their colony morphology, sensitivity to optochin (ethylhydrocupreine hydrochloride), bile-solubility, and catalase negative test. The 134 invasive strains of *S. pneumoniae* were isolated from blood and CSF of patients aged 4 months to 90 years. All isolates were stored in cryovials at –80°C until further analysis. Hamad Medical Corporation Research and Ethics Committee (Ref #7072/07) granted ethical approval to conduct this study and they waived the need for patient consent.

Antimicrobial susceptibility

For 118 isolates, susceptibility to chloramphenicol, cotrimoxazole erythromycin, and vancomycin was determined by the Kirby–Bauer disc diffusion method, and the results were interpreted according to CLSI 2012 guidelines.²⁷ The minimum inhibitory concentration (MIC) of penicillin and cefotaxime were determined using E-test method (AB Biodisk, Solna, Sweden) as recommended by CLSI. Reference strain with known susceptibility (*S. pneumoniae* ATCC 49619) was included as control. The MDR organism phenotype was defined as having diminished susceptibility to three or more antibiotics. Interpretation of the results was done according to CLSI 2012 recommendation for meningitis and nonmeningitis cases.

Serotyping

Serotyping of the isolates was done at the Naval American Medical Research Unit Number 3 (NAMRU3) in Cairo, Egypt, using the Quellung reaction.

Results

The serotype was determined for 122/134 (91%) invasive pneumococcal isolates (Table 1 and Figure 1); the most common seven serotypes were 3 (12.7%), 14 (11.9%), 1 (11.9%), 19A (9%), 9V (5.2%), 23F (5.2%), and 19F (4.5%). The most

Table 1 Serotype distribution of 134 invasive pneumococcal isolates by age group

Serotypes	Number of isolates per age group			
	<2 years	2–5 years	6–64 years	≥65 years
4	0	0	3	1
6B	1	1	1	0
9V	0	1	4	2
14	5	6	3	2
18C	0	1	0	0
19F	2	2	1	1
23F	0	3	4	0
1	2	4	7	2
5	1	1	1	0
7F	1	0	0	0
3	2	1	11	3
6A	0	0	0	1
19A	4	1	3	4
G	0	0	0	1
9A	0	1	0	0
12F	0	1	2	1
18F	0	0	1	1
23 A	0	0	0	1
24F	1	0	0	0
8	0	1	2	0
6A/B	0	0	0	2
22F	0	0	1	0
34	0	0	2	0
15A	0	0	0	1
35B	2	1	0	0
31	0	0	1	0
18	0	1	1	0
7C	0	0	1	1
33F	0	0	1	0
6C	0	0	1	0
Not determined	2	2	7	1
Total	23	28	58	25

common serotypes in children ≤5 years were 14, 19F, 23F, 19A, 1, and 3, and for the 6–64 years age group were 14, 1, 19A, 12F, 3, 9V, 8, and 23F, while in elderly patients ≥65 years, the most common serotypes were 19A, 3, 1, 14, and 9V.

Vaccine coverage is shown in Figure 2. For patients <2 years, vaccine coverage rates for PCV-7, PCV-10, and PCV-13 were 34.78%, 52.17%, and 78.26%, respectively; coverage rates of these vaccines were 50%, 67.86%, and 75% for those aged 2–5 years age; 27.12%, 40.68%, 64.41% for those aged 6–64 years; and 25%, 33.33%, and 66.67% for the ≥65 years age group, respectively.

Susceptibility pattern is available for 118 strains against penicillin, cefotaxime, chloramphenicol, erythromycin, co-trimoxazole, tetracycline, and vancomycin and is listed in Table 2. Penicillin MIC for all isolates ranged from 0.008 to 4.0 µg, and, for cefotaxime, MIC ranged from 0.008 to 3.0 µg.

(Figure 3). On the basis of the oral penicillin breakpoints, 66 (55.93%) were susceptible to penicillin, 40 (33.90%) were intermediate resistant, and 12 (10.17%) resistant to penicillin. A total of 52 isolates (44.06%) were nonsusceptible to penicillin (resistant and intermediate resistant; penicillin-nonsusceptible *S. pneumoniae* [PNSP]). On the basis of the meningitis breakpoints, 97 (83.05%) were susceptible to cefotaxime, 19 (15.25%) were intermediate resistant, and 2 (1.69%) were resistant, ie, 17.80% cefotaxime nonsusceptible *S. pneumoniae* (CNSP). While applying the nonmeningitis breakpoints 107 (90.68%) were susceptible, 5 (4.24%) were intermediate resistant, and 2 (1.69%) were resistant to cefotaxime (5.93% CNSP). Resistance to chloramphenicol, co-trimoxazole, erythromycin, and tetracycline was 11.86%, 56.78%, 22.85%, and 38.14%, respectively. None of the isolates was resistant to vancomycin.

Table 2 shows that penicillin-nonsusceptible SP isolates were more likely to be resistant to other antibiotics than susceptible strains, with the exception of chloramphenicol, which showed good susceptibility to both penicillin-susceptible and penicillin-nonsusceptible isolates. Thirty-nine (33.05%) isolates were MDR. Serotyping was available only for 31 isolates. Serotypes distribution of these MDR isolates is shown in Figure 4. Predominant MDR serotypes were 14, 19A, 19F, and 23F (58.97% of all MDR isolates).

The susceptibility rate to penicillin, cefotaxime, and erythromycin of the isolates in this study was compared with the result of the previous study (1999–2000).²⁸ There was a significant reduction in PNSP from 66.67% to 43.86%, and a slight insignificant reduction in erythromycin nonsusceptible strains from 27.60% to 22.8%, while there was a significant increase in cefotaxime nonsusceptible strains from 3.55% to 17.80%.

Discussion

Diseases caused by *S. pneumoniae* are a major public health problem worldwide. The increasing difficulty in the management of pneumococcal disease has led to adoption of preventative measures against infection by *S. pneumoniae*. In the developing countries, young children and the elderly are most affected, and resistance to antimicrobials is a serious and rapidly increasing problem worldwide. In a previous study²⁸ conducted in Qatar in 1999–2000, the PCV-7 vaccine provided only 52% coverage for infants <2 years. The most prevalent serotypes in this age group during that study were 4, 6A, 6B, 11A, 14, 19A, 19F, and 23F. In this study, there is increased isolation of serotypes 1 and 3 that are covered by PCV-13, and PCV-10 covers only serotype 1, whereas PCV-7

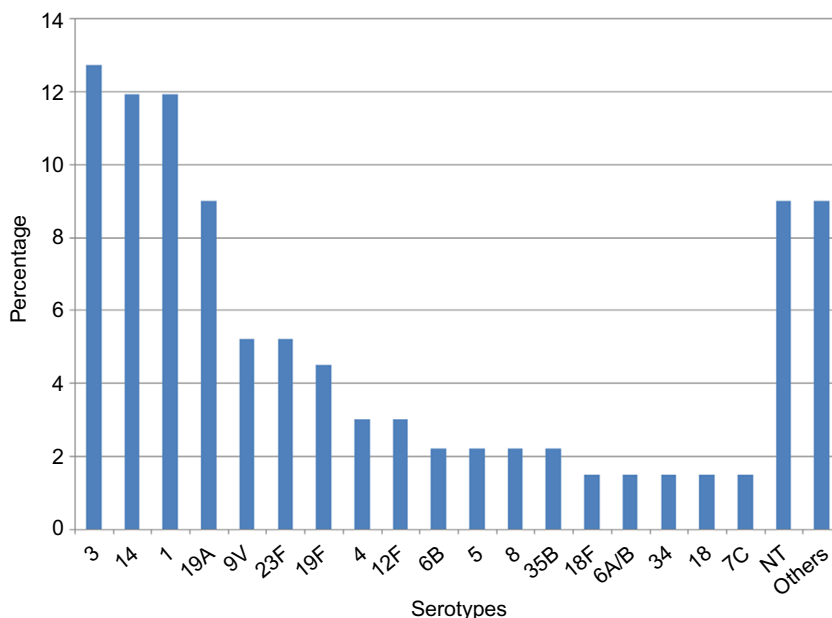


Figure 1 Serotype distribution of 134 invasive pneumococcal isolates.
Note: Others: 18C, 7F, 6A, G, 9A, 23A, 24F, 22F, 15A, 31, 33F, and 6C.
Abbreviation: NT, not determined.

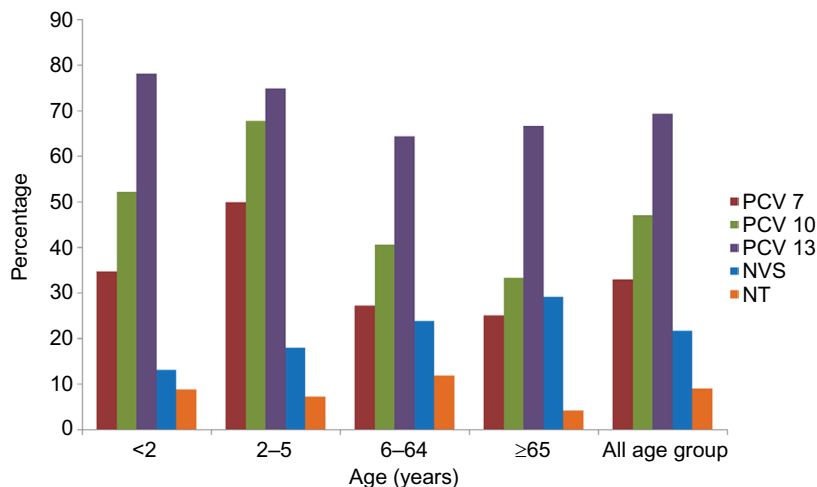


Figure 2 Vaccine coverage of 134 invasive pneumococcal isolates.
Abbreviations: NVS, nonvaccine serotypes; PCV-7, pneumococcal 7-valent conjugated vaccine; PCV-10, 10-valent conjugated vaccine; PCV-13, 13-valent conjugated vaccine; NT, not determined.

Table 2 Antimicrobial susceptibility of 118 invasive pneumococcal isolates

Antibiotics	All isolates (n=118)			Penicillin-susceptible isolates (n=66)			Penicillin-nonsusceptible isolates (n=52)		
	S	I	R	S	I	R	S	I	R
Penicillin	66 (55.93%)	40 (33.90%)	12 (10.17%)						
Cefotaxime	97 (83.05%)	19 (15.25%)	2 (1.69%)	66 (100%)	0 (0%)	0 (0%)	32 (61.54%)	18 (34.62%)	2 (3.85%)
Chloramphenicol	104 (88.13%)	0 (0%)	14 (11.86%)	56 (84.85%)	0 (0%)	10 (15.15%)	48 (92.31%)	0 (0%)	4 (7.69%)
Co-trimoxazole	39 (33.05%)	12 (10.17%)	67 (56.78%)	31 (46.97%)	8 (12.12%)	27 (40.91%)	7 (13.46%)	4 (7.70%)	35 (67.31%)
Erythromycin	90 (76.27%)	1 (0.85%)	27 (22.85%)	62 (93.94%)	0 (0%)	4 (6.06%)	28 (53.84%)	1 (1.92%)	17 (32.70%)
Tetracycline	68 (57.63%)	5 (4.24%)	45 (38.14%)	48 (72.73%)	4 (6.06%)	14 (21.21%)	19 (36.54%)	1 (1.92%)	32 (61.54%)
Vancomycin	118 (100%)	0 (0%)	0 (0%)	66 (100%)	0 (0%)	0 (0%)	52 (100%)	0 (0%)	0 (0%)

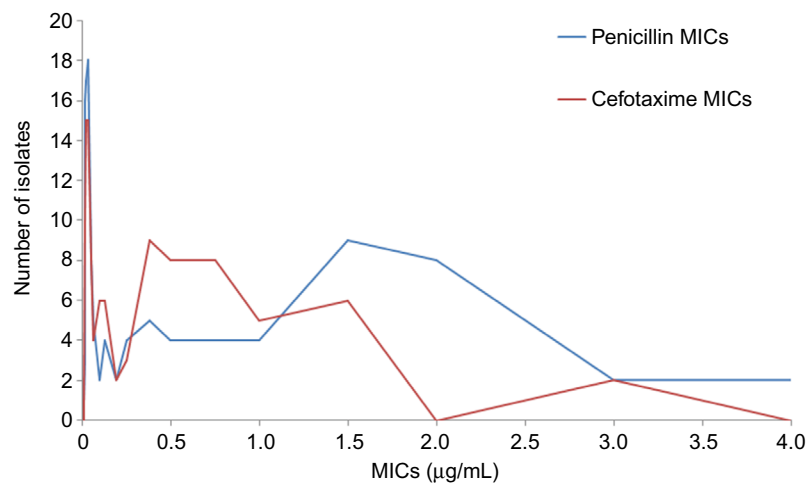


Figure 3 MICs (µg/mL) of 118 invasive pneumococcal isolates for penicillin and cefotaxime.

Abbreviation: MICs, minimum inhibitory concentrations.

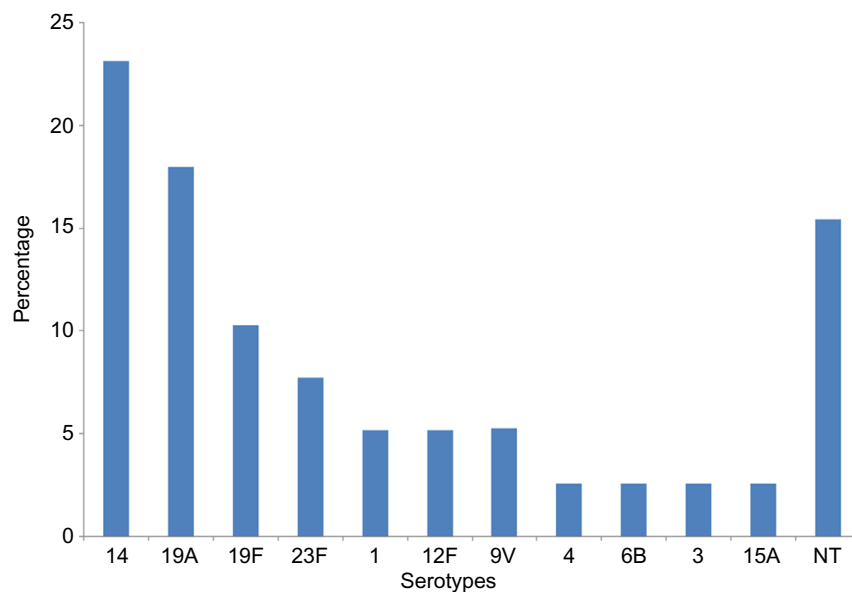


Figure 4 Percentage distribution of 31 MDR serotypes of invasive pneumococcal isolates.

Abbreviations: MDR, multidrug resistant; NT, not determined.

does not cover serotypes 1 and 3. The most common serotypes reported in the region from infants <2 years were 6B, 6A, 14, 5, and 23F in Egypt, and 3, 4, 6, 14, and 23 in Saudi Arabia,^{29,30} while in this study, the most common serotypes seen in infants <2 years were 14, 19A, 19F, 1, and 3. The heptavalent vaccine only covers the second most common serotype of *S. pneumoniae*.

Interestingly, this study also showed marked reduction of PCV-7 coverage in the <2 years age group from 52% to 34.78%. After mass vaccination has been introduced, IPD due to the vaccine serotypes, such as 6A, have tended to decrease in both young children and other age groups due to herd immunity. This marked decrease in PCV-7 serotypes

was reported recently in France.³¹ This suggests that the use of the PCV-7 may have prevented infection caused by the vaccine serotypes. However, reduction of PCV-7 types was accompanied by the emergence of non-PCV-7 serotypes, a phenomenon that was observed by others.^{30,32–35} This information supports the switch from PCV-7 vaccination to PCV-13 immunization program. The rise in non-PCV-7 types may have been a consequence of the disappearance of the PCV-7 types. Nonetheless, this approach has the advantage of acknowledging that the serotype epidemiology has changed, as well as indicating that continued prevention of PCV-7 types is important.³⁶ Overall coverage of PCV-13 to all age groups is up to 69.40%, and for the <2 years age group it was 78.26%.

Prevalence of PNSP varied widely among countries. In Asia, PNSP rates in 2000–2001 ranged from 78% to 92%,³⁷ and markedly decreased to 25% during 2008–2009.³⁸ Other regions also showed moderately high rates of PNSP based on the pre-2008 breakpoints. In Southern and Eastern Mediterranean regions, the overall PNSP rate was 25% in 2003–2005,³⁹ and in Latin America, PNSP rates was 30.5%–38.8%.⁴⁰

In Qatar, the rate of PNSP was reported previously in 1999–2000 as 60%²⁸ (applying the 2012 CLSI interpretative criteria, it will be 66.67%), while in this study the rate was 44.07%. During the study period, injectable antibiotics were used only in the inpatients, but for the outpatients oral antibiotics were over the counter, which may have some impact on resistance. This decline in penicillin nonsusceptibility may be due to the introduction of the PCV-7 vaccine in 2005. This phenomenon was reported by others who demonstrated a substantial decline in PNSP prevalence after the introduction of PCV-7 vaccine, followed by an increase in prevalence as vaccine serotypes were replaced by nonvaccine serotypes.^{41–45} The use of cefotaxime/ceftriaxone instead of penicillin for empirical treatment of *S. pneumoniae* infections and the replacement of penicillin prophylaxis with vaccination may have also contributed to this significant reduction in PNSP.

Erythromycin nonsusceptible *S. pneumoniae* (ENSP) levels decreased slightly from 30% in 1999–2000 to 23.73% in this study. Stable or increasing ENSP were observed in countries without introduction of PCV-7 vaccine in the national immunization program,^{28,46,47} but declined in countries like Germany following the introduction of PCV-7 in 2006.⁴⁸ CNSP increased from 2.6% (or 3.55% using the 2012 meningitis breakpoints) to 17.80% in this study.

Previous studies have shown that levels of antimicrobial resistance are directly proportional to antibiotic consumption in the community.^{38,49} Some of the first isolates of PRSP were found in a village in New Guinea after prophylactic penicillin had been used in an attempt to reduce the incidence of pneumococcal disease.⁵⁰ Additional associations were found between uses of other antibiotics, ie, cross-resistance between antibiotic classes, eg, macrolide, other β -lactams.⁴⁰

MDR *S. pneumoniae* infection developed in two vaccinated patients. One was a 12 years old female who received the 23-valent vaccine; she developed severe sinusitis with septicemia due to serotype 12F. The second patient was a 4 months old female vaccinated with the PCV-7 vaccine; she developed severe meningitis due to serotype 19A, which is not included in the PVC-7 but included in the PVC-13.

An alarming situation is the increase in resistance to third-generation cephalosporins – the mainstay agent for

the treatment of meningitis, severe cases of pneumonia, and other serious diseases. The important strategy to minimize the development of resistance is to further decrease the indiscriminate use of antibiotics,⁵¹ and introduce a vaccine that covers more serotypes, eg, PVC-13.

The introduction of PCV-7 conjugate vaccine not only resulted in reduction of the prevalence of covered serotypes but also resulted in the emergence of serotypes not included in the PCV-7. The usage of antibiotics and the uptake of PCV-7 vaccine were associated with changes in the prevalence of antibiotic-resistant *S. pneumoniae* as well. Most countries reported a decrease in antibiotic-resistant *S. pneumoniae* after the introduction of the PCV-7, and at the same time, the overall usage of antibiotics decreased. Although penicillin and erythromycin resistance is decreasing, cefotaxime resistance is on the rise due to the increased use of this antibiotic. The introduction of the PCV-13, which offers 78.26% protection for infants younger than 2 years, and 66.67% for elderly patients ≥ 65 years of age, is recommended. Routine immunization in children may also give protection to others by herd immunity.⁵²

Regular surveillance is necessary since changes in serotypes may occur naturally with time as well as with replacement by nonvaccine serotypes in response to vaccine pressure. After the introduction of PCV-7, a serotype replacement phenomena and persistence of PCV-7 serology were documented.⁵³

Conclusion

In conclusion, IPD and the development of emerging antibiotic-resistant serotypes of *S. pneumoniae* is a global burden that must be addressed through multiple strategies, including national immunization program, antibiotic stewardship, and ongoing surveillance.

Disclosure

The authors report no conflicts of interest in this work.

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