International Journal of Infectious Diseases 40 (2015) 95–101

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Invasive pneumococcal disease among children younger than 5 years of age before and after introduction of pneumococcal conjugate vaccine in Casablanca, Morocco

Idrissa Diawara [a,b,*], Khalid Zerouali [a,b], Khalid Katfy [a,b], Bahija Zaki [b], Houria Belabbes [a,b], Jillali Najib [c], Naima Elmdaghri [a,b,d]

[a] Department of Microbiology, Faculty of Medicine and Pharmacy, 19 rue Tarik Bnou Zyad, Casablanca, Morocco
[b] Bacteriology-Virology and Hospital Hygiene Laboratory, University Hospital Centre Ibn Rochd, 1, Rue des Hôpitaux, Casablanca, Morocco
[c] Department of Pediatric Infectious Diseases and Clinical Immunology, University Hospital Centre Ibn Rochd, Casablanca, Morocco
[d] Pasteur Institute of Morocco, 1 Louis Pasteur place, Casablanca, Morocco

ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 27 August 2015
Accepted 25 September 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Streptococcus pneumoniae
Invasive pneumococcal disease
Pneumococcal conjugate vaccine
Antibiotic resistance

ABSTRACT

Objectives: The purpose of this study was to compare the incidence rate of invasive pneumococcal disease, the rates of antibiotic resistance and serotype distribution among children ≤5 years old before and after PCVs introduction in Casablanca, Morocco.
Methods: This study was conducted at the Ibn Rochd University Hospital Centre of Casablanca during two periods encompassing pre-and post-implementation of PCVs, respectively from January 2007 to October 2010 and from January 2011 to December 2014. All the non-duplicate invasive *S. pneumoniae* isolates recovered during the study periods were included.
Results: There were 136 cases of IPD, 91 before and 45 after PCVs introduction. The greatest decrease in incidence rate of IPD occurred in children ≤ 2 years of age declining from 34.6 to 13.5 per 100,000 populations ($p < 0.0001$) before and after vaccination, respectively. The incidence rate of PCV-7, PCV-10 non-PCV-7 and PCV-13 non-PCV-10 serotypes decrease significantly from 18.0 to 4.6, from 5.7 to 1.3 and from 5.7 to 0.8/100,000 population ($p < 0.001$) in the same age, respectively.
Conclusion: Shifts in the distribution of IPD serotypes and reductions in the incidence rate of disease suggest an effective reduction of the burden of IPD in children, but continued high quality surveillance is critical to assess the changes in serotype distributions.

1. Background

Streptococcus pneumoniae (*S. pneumoniae*), an encapsulated, Gram-positive cocci, is a member of the normal upper airway flora but can cause life-threatening illnesses.[1] *S. pneumoniae* is one of the leading bacterial causes of community-acquired pneumonia, acute otitis media, sinusitis, bloodstream infection (bacteraemia), sepsis and meningitis. Invasive pneumococcal diseases (IPD) like meningitis and bacteraemia are major causes of morbidity and mortality, especially among young children (≤ 5 years old) and elderly adults (≥60 years old). Disease rates and mortality are higher in developing than in industrialized settings, with the majority of deaths occurring in Africa and Asia.[2–3] The World Health Organization (WHO) estimated that 1.6 million persons are dying of pneumococcal diseases each year, of which 0.7–1 million are children less than five years of age living in the developing world.[4]

Furthermore, there has been a consistent worldwide increase in antibiotic resistance of *S. pneumoniae* isolates, making therapeutic options more difficult.[5] Currently, 94 capsular serotypes (grouped into 46 serogroups based on immunologic similarities), including the recently reported serotypes 6C, 6D, 11E, and 20A/20B, have been identified.[6–9] The serotype distribution can vary by patient age, geographic region, and time period of surveillance. However, many of these serotypes are rarely recovered from serious diseases,

* Corresponding author. Department of Microbiology, Faculty of Medicine and Pharmacy, 19, Tarik Ibnou Ziad, Casablanca.
Tel.: +212 648490217 / +212 681008541; fax: +212 522271630, Casablanca, Morocco.
E-mail addresses: diawaraidris@gmail.com (I. Diawara), khalid.zerouali2000@gmail.com (K. Zerouali), khalidkatfy@hotmail.com (K. Katfy), zaki.bahija@yahoo.fr (B. Zaki), belhor2001@yahoo.fr (H. Belabbes), jilalinajib@hotmail.com (J. Najib), naimaelmdaghri@yahoo.fr (N. Elmdaghri).

http://dx.doi.org/10.1016/j.ijid.2015.09.019

and only around twenty serotypes cause the majority of IPD worldwide.[10–11]

Currently, there are two different types of pneumococcal vaccines marketed: 23-valent pneumococcal polysaccharide vaccines (PPV-23) available since the early 1980s and pneumococcal conjugate vaccines (PCV). The first one of the PCVs, 7-valent PCV (PCV-7), including capsular polysaccharides of serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F, was first licensed in the United States (US) in 2000.[12] All PCVs under development target these same seven serotypes, but with different additional serotypes added to subsequent formulations to expand the serotype coverage.[3] For example, PCV-7 did not include serotypes 1, 3, and 5, which are common in Europe, Asia, and Africa.[10,13] Thus, new conjugate vaccines PCV-10 (4, 6B, 9 V, 14, 18C, 19F, 23F, 1, 5, and 7F) and PCV-13 (4, 6B, 9 V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A, and 19A) have been introduced to replace PCV-7 and expand protective coverage. WHO recommends the inclusion of PCVs in childhood immunization programs worldwide.[4] Serotyping can be used to monitor epidemiological trends following the introduction of serotype-specific PCVs, which include serotypes commonly encountered in carriage and disease.

In Morocco, the PCV-13 was introduced in the national immunization program (NIP) in October 2010 in 2 + 1 schedule and replaced, based on the cost, by the PCV-10 in July 2012. Before the PCV introduction, serotypes included in PCV-10 and PCV-13 covered 71.6 and 82.4% of serotypes associated with IPD among children under five in Casablanca, respectively.[14] The objective of this present study was to compare the incidence rate of IPD as well as the rates of antibiotic resistance and serotype distribution among children under five before and after PCVs introduction in Casablanca, Morocco.

2. Materiel and methods

2.1. Study design and population

This study is based on a laboratory-based surveillance of IPD among hospitalized children ($\leq$ 5 years old) living in Casablanca. In order to clearly analyze the target population, we stratified the children in two age groups: children $\leq$ 2 years old and children >2-5 years old. The study was conducted during two periods encompassing pre-and post-implementation of PCVs from January 2007 to October 2010 and from January 2011 to December 2014, respectively.

The months of November and December 2010 were arbitrarily chosen as a transitional period. During which there was the awareness campaign for vaccination and the distribution of vaccination materials in the different health centers of Grand Casablanca. We also considered these two months to allowing the establishment of a post vaccination surveillance program at Grand Casablanca.

The study was performed at the Microbiology Laboratory of Ibn Rochd University Hospital Centre of Casablanca (IR-UHC), which is a tertiary care hospital, comprising 1451 beds (this bed capacity represents 52% of capacity management of patients living in Grand Casablanca). In the IR-UHC, pediatric patients are managed at the Abderrahim Harrouchi Children University Hospital, which is a department comprising 240 beds. Additionally, the IR-UHC is the only tertiary hospital covering the entire region of Grand Casablanca, which is a Moroccan region located on the Atlantic coast, west-central Morocco. This region covers an area of 1,140.54 km^2. The Grand Casablanca population was estimated 4,270,750 in 2014, which represents 12.6% of the national population of Morocco as provided by the High Commission for Planning of the National Centre of Documentation in Morocco. In Grand Casablanca, all cases of serious diseases such as meningitidis and complicated diseases in other hospitals are systematically transferred to the IR-UHC. The microbiology laboratory of this tertiary care hospital started laboratory-based surveillance of f IPD since 1994 and it is the only laboratory of which the capacity of pneumococcal serotyping is established in Casablanca. The microbiology laboratory of IR-UHC collaborates strongly with regional hospitals, clinics and private medical laboratories for confirmation of invasive pneumococcal strains and for serotyping.

For the specific target group of this study, Census data were available for 2008, 2013 and 2014 while values estimated from a standard curve determined by linear regression were used for the remaining years with the population growth rate of 0.9% for the study period.

2.2. Isolate collection

All the non-duplicate invasive *S. pneumoniae* isolates recovered during the study periods were included. Isolates obtained from normally sterile sites (cerebrospinal fluid [CSF], blood, pleural fluids, and articular fluids) were considered invasive. When an isolate was recovered from CSF and blood, the case was categorized as meningitis. *S. pneumoniae* isolates were identified following standard procedures of bacteriology (i.e., α- hemolysis, optochin susceptibility and bile solubility).

Each pneumococcal isolate is reported on a laboratory report form including demographic and medical information on the patient. For post-vaccination period, the vaccination history: vaccinated or not by a PCV, number of doses was added to describe immunization status. Data collected on the laboratory report forms are entered on WHONET 5.6 Software.

2.3. Pneumococcal serotyping

Serogrouping was done by the checkerboard method with Pneumotest-latex (Statens Serum Institute antisera, Copenhague, Denmark) and serotyping was performed by Quellung capsule swelling using Statens Serum Institute antisera (Copenhague, Denmark).

2.4. Antimicrobial susceptibility

Antibiotic susceptibility testing was done following Clinical Laboratory Standard Institute guidelines.[15] Erythromycin, tetracycline, choramphenicol and trimethoprim-sulfamethoxazole (cotrimoxazole) were tested by disk diffusion with antibiotic disks from Oxoid (Basingstoke, United Kingdom) on Mueller Hinton Agar supplemented with 5% sheep blood (BioMèrieux, Lyon, France). Oxacillin (1 μg) was used for screening of penicillin non-susceptible *S. pneumoniae* (PNSP). A minimal inhibitory concentration (MIC) for penicillin G and ceftriaxone was determined on 5% sheep blood Mueller Hinton agar with E-tests from Oxoid (Oxoid, Basingstoke, UK). The breakpoints used for interpretation were those recommended by the CLSI in 2012: $\leq$ 0.06 μg/ml and $\geq$2 μg/ml for penicillin; for ceftriaxone, $\leq$ 0.5 μg/ml and $\geq$2 μg/ml for meningeal isolates and $\leq$1 μg/ml and $\geq$4 μg/ml for non-meningeal isolates. Quality control was conducted using *S. pneumoniae* ATCC 49619.

2.5. Estimation of incidence rate and statistical analysis

We estimated the annual incidence rate of IPD, vaccine and non-vaccine serotypes per 100,000 populations by dividing the number of laboratory confirmed IPD, vaccine and non-vaccine serotypes by the number of population of the target group ($\leq$ 2 years old or >2 – 5 years) per year. Computerized data were analyzed with WHONET5.6, EpiInfo 7 (Centers for Disease Control, Atlanta, Georgia, USA) and Microsoft Excel. The chi square test or

Fisher's exact test was performed to compare proportion between different age groups and different collection periods. Statistical significance was determined with 95% confidence internals (CI). Change in incidence rate between the pre- and post- vaccination periods were assessed by calculating absolute risks reduction and relative risks reduction (ARR and RRR).[27] Differences were considered significant if the p-value was < 0.05.

2.6. Ethics statement

This study was approved by the Ethical committee for biomedical research of the University Mohammed V - Soussi, Faculty of Medicine, Pharmacy and Dental Medicine of Rabat, Morocco. The patients/legal guardians were informed about the study; they signed a consent form, and the study was carried out in an anonymous way.

3. Results

3.1. Isolate information and incidence rate of IPD

The number of isolates per year, by clinical presentation, and by age group are summarized in Table 1. A total of 136 *S. pneumoniae* isolates were recovered from 136 children (sex ratio 1): there were 91 during the pre-implementation period (79 from children $\leq$ 2 years old and 12 from children >2-5 years old) and 45 during the post-implementation period (32 from children $\leq$ 2 years old and 13 from children >2-5 years old). The incidence rate of IPD changed after the introduction of PCV. The overall annual incidence rate of IPD decreased significantly from 34.6 (95% CI, 27.8 – 43.1) to 13.5 (95% CI, 9.6 – 19.1; RRR = – 60.9%; $p < 0.0001$) per 100,000 populations among children $\leq$ 2 years of age before and after vaccination, respectively (Table 1). The rate of IPD for the pre- and post-vaccination period showed a non-significant change for children >2 - 5 years of age.

3.2. Serotype of S. pneumoniae recovered from IPD

The serotypes were determined for the 136 strains of *S. pneumoniae*. The incidence rate of serotype before and after PCV-13 and PCV-10 vaccines introduction in Casablanca (Table 2) showed that the overall incidence rate of IPD caused by PCV-7, PCV10-nonPCV7 serotypes and PCV13-nonPCV7 serotypes declined significantly among children $\leq$ 2 years of age from 18.0 to 4.6 ($p < 0.0001$); from 5.7 to 1.3 ($p = 0.02$) and from 5.7 to 0.8 ($p = 0.003$) per 100,000 populations, respectively. In the same age group, we observed a slight increase but non-significant of the non-vaccine type from 5.3 to 6.8 per 100,000 populations (RRR = + 28.6%). In children >2 – 5 years old, we observed a non-significant change in PCV-7, PCV-13 and PCV-10 serotypes according to the two periods of this study.

Overall, the leading serotypes causing IPD in children $\leq$ 2 years of age were 14 (16.1%), 6B (13.9%), 19A (11.4%), 19F (8.9%), 23F (8.9%) and 5 (7.6%) before vaccination. Only serotype 6B (18.7), 14 (12.5) and 1 (6.25) persist after vaccination for the same age group. The major serotype isolated from children >2 – 5 years of age were: 19F (25%) and 1(8.33%). There were no major changes in the incidence rate of relative-vaccine and non-vaccine serotypes before and after vaccine implementation **(data not shown, see additional file)**.

Analysis of the immunization status of our patients in the post-vaccination period showed that 40% (10/25) of the children under five received at least one dose on PCVs (Table 3) and 60% are unvaccinated. Immunization schedule was incomplete for all cases. Serotypes found in vaccinated children were: 6B (3), 14 (2), 1 (2), 7F (1), 3 (1) and 9 V (1). Clinical diagnosis of these vaccine serotypes was mainly from meningitis (3), bacteraemia (3), and pneumonia (2).

3.3. Antimicrobial resistance

The rate of resistance of pneumococcal strains isolated in the two age groups before and after vaccine introduction is shown in the Table 4. We observe significant decrease of PNSP and cotrimoxazole non-susceptible strains.

There were no significant changes in antimicrobial resistance rates between pre and post-vaccination periods for erythromycin, tetracycline, chloramphenicol ($p > 0.05$) for the two age groups. A significant reduction of penicillin and cotrimoxazole non-susceptible strains occurred in children under 2 years old. The proportion changed from 50.6% to 21.9% ($p = 0.005$) and from 39.2% to 6.3%

Table 1
Sample origin and IPD incidence according to age groups before and after introduction of pneumococcal conjugate vaccine in Casablanca (Morocco)

Age and specimen sources	Pre-implementation[a] 2007-2010 no. of cases/ 100,000 populations	Post-implementation[b] no. of cases/100,000 populations					Baseline (2007 - 2010) vs Post period (2011-2014)		
		2011	2012	2013	2014	Total (2011-2014)	Absolute Risk Reduction cases/100,000 populations (95% CI)	Relative Risk Reduction % (95% CI)	p-value
≤ 2 years									
CSF[c]	12.3	8.6	5.1	8.4	5.0	6.8	−5.5 (−11.5 to 0.2)	−44.9 (−94.2 to 1.3)	0.05
Blood	17.9	6.9	6.8	6.7	5.0	6.3	−11.6 (−18.5 to −5.4)	−64.7 (−100.0 to – 29.8)	0.003
Pleural fluid	2.2	0.0	0.0	0.0	0.0	0.0	−2.19 (−5.1 to −0.1)	−100.0	0.02
Other[d]	2.2	0.0	1.7	0.0	0.0	0.04	−1.8 (−4.7 to 0.6)	−80.7 (−97.7 to 65.1)	NS
Total	34.6	15.4	13.6	15.1	0.0	13.5	−21.1 (−30.5 to −12.3)	−60.9 (−88.1 to −35.5)	<0.0001
> 2 − 5 years									
CSF	0.6	0.4	0.4	0.0	0.4	0.3	−0.3 (−1.1 to 0.4)	−51.8 (−87.7 to 92.8)	NS
Blood	0.5	0.4	0.4	0.4	1.2	0.6	0.08 (−0.7 to 0.9)	15.74 (−64.7 to 279.2)	NS
Pleural fluid	0.1	0.4	0.0	0.8	0.4	0.4	0.3 (−0.2 to 0.9)	−285.0 (−56.9 to 335.2)	NS
Other	0.0	0.0	0.0	0.0	0.0	0.0	0 (−0.4 to 0.4)	–	NS
Total	1.2	1.2	0.8	1.2	2.0	1.3	0.06 (−1.0 to 1.1)	4.5 (−52.3 to 128.9)	NS

[a] Pre-vaccination periods were from January 2007 to October 2010. The year 2010 begins in January 2010 and ends in October 2010.
[b] Post vaccination periods were from January 2011 to December 2014.
[c] Cerebrospinal fluid.
[d] Other sterile sites (articular fluid, pus/tissues).
Incidences were calculated as incidence = Number of IPD cases x 100,000/population (≤ 2 years old or 2-5 years old) during the years of surveillance at Casablanca.
The Grand Casablanca population was estimated at 56,319 in 2007 and 59,993 in 2014 for children ≤ 2 years, and 237,278 in 2007 and 252,750 in 2014 for children >2 – 5 years old.
NS: not significant.

Table 2
Incidence of vaccine and non-vaccine serotypes according to age groups before and after introduction of PCVs in Casablanca, Morocco

Age and serotypes	Pre-implementation[a] 2007-2010 no. of cases/100,000 populations	Post-implementation[a] 2011 - 2014 no. of cases/100,000 populations					Baseline (2007 - 2010) vs Post period (2011-2014		
		2011	2012	2013	2014	Total (2011-2014)	Absolute Risk Reduction cases/100,000 populations (95% CI)	Relative Risk Reduction % (95% CI)	p-value
≤ 2 years old									
PCV7 serotypes[b]	18.0	1.7	8.5	8.4	0.0	4.6	−13.3 (−20.0 to −7.3)	−74.1 (−100.1 to −40.8)	<0.0001
PCV10-nonPCV7 serotypes[c]	5.7	1.7	0.0	0.0	3.3	1.3	−4.4 (−8.6 to −1.0)	−77.7 (−93.6 to −22.0)	0.02
PCV13-nonPCV10 serotypes[d]	5.7	1.7	1.7	0.0	0.0	0.8	−4.8 (−8.9 to −1.6)	−85.2 (−100.6 to −27.9)	0.003
Non-PCV13 serotypes[e]	5.3	10.3	3.4	6.7	6.7	6.8	1.5 (−3.2 to 6.3)	28.6 (−61.1 to 100.2)	NS
TOTAL	34.6	15.4	13.6	15.1	10.0	13.5	−21.1 (−30.5 to − 12.3)	−60.9 (−88.1 to −35.5)	<0.0001
> 2 - 5 years old									
PCV7 serotypes	0.6	0.04	0.4	0.4	0.4	0.4	−0.2 (−1.0 to 0.5)	−53.7 (−81.8 to 128.0)	**NS**
PCV10-nonPCV7 serotypes	0.3	0.0	0.4	0.8	0.0	0.3	−0.01 (−0.6 to 0.6)	−3.5 (−80.5 to 300.7)	**NS**
PCV13-nonPCV10 serotypes	0.2	0.08	0.0	0.0	0.0	0.2	−0.01 (−0.6 to 0.5)	−3.5 (−86.4 to 584.7)	**NS**
Non-PCV13 serotypes	0.2	0.0	0.0	0.0	1.6	0.4	0.3 (−0.2 to 0.9)	285.8 (−56.9 to 335.2)	**NS**
TOTAL	1.2	0.1	0.8	1.2	2.0	1.3	0.06 (−1.0 to −1.1)	4.5 (−52.3 to −128.9)	**NS**

[a] Vaccination periods were 2007-2010 (pre) and 2011-2014 (post). The year 2010 begins in January 2010 and ends in October 2010.
Incidences were calculated as incidence = Number of serotype x 100,000/ populations (≤ 2 years old or 2 - 5 years old) during the years of surveillance at Casablanca. The Grand Casablanca population was estimated at 56,319 in 2007 and 59,993 in 2014 for children ≤ 2 years, and 237,278 in 2007 and 252,750 in 2014 for children >2 – 5 years old.
[b] PCV7 vaccine serotypes are: 4, 6B, 9V, 14, 18C, 19F and 23F.
[c] PCV10-nonPCV7 are: 1, 5 and 7F.
[d] PCV13-nonPCV10 are 3, 6A and 19A.
[e] Non-PCV13 serotypes are: 11A/11E, 15A, 18F, 10F, 10A, 8, 2, 7A, 22F, 24F and Non-typables.
NS: not significant.

($p = 0.0004$) for PNSP and cotrimoxazole non-susceptible strains respectively. All the strains were susceptible to ceftriaxone.

4. Discussion

Pneumococcal disease remains the number one vaccine preventable cause of death in children less than 5 years worldwide.[16] *S. pneumoniae* is a constantly evolving species[17] and its epidemiology is complex, since different serotypes show different carriage and invasiveness properties[1,18]. The use of vaccine for prevention of pneumococcal disease is crucial given that PCV has proved to limit the spread of and infections caused by resistant pneumococcal strains. The PCV-7 vaccine's impact on IPD is well described, but few reports exist on the additional impact of the PCV-13 and PCV-10.

This study describes the serotype distribution and antimicrobial resistance of *S. pneumoniae* isolates collected in Casablanca before and after PCV-13 and PCV-10 introduction in Moroccan NIP. Vaccine coverage of PCVs vaccines in children aged to 2 years of was estimated to 88% at the Grand Casablanca in 2014 as declared by the observatory regional of epidemiology service of health of Casablanca.

In the present study, which covered the period before (from January 2007 to October 2010) and after (from January 2011 to December 2014), we observed a change in the IPD cases since the vaccine introduction.

As expected, the incidence rate of IPD associated with vaccine serotype declined after vaccine implementation. The observed decline was only significant in children < 2 years. In fact, following the introduction of PCVs in Casablanca, the incidence rate of IPD declined from 34.6 to 13.5/ 100,000 populations with a reduction of 61%. This reduction is dominated by bacteraemia cases followed by meningitis cases, according to the Table 1.

In many countries, routine use of PCVs has dramatically reduced the incidence of IPD, particularly among children aged ≤ 2 years,[23] as already reported in several studies.[24–25] In Europe the proportion of PCV-13 serotypes decreased from representing 76% of IPD reported during 2004–2009 to approximately 60% in 2010.[26] The same trend was found in several previously study after PCVs vaccines implementation in Canada and South Africa.[20,27]

In contrast to children under 2 years old, we have not observed incidence rate reductions in children of > 2 - 5 years (from 1.2 to 1.3/100,000 populations). The vaccination program was not fully implemented in all Moroccan children. In fact, in October 2010 only children ≤ 2 months was included in the vaccine program.

Table 3
Immunization status of children under 5 years after introduction of PCVs in Casablanca

Years	Cases	Age (month)	Serotype	Immunization status	Clinical diagnosis
2011					
	1	12	14	NV	Meningitis
	2	11	6A	NV	Pneumonia
	3	36	6A	NV	Bacteraemia
	4	36	3	NV	Meningitis
	5	3	7F	1 dose PCV13	Meningitis
	6	36	14	NV	Pleuro-pneumonia
2012					
	1	48	5	NV	Septicemia
	2	12	3	2 doses PCV13	Bacteraemia
	3	12	6B	2 doses PCV13	Meningitis
	4	24	14	NV	Meningitis
	5	12	9V	2 doses PCV13	Meningitis
	6	1	6B	NV	Bacteraemia
	7	36	14	NV	Polytrauma
	8	24	6B	NV	Peritonitis
2013					
	1	60	1	NV	Pleuro-pneumonia
	2	48	5	NV	Pleurisy
	3	6	14	2 doses PCV10	Pneumonia
	4	36	14	NV	Bacteraemia
	5	7	6B	2 doses PCV10	Pneumonia
	6	24	14	2 doses PCV13	Bacteraemia
	7	1	6B	NV	Polytrauma
	8	4	6B	1 dose PCV10	Polytrauma
2014					
	1	5	1	2 doses PCV10	Polytrauma
	2	56	14	NV	Bacteraemia
	3	29	1	1 dose PCV10	Bacteraemia

NV: Not vaccinated.

Table 4
Distribution of Penicillin G-, Erythromycin-,Cotrimoxazole-, Tetracycline-, Chloramphenicol- nonsusceptible (I + R) strains among children ≤ 5 years old during pre- and post-vaccination periods in Casablanca, Morocco

Antibiotics	≤ 2 years			> 2 - 5 years old		
	[a]Pre-period *n (%)*	[b]Post- period *n (%)*	p-value	[a]Pre-period *n (%)*	[b]Post- period *n (%)*	p-value
Penicillin G	40 (50.6)	7 (21.9)	0.005	5 (41.7)	4 (30.8)	NS
Erythromycin	13 (16.5)	8 (25.0)	NS	3 (25.0)	2 (15.4)	NS
Cotrimoxazole	31 (39.2)	2 (6.3)	0.0004	4 (33.3)	2 (15.4)	NS
Tetracycline	25 (31.6)	7 (21.9)	NS	6 (50.0)	3 (23.1)	NS
Chloramphenicol	8 (10.1)	1 (3.1)	NS	1 (8.3)	1 (7.7)	NS

I = Intermediate, R= Resistant.
[a] The pre-period was from January 2007 to October 2010 (n=79 for children ≤ 2 years and n=12 for >2 - 5 years old).
[b] The post-period was from January 2011 to December 2014 (n=32 for children ≤ 2 years and n=13 for >2 - 5 years old).
n = number of (I + R).
The percentage were calculated as = Number of (I + R) x 100/ total number of strains.

Therefore it may be expected that reductions in IPD incidence rate among children older than 2 years will be observed in the years to come. Reductions in older age groups were also lagged in the US,[33] Canada,[20] United Kingdom and other European countries[26] and South Africa.[27]

Following the introduction of PCVs to all Grand Casablanca provinces, all vaccine serotypes decline as previously described.[19–20] In 2012, the PCV-13 vaccine was replaced by the PCV-10 vaccine in the Moroccan's NIP. The effect of this latter vaccine is illustrated in 2013 and 2014. In this study, we examined 18 months periods to analyze PCV-13 impact on IPD. The monitoring period for the PCV-10 vaccine, which received a decreasing trend already initiated by the PCV-13 vaccine, was from July 2012 to December 2014. PCV-7 serotype common to the two vaccines declined significantly from 18.0 to 4.6/ 100,000 populations in children under 2 years. Comparing these results with the latest publication on the impact of PCVs, we find that our results, although with a certain particularity, follow the same trend as in other countries. The Canadian study on the impact of PCV-13, showed a significant decrease within the first year of PCV-13 introduction in the proportion of PCV-13 serotype among children ≤ 2 years old, from 63% to 43% ($p < 0.001$).[22] Considering the Brazilian study on the impact of PCV-10, it's clearly demonstrated a significant decrease in the incidence rate of serotype included in the PCV-10 from 16.47 to 0.44 cases/1000 peoples.[19] In South Africa, where the PCV-13 is also used, the incidence rate of IPD in children aged of 2 years was declined from 54.8 to 17.0 cases per 100,000 person-years.[27]

The impact of PCVs was least for serotype 6B and 14 and greatest for 19A, 19F and 23F. Although the PCV-13 vaccine was replaced in Moroccan NIP, the frequency of additional PCV-13 serotypes such as 19A, 3 and 6A remains virtually very low. The overall incidence rate of IPD in children aged to 2-5 years old is relatively low in Casablanca. This could be explained in part by the type of monitoring system. Indeed, in passive surveillance, health authorities do not stimulate reporting by reminding health care workers to report disease or providing feedback to individual health workers. Passive surveillance is less expensive than other surveillance strategies, such as an active surveillance, and covers wide areas (whole countries or provinces); however, because it relies on an extensive network of health workers, it can be difficult to ensure completeness and timeliness of data. These statements are considered as a limitation of this study.

The examination of vaccine status showed that 40% of vaccine serotypes vaccine were isolated among children who received at least one dose of PCV-10 or PCV-13 (Table 3). None of them had completed the immunization schedule. Vaccine failure has been reported in fully immunized children, most notably due to serotype 3 after PCV-13, but also serotype 19A after PCV-10, and after receipt of one dose of PCV-13.[34–36] Here, we reported vaccine failures after one or two doses of PCV. Vaccination schedule was incomplete for all vaccine failures described in this study. Indeed, the booster dose is performed at 12 months and sometime many parents are struggling to make the booster dose which may cause a decrease of immunity against vaccine serotypes.

After vaccine use, several studies reported a substantial increase of the non-vaccine serotype (NVS), known as serotype replacement[1,28] or no change in non-vaccine serotype incidence rate.[19] The overall serotype fluctuations have been investigated are probably multifactorial, as previously reported.[14] Vaccination likely contributes to serotype replacement by elimination of strains targeted by the new vaccines, allowing the NVS to fill the new vacant ecological niche.[29] Many studies have reported an augmentation or diminution of the NVS after vaccine introduction as reported by the strategic advisory group of experts on immunization of WHO.[30] For our study, there is no substantial change on NVS incidence rate before and after vaccination, even if a slight raise was observed after PCV-13 implementation.

One of the added benefits of the PCV-13 and PCV-10 immunization programs in Casablanca has been the decline in *S. pneumoniae* antibiotic resistance, most notably for the PNSP and cotrimoxazole non-susceptible strains in children under 2 years, as reported elsewhere.[31] This is not surprising since the majority of serotypes associated with penicillin resistance in Casablanca are serotypes found in the PCV-10 and PCV-13 (i.e. 9 V, 6B, 14, 19A, 19F and 23F). Antibiotic resistance in *S. pneumoniae* is a serious concern globally.[5] Vaccination has reduced the incidence rate of antibiotic resistant serotypes, but we reported here, a rebound due to the persistence of serotype 6B and 14. Based on a comparison of the two study periods, pneumococcal isolates in children have become less resistant to antibiotics in Casablanca, especially to penicillin and cotrimoxazole, both in terms of rates and levels of resistance. Apart from the significant reduction of penicillin and cotrimoxazole resistant strains, a reduction but no significant occurred for tetracycline, chloramphenicol and erythromycin resistant *S. pneumoniae* as previously described.[21,22,32]

In some countries IPD caused by vaccine serotypes has virtually disappeared, even in age groups not primarily targeted by the immunization program, the indirect (herd) effect.[4,25] For our study, monitoring over a long period is required for other age groups, including teenager, adults and elderly to study the herd effect.

The laboratory-based, passive surveillance of pneumococcal serotype distribution may be limited by variable regional standards, the preliminary nature of some data, the timeliness of testing and reporting, the availability of isolates for testing, and privacy concerns. There continues to be a need for a national enhanced IPD surveillance program that includes the collection of additional demographic and enhanced epidemiological patient information to improve estimates of mortality and morbidity rates, to determine their association with specific serotypes, and to guide

intervention strategies to specific populations. This enhanced system could also provide timely access to estimated vaccine coverage rates and vaccine effectiveness information as previously recommended.[20]

5. Conclusion

These data are the first from Morocco examining the effects of PCV-13 and PCV-10 on IPD. Moreover, these data are useful because the epidemiology of IPD in developing countries differs from that in other parts of the world. In this study, the incidence rate of IPD among children $\leq$ 2 years old decreased, the vaccine serotypes, PCV-13 and PCV-10, showed the same pattern of evolution during the monitoring period. The IPD incidence rate remained unchanged in children 2-5 years old. The PCVs contributed to the decrease in the antibiotic resistance rates, especially in PNSP and cotrimoxazole non-susceptibility strains. Moreover, the serotypes with high resistance rate were rarely isolated after vaccination. Continued high-quality surveillance is critical to assess the changes in serotype distributions of invasive and non-invasive *S. pneumoniae* after introduction of PCVs.

Competing interests

The authors declare that they have no competing interests

Financial competing interests

This work was supported by an unrestricted, investigator-initiated grant from Pfizer. The authors conceived the study and the study design was developed and agreed to by the authors without any input from the funding body. The study concept and design was peer reviewed prior to award of the grant from the funding body. The funding body was not involved in and, had no influence over, study design, data collection, data analyses, interpretation of results, report writing or in the decision to submit the paper for publication. All study data are held solely by the authors.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2015.09.019.

References

1. Isaacman D, McIntosh ED, Reinert RR. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal vaccine and considerations for future conjugate vaccines. *Int J Infect Dis* 2010;**14**:e197–209.
2. Advisory Committee on Immunization Practices. Preventing pneumococcal diseases among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2000;**49**(RR-9):1–35.
3. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Freimanis Hance L, Reithinger R, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS Med* 2010;**5**(10):7.
4. World Health Organization (b): Pneumococcal conjugate vaccine for childhood immunization-WHO position paper. *Wkly Epidemiol Rec* 2012;**87**:129–44.
5. Linares J, Ardanuy, Pallares R, Fenoll A. Changes in antimicrobial resistance, serotypes and genotypes in *Streptococcus pneumoniae* over a 30-year period. *Clin Microbiol Infect* 2010;**16**:402–10.
6. Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MC, Nahm MH. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. *J Clin Microbiol* 2007;**45**:1225–33.
7. Jin P, Kong F, Xiao M, Oftadeh S, Zhou F, Liu C, et al. First report of putative *Streptococcus pneumoniae* serotype 6D among nasopharyngeal isolates from Fijian children. *J Infect Dis* 2009;**200**:1375–80.
8. Calix JJ, Dagan R, Pelton SI, Porat N, Nahm MH. Differential occurrence of *Streptococcus pneumoniae* serotype 11E between asymptomatic carriage and invasive pneumococcal disease isolates reflects a unique model of pathogen microevolution. *Clin Infect Dis* 2012;**54**:794–9.
9. Calix JJ, Porambo RJ, Brady AM, Larson TR, Yother J, Abeygunwardana C, et al. Biochemical, genetic and serological characterization of two capsule subtypes among *Streptococcus pneumoniae* serotype 20 strains: discovery of a new pneumococcal serotype. *J BiolChem* 2012;**287**:27885–94.
10. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis* 2000;**30**(1):100–21.
11. Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, et al. Active Bacterial Core Surveillance (ABCs)/Emerging Infections Program Network. Epidemiology of invasive *Streptococcus pneumoniae* infections in the United States, 1995–1998: Opportunities for prevention in the conjugate vaccine era. *JAMA* 2001;**285**(13):1729–35.
12. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J* 2000;**19**:187–95.
13. Song JY, Nahm MH, Moseley MA. Clinical Implications of Pneumococcal Serotypes: Invasive Disease Potential, Clinical Presentations, and Antibiotic Resistance. *J Korean Med Sci* 2013;**28**:4–15.
14. Elmdaghri N, Benbachir M, Belabbes H, Zaki B, Benzaid H. Changing epidemiology of pediatric *Streptococcus pneumoniae* isolates before vaccine introduction in Casablanca (Morocco). *Vaccine* 2012;**30**(Suppl 6):G46–50.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility. Wayne, PA, USA: Twenty-first Informational Supplement M100-S21. CLSI; 2012.
16. WHO Initiative for Vaccine Research Division. Acute respiratory infections, *Streptococcus pneumoniae*; September 2009. Available at: http://www.who.int/vaccine research/diseases/ari/en/index3.html (accessed 23.09.12).
17. Harboe ZB, Benfield TL, Valentiner-Brananth V, Hjuler T, Lambertsen L, Kaltoft M, et al. Temporal trends in invasive pneumococcal disease and pneumococcal serotypes over 7 decades. *Clin Infect Dis* 2010;**50**:329–37.
18. Hausdorff WP, Fekin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. *Lancet Infect Dis* 2005;**2**:83–93.
19. Iroh Tam PY, Madoff LC, Coombes B, Pelton SI. Invasive pneumococcal disease after implementation of 13-valent conjugate vaccine. *Pediatrics* 2014;**134**(2):210–7.
20. Demczuk WH, Martin I, Griffith A, Lefebvre B, McGeer A, Lovgren M, et al. Toronto Bacterial Diseases Network, Canadian Public Health Laboratory Network: Serotype distribution of invasive *Streptococcus pneumoniae* in Canada after the introduction of the 13-valent pneumococcal conjugate vaccine, 2010–2012. *Can J Microbiol* 2013;**59**(12):778–88.
21. Tan TQ. Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. *Clin Microbiol Rev* 2012;**25**(3):409–19.
22. Burgos J, Lujan M, Falcó V, Sánchez A, Puig M, Borrego A, et al. The spectrum of pneumococcal empyema in adults in the early 21st century. *Clin Infect Dis* 2011;**53**:254–61.
23. American Academy of Pediatrics Committee on Infectious Diseases: Recommendations for the prevention of *Streptococcus pneumoniae* infections in infants and children: use of 13-valent pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). *Pediatrics* 2010;**126**(1):186–90.
24. dos Santos SR, Passadore LF, Takagi EH, Fujii CM, Yoshioka CR, Gilio AE, et al. Serotype distribution of *Streptococcus pneumoniae* isolated from patients with invasive pneumococcal disease in Brazil before and after ten-pneumococcal conjugate vaccine implementation. *Vaccine* 2013;**31**(51):6150–4.
25. Tyrrell GJ, Lovgren M, Chui N, Minion J, Garg S, Kellner JD, et al. Serotypes and antimicrobial susceptibilities of invasive *Streptococcus pneumoniae* pre- and post-seven valent pneumococcal conjugate vaccine introduction in Alberta, Canada, 2000–2006. *Vaccine* 2009;**27**(27):3553–60.
26. European Centre for Disease Prevention and Control: Annual Epidemiological Report 2012. Reporting on 2010 surveillance data and 2011 epidemic intelligence data. ECDC, Stockholm, Sweden. Available from http://www.ecdc.europa.eu/en/publications/Publications/Annual-Epidemiological-Report-2012.pdf [accessed 15 June 2013].
27. von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, et al. GERMS-SA Investigators: Effects of vaccination on invasive pneumococcal disease in South Africa. *N Engl J Med* 2014;**371**(20):1889–99.
28. Lynch JP, Zhanel GG. *Streptococcus pneumoniae*: epidemiology, risk factors and strategies for prevention. *Semin Respir Crit Care Med* 2009;**30**:189–209.
29. Bruggeman AB, Pai R, Crook DW, Beall B. Vaccine escape recombinants emerge after pneumococcal vaccination in the United States. *PLOS Pathog* 2007;**3**:1628–36.
30. World Health Organization (a). Meeting of the Strategic Advisory Group of Experts on Immunization, conclusions and recommendations. *Wkly Epidemiol Rec* 2012;**87**(1):1–16.
31. Dagan R, Klugman KP. Impact of conjugate pneumococcal vaccines on antibiotic resistance. *Lancet Infect Dis* 2008;**8**(12):785–95.
32. Shibl AM, Memish ZA, Al-Kattan KM. Antibiotic resistance and serotype distribution of invasive pneumococcal diseases before and after introduction of pneumococcal conjugate vaccine in the Kingdom of Saudi Arabia (KSA). *Vaccine* 2012;**30**(Suppl 6):G32–6.

33. Centers for Disease Control and Prevention (CDC). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease—United States, 1998–2003. *MMWR Morb Mortal Wkly Rep* 2005;**54**(36):893–7.
34. Madhi F, Godot C, Bidet P, Bahuaud M, Epaud R, Cohen R. Serotype 3 pneumococcal pleural empyema in an immunocompetent child after 13-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2014;**33**:545–6.
35. Ho PL, Chan MY, Chow KH, Chiu SS. *Streptococcus pneumoniae* serotype 19A bacteremia in a child fully immunized with 10-valent pneumococcal conjugate vaccine. *J Microbiol Immunol Infect* 2014;**47**:164–5.
36. Lu CY, Ting YT, Huang LM. Severe *Streptococcus pneumoniae 19A* pneumonia with empyema in children vaccinated with pneumococcal conjugate vaccines. *J Formos Med Assoc* 2014. http://dx.doi.org/10.1016/j.jfma.2014.02.007.