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Full Length Research Paper

Serotypes and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates from East Algeria (2005-2011)

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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 bacteremia. Antibiotic resistance in *S. pneumoniae* has increased worldwide but there are few data in Algeria and more information is needed about serotype distribution of invasive *S.pneumoniae* isolates. From 2005 to 2011, a total of 100 non-duplicate invasive *S. pneumoniae* isolates were identified at the University Hospital from East Algeria. Antibiotic resistance was determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion test and the minimum inhibitory concentration of beta-lactams and erythromycin were determined using the E test method (AB BIODISK). Eighty three (83) serotypes were determined by agglutination by latex particles and/or by the Neufeld test using monovalent antisera (Statens Serum Institute). Among the 100 isolates, 57% were non-susceptible to penicillin (PNSP), 46% were intermediate and 11% were resistant (MIC range 2-4 µg/ml). Resistance rates to other antibiotics were as follow: erythromycin (22%), tetracycline (20%), cotrimoxazol (51%). All the strains were susceptible to chloramphenicol, vancomycin and levofloxacin. The predominant serotypes were 14, 19F, 23F, and 6B accounting for 50.6% of tested strains. Non-penicillin susceptibility was associated with serotype 14 (88.23%), 6B (80.00%), 19F (61. 53%), and 23F (57.14%). In children ≤ 5 years of age, the rate of this serotypes were 14 (23.33%), 19F (13.33%), 23F (13.33%) and 6B (10%). Pneumococcal vaccination is not compulsory in Algeria. The theoretical coverage of PCV13 added up to 74.19%. Continual surveillance of antibiotic susceptibility and serotype distribution is recommended in order to plan future treatment and preventive strategies.**

Keywords: *Streptococcus pneumoniae,* serotype distribution, antibiotic resistance, invasive infection, pneumococcal conjugate vaccine.

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is one of the most frequent causes of serious invasive infections, such as, meningitis, bacteremia and pneumonia and is the major cause of morbidity and mortality worldwide. In 2005, WHO estimated that 1.6 million people die of pneumococcal diseases every year, including the deaths

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of nearly one million children aged < 5 years, most of whom live in developing countries (vaccine for childhood immunization-WHO position paper, 2007). The capsule is the main virulence factor and there are 93 known antigenically distinct capsular polysaccharide serotypes of *S. pneumoniae* (Henrichsen, 1995; Bentley et al., 2006; Calix and Nahm, 2010). The prevalence of penicillin resistance has been increasing worldwide (Jenkins et al., 2005; Yang et al., 2008; Hoban et al., 2005; Varon, 2012). Penicillin resistance is usually associated with resistance to other antibiotics, particularly, macrolide and the emergence of multidrug resistance *S. pneumoniae* has been observed in various countries making therapeutic options more difficult (Song et al., 2004b; Jenkins et al., 2005; Johnson et al., 2006; Zhou et al., 2011; Charfi et al., 2012). Many studies have shown that levels of antibiotic resistance are directly proportional to antibiotic consumption in the community (Bronzwaer, et al., 2002; van de Sande-Bruinsma et al., 2008).

The resistance of *S. pneumoniae* to antibiotics is gradually becoming a serious problem, which underlines the urgent need for vaccines to control pneumococcal diseases. At present, three pneumococcal conjugate vaccines are available for children. Introduction of heptavalent pneumococcal conjugate vaccine (PCV7) for infants led to substantial reductions in the incidence of invasive pneumococcal disease (IPD) in the United States and other industrialized countries (Varon, 2012; Myint et al., 2013). However, the increase in the rate of invasive pneumococcal disease (IPD) cases caused by non-vaccine strains has been a concern (Ingels et al., 2012; Van der Linden et al., 2012). Although PCV7 continues to effectively decrease the pneumococcal disease burden, the incidence of IPD caused by non-PCV serotypes has increased among vaccinated children, and these strains are often highly resistant to commonly used antimicrobials (Tyrrell et al., 2009; Azzari et al., 2012; Gant et al., 2012; Pichon et al., 2013, Tóthpál et al., 2012).

The aim of this study was to characterize the epidemiology of children and adult IPD in University Hospital from Constantine. 100 strains of *S. pneumoniae* were isolated from patients with invasive infections across the period of 2005-2011. In Algeria, the pneumococcal conjugate vaccine was not introduced yet in the national program of immunization. In order to evaluate the potential contribution of a pneumococcal conjugate vaccine, antibiotic susceptibility and multi-drug resistance were investigated and serotype distribution was analyzed. Furthermore, the theoretical coverage of the 7-, 10- and 13-valent conjugate vaccines was evaluated.

MATERIALS AND METHODS

Bacterial strains and species identification

A total of 100 *S. pneumoniae* clinical isolates were collected from January 2005 to December 2011 in the University Hospital Ibnbadis from Constantine, Algeria. All the non-duplicate invasive *S.* pneumoniae isolates recovered from adults and children were included. Isolates were obtained from cerebrospinal fluid (CSF), blood and pleural fluid and when an isolate was recovered from CSF and blood, it was categorized as meningitis. Bacterial strains were grown on Columbia sheep blood agar and incubated at 37°C under a 5% CO_2 atmosphere for 20-24 h. All isolates were originally identified as *S. pneumoniae* based on colony morphology, Gram staining, α-hemolysis and optochin susceptibility.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was determined on Mueller-Hinton agar by standard disk diffusion procedure according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). A total of 15 antibiotics were tested including oxacillin (screening), penicillin, amoxicillin, cefotaxime, imipenem, erythromycin, clindamycin/lincomycin, tetracycline, chloramphenicol, cotrimoxazole, vancomycin, rifampicin, levofloxacin, ciprofloxacin and linezolid. Minimum inhibitory concentration (MIC) is determined using E test method ((AB BIODISK) for penicillin, amoxicillin, cefotaxime, imipenem and erythromycin. The CLSI criteria for MIC were applied to classify the isolates as susceptible (S), intermediate (I), or resistant (R) (both the CLSI 2007 and the CLSI 2011 criteria for penicillin) (CLSI, 2007; CLSI, 2011).

S. pneumoniae ATCC 49619 was used as the quality control strain and was included in each set of tests to ensure the accuracy of the results. Multi-drug resistant (MDR) was defined as resistance to three or more classes of antibiotics used in this study.

Serotyping

Eighty three (83) isolates were serotyped using rapid latex agglutination (Pneumotest kits) and the capsule reaction test used antisera from the Statens Serum Institut (Copenhagen, Denmark). The isolates that reacted negatively with the antisera were classified as non-typeable.

The coverage of the PCV7, PCV10 and PCV13 vaccines was estimated by calculating the percentage of isolates that expressed the serotypes included in the vaccine.

Statistical methods

All data was analyzed with the software WHONET 5. The x^2 test was used for comparing proportion of PNSP in the two age groups; P value of < 0.05 was considered to be statistically significant.

RESULTS

Antimicrobial-susceptibility

100 clinical isolates responsible from invasive pneumococcal diseases (IPD) (cerebrospinal fluid n= 75, blood n=22, pleural fluid n= 3) were analyzed and the more clinical presentation was meningitis (75%). Of 100 isolates, sex ratio was 2.8 (74 males and 26 females). 54 strains were isolated from adults (≥ 18 years) and 46 were from children (≤ 17 years), among them, 31 were under 5 years of age (31/46, 67.39%) (Table 1).

The global non-susceptible rate of *S. pneumoniae* to

Table 1. Distribution of 100 pneumococcal strains according to type of sampling and age.

Sample	≤5years	6 to17 years	18 to 40 years	>41 years	Total
CSF	24	10	19	8	61
CSF + Blood	0	3	8	3	14
Blood	7	2	6	7	22
Pleural fluid	0	0	0	3	3
Total	31	15	33	21	100

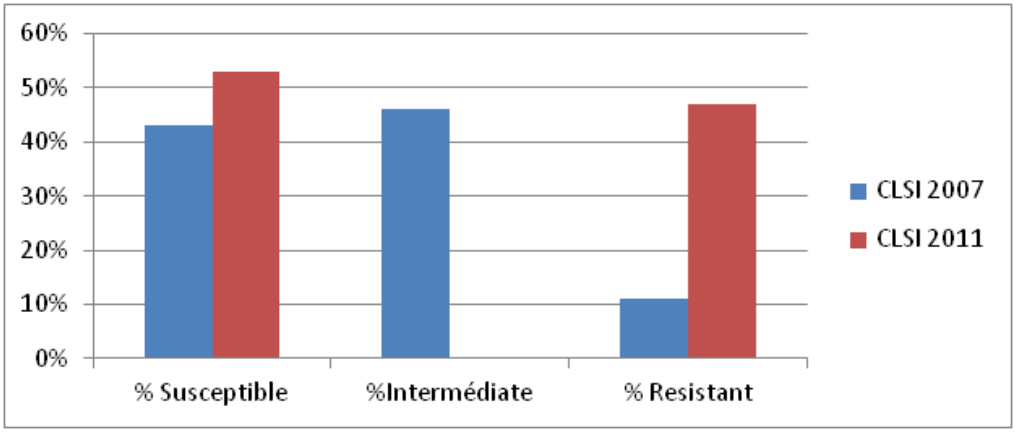

Figure 1. Frequency of resistance to penicillin according to CLSI standards.

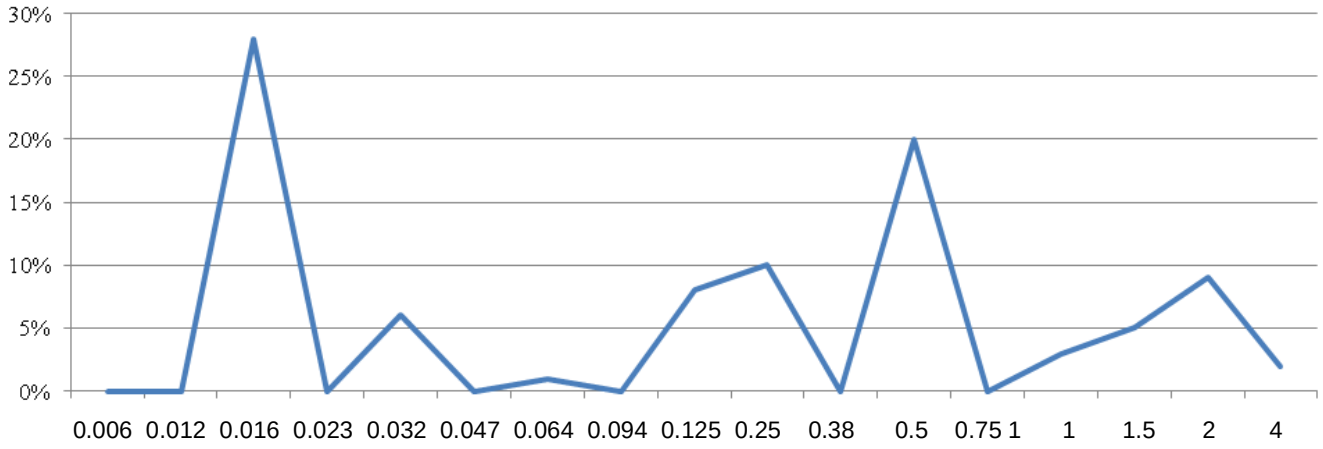

Figure 2. MICs (µg/ml) of 100 pneumococcal isolates for penicillin.

penicillin (R+I) was 57%, using the CLSI 2007 criteria, the penicillin intermediate rate was 46% and resistant rate was 11% (Figure 1).

The MICs of penicillin to most *S. pneumoniae* strains ranged from 0.012 µg/ml to 0.023 µg/ml and from 0.38µg/ml to 0.75 µg/ml (Figure 2). The penicillin non-susceptible rate of pediatric isolates was 80. 42% (37/46) with 15.21% (7/46) penicillin-resistant strains (MIC ranged between 2 - 4 µg/ml). There were 29.62% penicillin-intermediate strains and 7.4% penicillin-resistant strains among adult isolates (MIC = 2 µg/ml).

The non-susceptible rates to amoxicillin and cefotaxime were 9 (2% of resistant strains), and 8% respectively without any identified resistant strain for cefotaxime. All the isolates were susceptible to imipenem (Table 2).

The non-susceptible rates to erythromycin, tetracycline

Table 2. Susceptibility and MICs of 100 pneumococcal isolates to 5 antibiotics.

Antimicrobial agent	Sample source age	Range	MIC (µg/ml) CLSI 2007			MIC (µg/ml) CLSI 2011		
			%S	%I	%R	%S	%I	%R
Penicillin	Adults	0.012- 2	62.96	29.62	7.4	72.22	/	27.77
	Children	0.064- 4	19.56	65.21	15.21	30.43	/	69.56
Amoxicillin	Adults	0.016- 1.5	92.59	7.4	0			
	Children	0.064- 4	89.13	6.52	4.34			
Cefotaxime	Adults	0.016- 1.5	94.44	5.55	0			
	Children	0.064- 1.5	89.13	10.86	0			
Imipenem	Adults	0.002- 0.38	100	0	0			
	Children	0.002- 0.12	100	0	0			
Erythromycin	Adults	0.016- > 256	79.62	0	20.37			
	Children	0.064- > 256	76.08	0	23.91			

MIC, Minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute.

and trimethoprim-sulfamethoxazole were respectively 22, 20 and 51% (Figure 3).

The MICs of 100% resistant *S. pneumoniae* for erythromycin (22 strains) were above 256 µg/ml and 100% were resistant to clindamycin (MLS_B phenotype). All the isolates were susceptible to chloramphenicol, vancomycin, rifampicin, levofloxacin and linezolid.

Of 100 isolates, the rate of MDR was 20% and among PNSP isolates (57 strains), 29.8% (17/57) were resistant to erythromycin and 33.33% (19/57) were MDR. Among erythromycin resistant strains, 77.27% (17/22) were PNSP and 77.27% (17/22) were MDR.

Serotype distribution

The serotype distribution of 83 clinical isolates is shown in Figure 4. 82 serotypes were identified and one strain was non-typeable. The most prevalent serotypes were 14, 19F, 23F, and 6B accounting for 50.60% (42/83) of the tested strains.

In children ≤ 5 years of age, the rate of these serotypes were 14 (22.58%), 23F (12.90%), 19F (12.90%), and 6B (9.60%), and a total of 19 pneumococcal isolates expressed the serotypes included in PCV7, so the coverage of PCV7 was 61.29% (19/31).

In this group of age, there were 2 strains expressing serotypes 1 and 7F (3.2% for each). There were no strains expressing serotype 5, furthermore, the coverage of PCV10 was 67.74% (21/31). On the other hand, the coverage of PCV13 added up to 74.19% (23/31).

Non-vaccine serotypes, such as serotypes 9A, 10A, 11, 12A, 24F, 33F and 35B are expressed in small proportions (3.22% each) (Figure 5).

Non-penicillin susceptibility was associated with serotypes 14 (88.23%), 23F (80%), 6B (80. 00%) and19F (61.53%) whilst serotype 18C was identified in three strains which were PNSP.

Serotype 19A was identified in three strains and two of them were PNSP isolated from meningitis, among them, one strain was isolated in a 2 year old children. Serotype 1 was identified for three strains; one strain was isolated in children under 5 years of age and was PNSP. Serotype 35B was found mostly in meningitis and none was PNSP. Non-susceptibility to penicillin was observed in other serotypes such as serotypes 9N, 16, 29, 12A, 47F and 24F, rarely isolated (Table 3).

The most resistant isolates to erythromycin were serotype 19F (31.81%, 7/22) and 14 (22.72%, 5/22). MDR were most frequent among serotype 14 and 19F (35%, 7/20 and 30%, 6/20 respectively). There were other MDR serotypes such as serotype 19A (2/20), and serotypes 7F, 6B, 3, 10A and 9N (1/20 for each).

DISCUSSION

The resistance of *S. pneumoniae* to antibiotics varied over time, among different regions, age, serotypes, sources of the strains, and treatment of IPD presents a difficult challenge because of the fast distribution of the penicillin non-susceptible strains worldwide (Felmingham et al., 2002; Reinert, 2009). Despite its importance, a few studies on the serotype distribution and antimicrobial resistance of invasive *S. pneumoniae* diseases (IPD) were investigated in Algeria.

In the present study, the penicillin non-susceptible rate was very high and increased to 57%, when the breakpoint of CLSI 2007 was adopted, the penicillin intermediate rate was 46% and resistant rate was 11% whereas, no isolate was found to have intermediate susceptibility to penicillin and resistance rate was 47% based on the 2011 CLSI criteria. These rates of PNSP placed Algeria among countries with the highest levels of penicillin resistance, due at least in part to the misuse of this antibiotic over an extended period of time.

Application of the 2011 breakpoints showed higher resistance rates for meningitis than non-meningitis in our

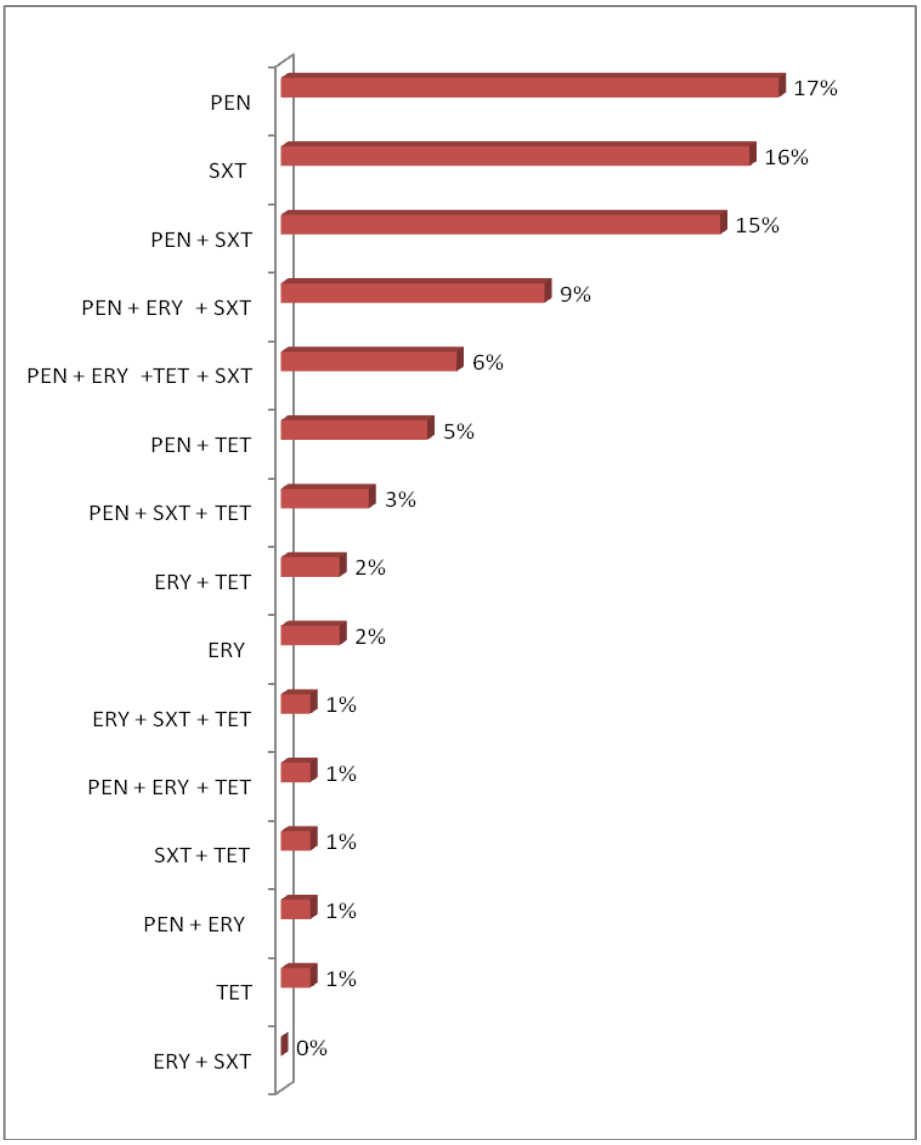

Figure 3. Antibiotic resistant pattern of 100 pneumococcal isolates. PEN, penicillin; ERY, erythromycin; TET, tetracycline; SXT, cotrimoxazole.

study. In the USA, the proportion of resistant meningeal isolates increased from 10.7% under the pre-2008 break-points to 27.5% under the 2008 breakpoints. However, according to the new non-meningeal breakpoints, all isolates were susceptible to penicillin while the majority expressed intermediate resistance (CDC, 2008). There was a significant difference of resistance to penicillin in the two age groups ($P<0.05$) in present study. Such antimicrobial susceptibility differences between isolates from children and adults have previously been reported in other studies (Hoban et al., 2005; Varon, 2012).

Previously reported rates of PNSP in Algeria cannot be compared with our results, because the criteria were different. Generally, prevalence of PNSP increased over time and the rate observed in our study was higher than those reported by Smati et al. (1994) and Tali-Maamar et al. (2012) (12.5% in all isolates and 23.5% in meningitis respectively).

Prevalence rates of penicillin non-susceptible varied widely among countries that did not include PCV7. In Asia, Song et al. (2004a) demonstrated that Asians had the world's highest level of antimicrobial resistance in *S.pneumoniae*. The rates of penicillin resistance amongst clinical strains were 71.4% in Vietnam, 68.8% in Thailand and 54.8% in Korea. In France in 2002, the rate of penicillin non-susceptible pneumococci (PNSP) reached up to 50% of all strains isolated (Varon, 2012). Moderately high rates of PNSP were showed in southern and eastern Mediterranean region (25% in 2003-2005) (Borg et al., 2009) and reached 40.5% in Spain (García-

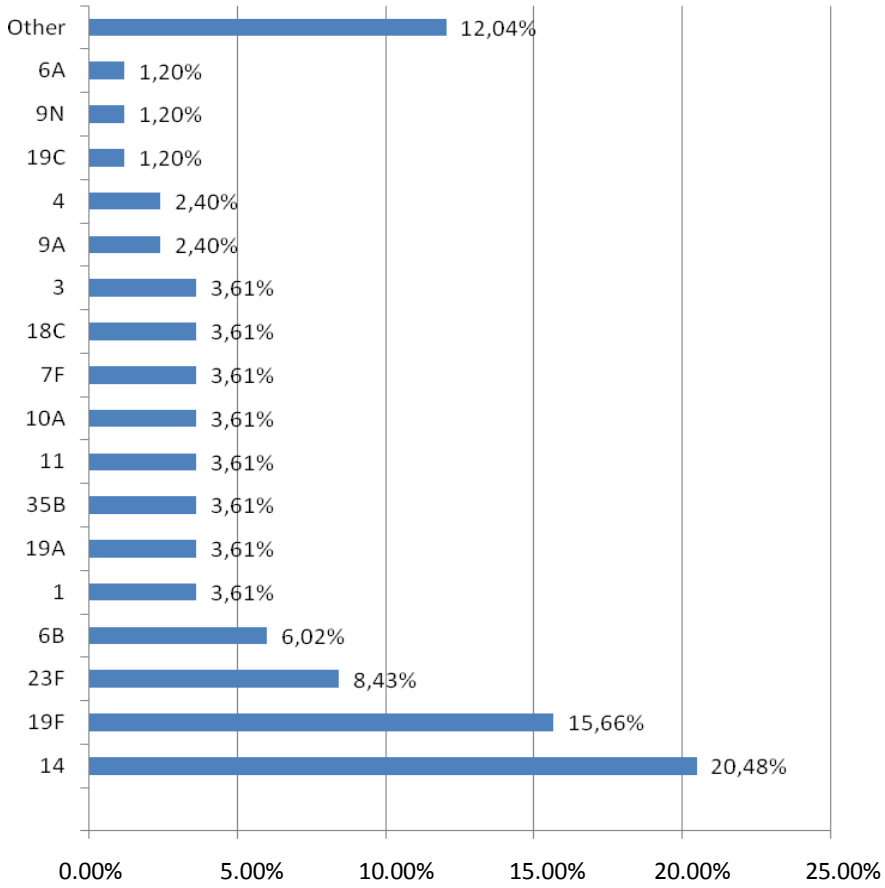

Figure 4. Serotype distribution of 83 pneumococcal isolates. Other includes serotypes 12A, 16, 24F, 47F, 33F, 39, 29, 21, 48 and one non-typeable strain.

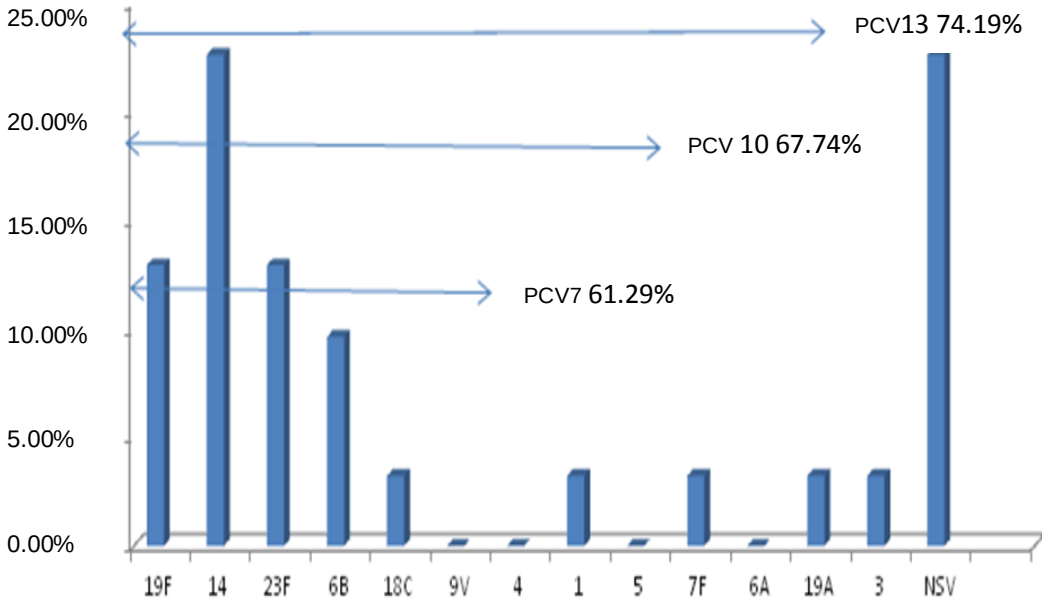

Figure 5. Distribution of serotypes in children ≤5 years of age and vaccine coverage (n=30/31). NSV, Non-serotype-vaccine; PCV, Pneumococcal vaccine; PCV7, 4, 6B, 9V, 14, 18C, 19F, 23F; PCV10, 4, 6B, 9V, 14, 18C, 19F, 23F + 1, 5, 7F; PCV13, 4, 6B, 9V, 14, 18C, 19F, 23F + 1, 3, 5, 6A, 7F, 19A.

Table 3. Distribution of the predominant serotypes of S.pneumoniae PNSP, ENSP, and MDR from adults and children.

Serotype	Number of isolates %	PNSP%		ENSP		MDR%	
		Adults	Children	Adults	Children	Adults	Children
14	17	23.52	64.70	11.76	17.64	11.763	23.52
19F	13	23.07	38.46	23.07	30.76	23.07	23.07
23F	7	0	54.14	0	0	0	0
6B	5	20	60	40	0	0	25
1	3	0	33.33	0	0	0	0
3	3	0	33.33	0	0	0	33.33
19A	3	0	66.66	0	66.66	0	66.66
35B	3	0	0	0	0	0	0
11	3	66.66	33.33	0	0	0	0
10A	3	0	66.66	0	33.33	0	33.33
7F	3	33.33	33.33	0	33.33	0	33.33
18C	3	33.33	66.66	0	0	0	0

PNSP: penicillin non-susceptiptible pneumococcus, ENSP: erythromycin non-susceptible pneumococcus, MDR: multidrug resistance.

Suárez et al., 2006). In African countries, PNSP rates also varied. Rates of 48.5 and 50.4% have been reported respectively in Morocco, and Tunisia as in Algeria (Elmdaghri et al., 2012 ; Smaoui et al., 2009). PNSP rates reported for Kenya, Uganda, Tanzania and Ethiopia were as low as 0% in 2003-2007 (Mudhune et al., 2009), rates of 0.5, 12 and 27.3% have been reported respectively in South Africa (Silberbauer et al., 2011),Ghana (Holliman et al., 2007) and Senegal (Manga et al., 2008). Differences in rates of pneumococcal penicillin resistance between countries have been shown to be associated with levels of antimicrobial consumption (van de Sande-Bruinsma et al., 2008).

Introduction of PCV7 was associated with substantial declines in PNSP prevalence (Farrell et al., 2007; van de Sande-Bruinsma et al., 2008; Varon, 2012). In France, the rate of the strains with decreased susceptibility to penicillin decreased from 50 to 30% over a 6-years study (2002-2007). The decrease was even more marked in children less than 2 years of age: 64% of PNSP in 2002 to 41% in 2007 (Varon, 2012). In agreement, our study shows higher rates of resistance strains relative to countries that implement the vaccine

The rates of strains with decreased susceptibility to other beta-lactams in our study were higher than those showed in two previous Algerian's studies. An Algerian's study in 2003 did not identify any strains resistant to amoxicillin or cefotaxime (Ramdani-Bouguessa and Rahal, 2003). A later study, published in 2012, identified 4.2% as cefotaxime resistant in meningitis (Tali-Maamar et al., 2012). In Tunisia (Smaoui et al., 2009), these rates were a bit higher, in IPD of children under 5 years of age, the rates of resistance to amoxicillin and cefotaxime were 11.4 and 5.7% respectively.

In post vaccine period, a study on the antimicrobial susceptibility of *S. pneumoniae* in eight European countries indicated that the resistance rate to cefotaxime was 5.1% (Reinert et al.,2005) while, the rate of strains with decreased susceptibility to other beta lactams remained high in France (20% to amoxicillin and 10% to cefotaxime) (Varon, 2012)

The rates of resistance to erythromycin, was 22% in this study, While resistance rate was higher for erythromycin (31%) in a previous Algerian's study because criteria were different (Tali-Maamar et al., 2012) whereas the rate of resistance reported in 1994 was low (Smati et al., 1994).

The prevalence of macrolide resistance in *S. pneumoniae* increased worldwide but was highly variable between countries, and was mainly due to widespread use of macrolides, mostly azythromycine (Hyde et al., 2001; Dias and Canica, 2004).

Many Asian countries showed extremely high prevalence rates of macrolide resistance (> 88,3%° during 2000-2002 (Song et al., 2004b). In the United States, the rate of resistance to erythromycin was 25% in 2000 (Whitney et al., 2000). The highest rates of 43.6, 46.1, and 53.7% respectively has also been reported from Spain, France and Greece (Reinert et al., 2005; Daikosa et al., 2008). In Australia, an increase in resistance was remarkable for erythromycin (3.5% in 2000, 11% in 2008) (Hoenigl et al., 2010). In Africa, an increase of resistance rates was also noticed for erythromycin in Morroco (9.4% in 1998-2001, 12.2% in 2002-2005 and 14.4% in 2006-2008) (Benbachir et al., 2012).

Introduction of PCV7 was followed by declines in erythromycin non-susceptible pneumococci (Kyaw et al. 2006; Farrell et al., 2008; Tyrrell et al., 2009; Varon, 2012). In France in 2007, around 30% of isolated *S. pneumoniae* strains were resistant to macrolides compared to 50% in 2001 (Varon, 2012) whereas, erythromycin resistance continued to rise in the post PCV7 years (Horacio et al., 2012).

In this study, rates of resistance to tetracycline, cotrimoxazole and chloramphenicol were respectively 20, 51 and 0% and the rates of 30, 43 and 5.8% respectively, were observed in recent Algerian study (Tali-Maamar et al., 2012). Highest rates of resistance were seen in African's and Asian's countries (Holliman et al., 2007; Liu et al., 2008; Charfi et al., 2012; Thomas et al., 2013). In Ghana, although most isolates of IPD were resistant to tetracycline and cotrimoxazole (85% and 63%), resistance to tetracycline and cotrimoxazole remained high even though these agents were no longer used for empirical treatment of chest infection in the region (Holliman et al., 2007).

The resistance to cotrimoxazole was noted to be high in Asia (> 85%), probably because of its widespread use for presumptive treatment of pneumonia (Thomas et al., 2013). In our study, cotrimoxazole resistance was high; nevertheless this antibiotic was not delivered without prescription in Algeria and is used as the second line of defense in treating bacterial acute lower respiratory tract infections after amoxicillin failure. While, all strains were susceptible to chloramphenicol, this antibiotic is rarely used nowadays. The resistant rates to chloramphenicol, in Morocco and Tunisia were relatively low (8.1 and 17.2% respectively) (Elmdaghri et al., 2012; Smaoui et al., 2009). The resis-tant rates to chloramphenicol increased to 68.2% in Senegal; chloramphenicol was the most frequently used antibiotics (Manga et al., 2008).

Vancomycin and levofloxacin showed 100% of efficacy in the present study; vancomycin is not recommended for monotherapy in meningitis and fluoroquinolones are rarely used for empiric therapy of community-acquired pneumonia in Algeria. These drugs may be important alternatives for use in the treatment of infections caused by multidrug-resistant *S. pneumoniae*, but the spread of fluoroquinolone-resistant clones may cause rapid increase in resistance with widespread use of these agents as has been reported from Honk Kong (Ho et al., 2004).

The rate of multidrug resistance strains (MDR) is relatively low in our study compared to those found in Asian countries (up to 71.4%) (Lee et al., 2010), and the rate of MDR in PNSP was so low, whereas penicillin resistance is an important marker for the presence of MDR. Introduction of PCV7 in several countries was followed by decline in prevalence in PNSP and in MDR; the overall rate of invasive MDR isolates declined by 59% in USA (Kyaw et al, 2006).

Natural fluctuations in serotypes responsible for IPD occurred over time. The pattern of predominant IPD associated serotypes varied with age and country (Mehr and Wood, 2012). Globally, seven serotypes account for the bulk of IPD disease (1, 5, 6A, 6B, 14, 19F and 23F).

S. pneumoniae serotypes identified in our study were similar to those reported in some countries before introduction of PCV7 (Reinert et al., 2010). An Algerian's authors have reported the variation in time of circulating SP serotypes. A study from 1996 to 2000 showed that serotypes 1 and 5 were the most frequent in both adults and children, while serotype 19 and 23 were rare (Ramdani-Bouguessa and Rahal, 2003). In contrast, a study from 2001-2010 in children under 5 years of age, showed that serotypes 14, 23F,19F, 6B and 1 were common (Tali-Maamar et al., 2012).

In our study, a correlation between serotypes and antimicrobial resistance patterns was observed. The four most common serotypes (14, 23F, 6B and 19F) were associated with high rates of resistance to penicillin .The highest rates of resistance tented to occur in the most prevalent serotypes.

Similar results were also reported in many countries before introduction of PCV7. In Tunisia, the most prevalent serotypes for invasive pneumococcal isolates in children were 14, 23F, 4 and 19F; serotype 14 was the most prevalent serotypes in IPD and was highly penicillin non-susceptible (Charfi et al., 2012). A study from South Africa reported that the most common serotypes in IPD in children < 5 years of age were 14, 1, 6A/6B, 19F and 23F and penicillin non-susceptibility was observed in serotypes 14, 19F, 6A and 23F (Silberbauer et al., 2011).

In Australia, the most common serotypes causing IPD were 14, 19F, 6B, and 18C, and the most common PNSP IPD serotypes were serotypes 19F and 9V (Watson et al., 2007).

In China, the most prevalent serotypes were 19F, 14, 23F, 6B and 19A and the most prevalent serotypes of PNSP were 19F, 14, and 23F (Yang et al., 2008).

In Brazil, serotypes 14, 3, 23F, 19F, and 6B were the most prevalent serotypes and 86% of serotypes 14, 23F, 6B and19F were PNSP (de O Menezes et al., 2011).

Following introduction of PCV7, there has been a steady increase in the incidence of non-PCV7 serotypes. The replacement of vaccine serotypes by non-vaccine serotypes observed in invasive infections partly reflects the modified distribution of serotypes colonizing the nasopharynx of young children (Cohen et al., 2010). Most of the rise in non-PCV7 IPD is attributable to serotype 19A (Munoz-Almagro et al., 2009; Tyrrell et al., 2009; Azzari et al., 2012; Rosen et al., 2011; Horacio et al., 2012; Ingels et al., 2012; Bautista-Marquez et al., 2013).

In the USA, the incidence of 19A IPD in children < 5 years of age rose from 2.6 cases per 100,000 population (pre-PCV7 period; 1998-1999) to 9.3 cases per 100,000 population (post-PCV7 period; 2005) (MMWR, 2008). Serotype 19A is now one of the most common causes of IPD in young children from developed countries (Fenoll et al., 2009; Bettinger et al., 2010; Kaplan et al., 2010). Changes in *S. pneumoniae* serotype distribution after the introduction of PCV7 cannot be automatically assumed to be due to PCV7, because temporal changes in serotype distribution were observed in some countries pre-PCV7 (Jefferies et al., 2010). However, the emergence of serotype 19A was reported before the introduction or widespread use of PCV7 in some countries (Choi et al., 2008;

Shin et al., 2011).

Serotype 19A is particularly important in the epidemiology of IPD because of its potential for invasiveness and its propensity to acquire resistance and MDR (Kyaw et al., 2006; Farrell et al., 2007). Serotype 19A was greatly exposed to selection pressure of antibiotics: 85% of serotype 19A pneumococci were PNSP in French's study (Varon, 2012).

In USA, the proportion of IPD caused by PNSP 19A increased from 20.4 in 2004 to 43.7% in 2008 (Beall et al., 2011). Furthermore, most PNSP serotype 19A isolates were also resistant to other antibiotics or were MDR. One of the most significant findings from this study was the presence of serotype 19A (3.61%) and 66.6% of 19A showed high resistance rates to several antibiotics including penicillin.

We demonstrate in our study that PCV13 provided good coverage for invasive pneumococcal isolates for the children ≤5 years of age (74.19%). The theoretical vaccinal coverage for PNSP in children was evaluated at 62.1, 66.7and 72.4% for PCV7, PCV10 and PCV13 respectively in previous study in Algeria.

These results represented an additional contribution to our current understanding of burden invasive pneumococcal disease in one of developing countries. Continual surveillance of antibiotic susceptibility and serotype distribution is recommended. These results suggest that the expanded coverage offered by PCV13 will provide additional protection against pneumococcal disease in Algeria.

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