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Characteristics of *Streptococcus pneumoniae* serotype 19A isolates from children in the pre and post Conjugate Vaccine Era. Single center experience 1986–2015

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ABSTRACT

Background: The present study assessed the prevalence and characteristics of *S. pneumoniae* serotype 19A isolates from children with pneumococcal disease (PD), before and since introduction of pneumococcal conjugate vaccines (PCVs) in Greece.
Methods: *S. pneumoniae* isolates collected at one large pediatric hospital between 1986 and 2015 were serotyped by the Quellung reaction and MICs determined by Etest. Alterations of *pbp* genes and the presence of *mefA, mefE, ermB* genes were detected by polymerase chain reaction. Genotypes were assessed by multilocus sequence typing (MLST).
Results: Among 1875 isolates, 210 (11.2%) belonged to serotype 19A. The prevalence of PD caused by serotype 19A increased from 4.6% in the pre-PCV7 years (1986–2005) to 19.6% in the post-PCV7 years (2006–2010), peaking at 27% in 2009 ($p < 0.001$, 95% CI; 2.0, 18.2) with a significant upward trend ($p = 0.04$, 95% CI; 1.02, 12.66). Following the introduction of PCV13 in 2010, the rate decreased from 22% in 2011 to 11.4% in 2015 ($p = 0.08$, 95% CI; 0.92, 5.1) with a downward trend of borderline significance ($p = 0.05$, 95% CI; −6.8, 0.04). The multidrug resistant (MDR) serotype 19A isolates increased from 10.6% in 1986–2005 to 21.2% in 2006–2010 and to 71.8% in 2011–2015 ($P < 0.001$). Alterations in *pbp* genes were detected in all penicillin non-susceptible isolates. Of 110 erythromycin resistant isolates, 21 contained the *mefE* gene, 36 the *ermB* and 53 both the *mefE* and *ermB* genes. MLST analysis of 142 isolates revealed four dominant clonal complexes (CC); CC320, CC172, CC276 and CC199. The majority of CC320 and CC276 isolates displayed MDR phenotypes.
Conclusion: PD caused by serotype 19A increased significantly after the introduction of PCV7 followed by a decline after PCV13 use. The vast majority of persisting 19A isolates was MDR. Surveillance studies are necessary to monitor the changes in the pneumococcal population.

1. Introduction

Streptococcus pneumoniae remains an important pathogen causing invasive and non-invasive diseases such as bacteremia, meningitis, pneumonia, acute otitis media, mastoiditis and sinusitis [1–4]. Worldwide, pneumococci continue to cause more than one million deaths annually, mainly among children <5 years of age [5]. The epidemiology of *S. pneumoniae* is continually changing under the antibiotic selection pressure and the applied immunization practices. Indeed, the widespread use of heptavalent conjugate vaccine (PCV7) reduced pneumococcal disease associated with vaccine serotypes, however, the number of infections caused by non-vaccine serotypes have increased significantly after the introduction of this vaccine [6–9]. The PCV7 (serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F), was introduced in Greece in January 2006 and was replaced by PCV10 (serotypes PCV7 + 1, 5, 7F) in 2009 and PCV13 (serotypes PCV10 + 3, 6A, 19A) in 2010. The National Advisory Committee on Immunization Practices recommended that all Greek children under 5 years of age should receive pneumococcal conjugate vaccination according to immunization schedule (3 + 1 if initiated before the first year, 2 doses if initiated between 12 and 24 months and one dose after two years of age). PCV7 became

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available in 2006 and its cost was fully reimbursed in 2008 whereas the cost of PCV10 and PCV13 received full reimbursement in October 2010.

Serotype 19A emerged as a predominant serotype in many countries, after the wide use of PCV7 [6–9]. More importantly, serotype 19A has become increasingly resistant to antibiotics and has increased potential to cause invasive disease relative to other serotypes [10–12].

Herein we present the prevalence of *S. pneumoniae* serotype 19A recovered from invasive pneumococcal disease (IPD) and non-IPD in children from a tertiary care children's hospital located in Athens, Greece, in three time periods; i) before the introduction of conjugate vaccines (1986–2005), ii) after the introduction of PCV7 (2006–2010) and iii) after the introduction of PCV10 and PCV13 (2011–2015). We also describe the antimicrobial susceptibilities and the genetic lineages of serotype 19A as they evolved during the 30-year period in our setting.

2. Materials and methods

2.1. Study design and bacterial isolates

This study was conducted from January 1986 to December 2015, at Aghia Sophia Children's Hospital, an 800-bed tertiary care pediatric hospital located in Athens, Greece. Aghia Sophia Children's Hospital, serves approximately 45% of paediatric population of Athens Metropolitan area and accepts referrals from other health districts of the country. All *S. pneumoniae* isolates recovered from sterile sites and middle ear fluid, obtained by needle aspiration or from ear canal drainage, of children hospitalized or visiting the emergency room were stored at −75 °C at the infectious disease research laboratory. All isolates collected during the study period (one isolate per patient) were re-cultured for further testing. Identification of *S. pneumoniae* was confirmed by conventional microbiologic techniques including optochin sensitivity and bile solubility testing. Isolates exhibiting resistance to optochin were examined with polymerase chain reaction (PCR) for the *lytA* gene as previously reported [13].

2.2. Serotyping

Serogrouping was performed using the Pneumotest-latex agglutination kit and serotyping by the Quellung reaction using type-specific antisera (Statens Serum Institute, Copenhagen, Denmark).

2.3. Susceptibility testing

Susceptibility testing to penicillin, cefotaxime, erythromycin, clindamycin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, rifampin, moxifloxacin, vancomycin and linezolid was performed by Etest (bioMérieux, SA, RCS LYON) on Mueller–Hinton agar supplemented with 5% sheep blood incubated for 18–24 h at 35 ± 2 °C in 5% CO2. The results were interpreted using the CLSI 2014 criteria [14]. The isolates were classified as susceptible, intermediate, or resistant if the MICs were ≤0.06 μg/ml, 0.12–1.0 μg/ml or ≥2 μg/ml for penicillin and ≤0.5 μg/ml, 1.0 μg/ml or ≥2 μg/ml for cefotaxime respectively.

2.4. Detection of resistance genes in isolates of serotype 19A

Genomic DNA was extracted from isolated colonies using a standardized procedure (http://www.cdc.gov/streplab/protocol-emm-type.html). Non-susceptible isolates to penicillin were examined for alterations in the structure of *pbp1a, pbp2b and pbp2x* genes as previously described [15]. Macrolide resistance genes were detected by PCR using specific primers [16]. The distinction between *mefA and mefE* was made using a multiplex PCR as described by Bley et al. [16]. Products were run on a 1% agarose gel and were visualized by ethidium bromide staining.

2.5. Multilocus sequence typing (MLST) in isolates of serotype 19A

A subset of isolates were examined by MLST selected by antimicrobial susceptibility profile and the time period isolated. MLST was performed by amplifying seven housekeeping genes (*aroE, gdh, gki, recP, spi, xpt* and *ddl*) and PCR products were sequenced with an ABI PRISM 3500 Genetic Analyzer as previously described [17]. DNA alignment was performed using the BioEdit Sequence Alignment Editor v.7.1.9 (downloaded from http://www.mbio.ncsu.edu/bioedit/bioedit.html). Allele numbers and sequence types (STs) were assigned using the pneumococcal MLST web site (https://pubmlst.org/spneumoniae/). Clonal complexes were determined using the eBURST algorithm from the same website.

2.6. Statistical analysis

Data were analyzed using the Prism v6.01 software (GraphPad Software Inc, La Jolla, CA USA). Trends in the prevalence rate of pneumococcal disease caused by serotype 19A were calculated by linear regression analysis. The x^2 test was used for categorical variables and the Student *t* test or the Mann-Whitney *U* test for continuous variables. Statistical significance was set at 0.05.

3. Results

3.1. Study population

Between January 1986 and December 2015, 2603 cases of culture confirmed IPD and non-IPD in children ≤14 years of age were identified. Of 2603 isolates, 1875 (953 IPD and 922 non-IPD) were recovered for further examination; 1018 isolated between 1986 and 2005, 450 between 2006 and 2010 and 407 between 2011 and 2015. Of those, 210 (11.2%) belonged to serotype 19A. The characteristics of children with pneumococcal disease caused by serotype 19A are shown in Table 1. Males outnumbered females by a ratio of 1.2, the mean age was 32.9 months (median, 26;

Table 1
Characteristics of children with invasive or non-invasive pneumococcal disease caused by serotype 19A.

Variable	No. of patients
Gender	
Male	115
Female	95
Source of isolation	
CSF	7
Blood	68
Pleural fluid	6
Bone aspirate	1
Ear fluid	116
Mastoid pus	12
Vaccination (No. of doses)	
None	86
PCV7 ≥ 2	61
PCV10 ≥ 2	4
PCV13 ≥ 2	14
PCV7, PCV13 one of each	6
PCV7 = 1	15
PCV13 = 1	2
Unknown	22

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