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Short communication

Nationwide surveillance of *Streptococcus pneumoniae* in Greece: patterns of resistance and serotype epidemiology

G. Poulakou, I. Katsarolis, I. Matthaiopoulou, S. Tsiodras, S. Kanavaki, D. Hatzaki, E. Roilides, D. Sofianou, I. Kavaliotis, A. Kansouzidou, D.A. Kafetzis, I. Paraskakis, M. Foustoukou, G.L. Daikos, V. Syriopoulou, A. Pangalis, S. Leveidiotou, H. Giamarellou *,

on behalf of the Hellenic Study Group for the Susceptibility of *Streptococcus pneumoniae*

Fourth Department of Internal Medicine, Athens University School of Medicine, University General Hospital 'Attikon', 1 Rimini Street, 124 62 Athens, Greece

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Abstract

This nationwide study assessed the antimicrobial susceptibility and seroprevalence of *Streptococcus pneumoniae* in paediatric carriage isolates and in clinical isolates from adult pneumococcal disease in Greece during the years 2004–2006. Among 780 isolates recovered from the nasopharynx of children <6 years old attending day-care centres, non-susceptibility rates to penicillin, cefuroxime, ceftriaxone, erythromycin, tetracycline and trimethoprim/sulfamethoxazole were 34.7%, 25.1%, 1.0%, 33.5%, 26.4% and 44.2%, respectively. Among 89 adult clinical isolates, the respective rates were 48.3%, 46.1%, 5.6%, 48.3%, 32.6% and 40.4%. High-level resistance to penicillin, cefuroxime and ceftriaxone was recorded for 14.4%, 23.3% and 0.1% of paediatric carriage isolates, whereas for clinical adult isolates the respective rates were 25.8%, 38.2% and 2.2%. No resistance to levofloxacin and moxifloxacin was recorded, although 3.5% of paediatric carriage isolates and 23.2% of adult clinical isolates had minimum inhibitory concentrations of ciprofloxacin >2 mg/L. Serotypes 19F, 14, 23F and 6B were the most prevalent among carriage and clinical isolates. The 7-valent pneumococcal conjugate vaccine was estimated to provide coverage against 71.7% of paediatric carriage isolates and 51.3% of adult clinical isolates. Resistance rates among clinical isolates from adult sources were higher than those recorded among paediatric carriage *S. pneumoniae* isolates and displayed an increasingly resistant profile compared with previous reports from our country, warranting continuous vigilance.

Keywords: *Streptococcus pneumoniae*; Antimicrobial resistance; Nasopharyngeal carriage; Invasive disease; Pneumococcal vaccine coverage

1. Introduction

Streptococcus pneumoniae remains the most commonly identified bacterial pathogen in community-acquired pneumonia (CAP), acute sinusitis and acute otitis media in children as well as being a frequent cause of meningitis and primary bacteraemia. The increasing problem of antimicrobial resistance in *S. pneumoniae* has emphasised the need for active epidemiological surveillance. Pneumococci colonise the nasopharynx of a large percentage of pre-school children, especially during winter months, and carriage isolates of *S. pneumoniae* are considered representative and predictive of isolates encountered in community invasive disease both in children and adults [1,2].

The geographical features of Greece, consisting of mountainous areas and numerous scattered islands, drives remote populations to empirical antimicrobial treatment against presumed pneumococcal infections and renders nationwide surveillance of clinical pneumococcal isolates an illusive target. The aim of this study was to collect a geographically and population-based representative sample of nasopharyngeal

* Corresponding author. Tel.: +30 210 583 1990; fax: +30 210 532 6446.
E-mail address: hgiama@ath.forthnet.gr (H. Giamarellou).

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colonising *S. pneumoniae* strains and to compare microbiological data for these isolates with those from clinical isolates derived from central hospitals.

2. Methods

2.1. Study group

A study group consisting of 12 co-operating hospitals was organised under the co-ordination of the 4th Department of Internal Medicine and the Infectious Diseases Laboratory of Athens University Medical School.

2.2. Distribution and performance of sampling for carriage strains

From February 2004 to May 2004, trained clinicians were responsible for nasopharyngeal sampling of healthy preschool children aged 1 month to 6 years attending local kindergarten and day-care centres. Children receiving antibiotics for active infection were excluded. Informed consent was obtained prior to sampling from the childrens' parents. The research protocol was approved by the Ministries of Health and Education as well as the Ethics Committees of all co-operating hospitals. The sampling distribution per geographic prefecture was representative of the specific population, according to the data of the National Statistical Service. Paediatric carriage isolates (PCIs) were collected pernasally with sterile swabs on flexible aluminium wire (Medical Wire & Equipment, Corsham, UK). Swabs were immediately plated on Columbia agar plates (Becton Dickinson, Sparks, MD) supplemented with 5% defibrinated horse blood and packed in 5% CO_2 Gas Pak Pouches (Becton Dickinson) [3].

2.3. Collection of clinical strains

Consecutive clinical strains of *S. pneumoniae* from adult patients (adult clinical isolates (ACIs)) with invasive pneumococcal disease (IPD) or pneumonia were collected during 2004–2006 from the participating hospitals and underwent a second identification and testing at the co-ordinating centre. History of vaccination with the 7-valent pneumococcal conjugate vaccine (PCV7) within the subject's family was an exclusion criterion. Non-invasive pneumococcal disease (NIPD) isolates were limited to those causing pneumonia, defined by clinical criteria (i.e. fever, leukocytosis, purulent sputum and consistent radiographic evidence) plus the recovery of *S. pneumoniae* as a single bacterial pathogen from the patient's sputum with concomitant depression of normal flora. Non-vaccine serotypes (NVS) were considered as all serotypes not included in the 23-valent polysaccharide pneumococcal vaccine [4].

2.4. Bacterial strains

Streptococcus pneumoniae isolates were identified on the basis of colony morphology, Gram stain, susceptibility to optochin by disk diffusion (5 μg disks; Becton Dickinson, Cockeysville, MD), bile solubility and latex agglutination test (Slidex pneumo-kit; bioMérieux, Marcy l'Etoile, France). Confirmed pneumococcal strains were stored in Todd Hewitt broth (Becton Dickinson) supplemented with bacteriological agar (0.3%, w/v) (Gibco BRL, Paisley, UK) and glycerine (3%, v/v) and kept at −70 °C until further processing for susceptibility testing and serotyping [3].

2.5. Antimicrobial agents and susceptibility testing

Penicillin (PEN), cefuroxime (CXM), ceftriaxone (CTX), erythromycin (ERY), trimethoprim/sulfamethoxazole (TMP/SXT), tetracycline (TET), moxifloxacin (MOX), levofloxacin (LEV) and ciprofloxacin (CIP) were tested by E-test on Mueller–Hinton agar. National Committee for Clinical Laboratory Standards methodology and breakpoints [5] for non-susceptibility and resistance were used for all antibiotics except CIP, for which the epidemiological cut-off of 2 mg/L was used as an indicator of resistance, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2006 recommendations [6]. *Streptococcus pneumoniae* ATCC 49619 was used for quality control. Multiresistance was defined as resistance to three or more classes of antibiotics [2].

2.6. Serotyping

All ACIs and PCIs displaying resistance to PEN, ERY or CIP were serotyped by the Quellung reaction using the 12 pooled antisera Pneumotest panel and selected factor sera (Statens Serum Institut, Copenhagen, Denmark).

2.7. Macrolide resistance phenotype

Macrolide resistance was determined by the double-disk diffusion method [7]. An M-phenotype was defined as resistance to ERY alone and an ML-phenotype as resistance to ERY and clindamycin, with the latter further distinguished as constitutive (cML) or inducible (iML/D-shape).

2.8. Statistical analysis

Data were compared using χ^2 or the Mann–Whitney *U*-test as appropriate. A two-tailed *P*-value of 0.05 was considered statistically significant. Statistical analysis was performed with SPSS® version 13.0 statistical software (SPSS Inc., Chicago, IL).