Spatial exploration of *Streptococcus pneumoniae* clonal clustering in São Paulo, Brazil

Authors
Amilton Mouro[1]
Carlos Kiffer[2]
Paula C.M. Koga[1]
Antonio M.V. Monteiro[3]
Eduardo Celso Gerbi Camargo[3]
Antonio Carlos Campos Pignatari[4]

[1]MSc; Special Laboratory of Clinical Microbiology, Universidade Federal de São Paulo (UNIFESP); Hospital Israelita Albert Einstein, São Paulo, Brazil
[2]PhD, MD; Special Laboratory of Clinical Microbiology, UNIFESP; GC-2 Gestão do Conhecimento Científico Ltd, São Paulo, Brazil
[3]PhD; Department of Image Processing, Instituto Nacional de Pesquisas Espaciais, São José dos Campos, Brazil
[4]Full Professor, PhD, MD; Special Laboratory of Clinical Microbiology, UNIFESP, São Paulo, Brazil

ABSTRACT

Objectives: To examine the spatial distribution of *Streptococcus pneumoniae* and its clonal patterns collected between 2002 and 2006 in São Paulo, Brazil. **Methods:** As part of an observational study in São Paulo city, Brazil, *S. pneumoniae* isolates routinely cultured from blood, respiratory specimens, or cerebrospinal and other profound fluids were selected. Additionally, only isolates with either penicillin (PEN) intermediate (I) or resistant (R) status on routine antibiogram were included, in order to obtain a higher probability of clonal isolates. A single I/R *S. pneumoniae* isolate per patient was included and submitted to genotypic determination by pulsed field gel electrophoresis (PFGE). Minimum inhibitory concentrations (MICs) were determined for the isolates by Etest® to PEN and other antimicrobials. Each isolate was geocoded in a digital map. The Kernel function and ratio methods between total isolates vs. clones were used in order to explore possible cluster formations. **Results:** Seventy-eight (78) *S. pneumoniae* community isolates from two major outpatient centers in São Paulo, Brazil, were selected from the databank according to their penicillin susceptibility profile, i.e. R or I to penicillin assessed by oxacillin disc diffusion. Of these, 69 were submitted to PFGE, 65 to MIC determination, and 48 to spatial analytical procedures. Preliminary spatial analysis method showed two possible cluster formation located in southwest and southeast regions of the city. **Conclusion:** Further analyses are required for precisely determining the existence of *S. pneumoniae* clusters and their related risk factors. Apparently there is a specific transmission pattern of *S. pneumoniae* clones within certain regions and populations. GIS and spatial methods can be applied to better understand epidemiological patterns and to identify target areas for public health interventions.

Keywords: *Streptococcus pneumoniae*; penicillin resistance; drug resistance, bacterial; molecular epidemiology.

INTRODUCTION

Streptococcus pneumoniae is the main bacterial agent of many respiratory tract infections (RTI). The importance of this pathogen is not only related to its prevalence, especially in the pediatric population, but also to the risks associated to resistance development and its therapeutic consequences.

Antimicrobial resistance in pathogens causing RTIs is a global problem and surveillance studies are of fundamental importance for identifying locations and patterns of these infections. If, on one hand, routine *in vitro* susceptibility tests are usually determined by a simple S, I, or R classification, on the other hand, this antibiogram method provides little information on the underlying level of susceptibility or resistance [i.e., the minimum inhibitory concentration (MIC)].[1] As an alternative, the determination of a pathogen's MIC allows better interpretation in terms of low or non-fully expressed resistance levels. Additionally, pulsed field gel electrophoresis (PFGE) technique holds a notorious discriminatory ability, high reproducibility, and well-determined interpretative criteria, although it is labor and cost-intensive. PFGE has been vastly applied and considered as one of the main tools for epidemiological and surveillance studies.[2,3] Furhermore, due to its ability in differentiating isolates of same species and correlating them with endemic clones, it has also been one of the most frequently used methods for *S. pneumoniae* molecular typing.[4-7]

Submitted on: 20/07/2011
Approved on: 03/09/2011

Correspondence to:
Carlos Kiffer
Rua Lenadro Dupret 188, 04025-010, São Paulo, Brazil
Phone: 11 55715180
kiffer@uninet.com.br

Financial Support:
FAPESP.

We declare no conflict of interest

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Spatial analysis based on geographical information systems (GIS) is useful for understanding disease epidemiology. However, it has not been frequently used for understanding the patterns of specific bacterial infections and their related risk factors, despite the fact that spatial aspects are probably linked to many factors influencing antimicrobial resistance patterns. It is well-known that antimicrobial resistance prevalence in community-acquired infections varies greatly depending on location and its related patterns (antimicrobial usage density, socio-economic level, health care system).

The present study aimed at using all three techniques (MICs, PFGE and GIS) in order to explore possible spatial patterns among *S. pneumoniae* clones with similar characteristics isolated from a higher penicillin resistance prevalence population submitted to routine cultures [respiratory or other invasive sample – blood or cerebrospinal fluid (CSF)], between 2002 and 2006 in São Paulo, Brazil.

MATERIALS AND METHODS

The present study was part of the EUREQA project[8] (FAPESP). As such, it has been submitted and approved by the Ethics Committee of the Universidade Federal de São Paulo (CEP process 545/08) and it was based on data observation without patient identification.

Population data

The observational EUREQA study stored on its database all *S. pneumoniae* events by individual address. *S. pneumoniae* events had the following case definition: routine culture (respiratory, blood, CSF or other profound fluids) results positive for *S. pneumoniae*, collected on two large healthcare outpatient facilities in São Paulo between 2002 and 2006, encompassing a public and a private sector unit. All cases had to reside in São Paulo (with address in clinical request form).

Identification and susceptibility testing procedures

Bacterial isolates were manually identified, with the GPI VITEK system card (bioMérieux,, Inc., Hazelwood, Missouri, USA) and conventional biochemical tests applied when indicated. Susceptibility testing was determined by disc diffusion with oxacillin (1 mg disc, Oxoid) and by agar diffusion with Etest® (AB BIODISK, Solna, Sweden) according to the manufacturer's procedures. Interpretative criteria used were those described in CLSI document M100-S20.[9]

Genotyping

Evaluation of chromosomal polymorphisms was performed by PFGE as described by Denton et al.[10] with minor modifications. Each plug was digested with 10 U of SmaI restriction endonuclease (Invitrogen, Carlsbad, CA) at 37°C for 12 hr. Electrophoresis was performed by 1% PFGE agarose gel run on CHEF-DR III system (Bio-Rad Laboratories, Richmond, CA) over 22 hr at 14°C with 5 to 35 s of linear ramping at 6 V/cm. Electrophoretic patterns were analyzed with GelCompar II v. 2.5 (Applied Maths, Kortrijik, Belgium) using the interpretative criteria by Dice similarity coefficient.

Geo-codification spatial analysis procedures

All spatial analytical procedures were performed with TerraView 4.01 software (Instituto Nacional de Pesquisas Espaciais, São José dos Campos, Brasil, 2003). Digital maps containing different layers with streets and districts information were used as the basis for including each individual case in the map by their addresses (point events, i.e. *S. pneumoniae* cases). In order to explore possible cluster formation, point events were submitted to Kernel function method,[11] which is an initial exploratory technique for interpolating and smoothing point events and is mainly used for identifying possible cluster formations. Point events were submitted to an adaptive radius with a quartic density Kernel. The analytical procedure was: (I) total point events distribution in a digital map; (II) Kernel function application on total point events; (III) Kernel function application on major clonal (A to D) point events; (IV) Kernel ratio application between total point events vs. total clonal events, in order to compare possible cluster formations and exclude bias from total sample distribution.

RESULTS

Seventy-eight (78) *S. pneumoniae* community isolates from two major outpatient centers in São Paulo, Brazil, were selected from the databank (with susceptibility R or I to penicillin). Of these, 69 were submitted to PFGE, 65 to MIC determination, and 48 to spatial analytical procedures (differences due to either isolate viability in the period studied or address loss during geocoding techniques). The median MIC of all isolates was 1.0 μg/mL, with an MIC range of 0.016-8.0 μg/mL (full MIC results are not shown in the present report). Based on CLSI criteria for invasive (CSF) isolates, 100% were R to penicillin. Based on non-invasive isolates, 18% were R or I to penicillin. From the 65 isolates, 43.1% (n = 28) were collected from respiratory tract (sputum, middle ear fluid, nasopharyngeal swab or bronchoalveolar lavage), 41.5% (n = 27) from blood, and 15.3% (n = 10) from CSF or other profound fluids.

Genotyping

All 69 isolates submitted to PFGE were compared by Dice similarity coefficient with 80% cutoff and 2% tolerance (BioNumerics v 5.1, Applied Maths, Kortrijk, Belgium).

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