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Original article

Phenotype, genotype, and serotype distribution of macrolide resistant invasive and non-invasive *Streptococcus pneumoniae* strains, in Sousse, Tunisia

Caractérisation phénotypique, génotypique et sérotypique des souches invasives et non invasives de Streptococcus pneumoniae *résistantes aux macrolides, à Sousse, Tunisie*

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Abstract

Objective. – We determined the macrolide resistance phenotypes and genotypes in *Streptococcus pneumoniae* isolates in Sousse and assessed the serotype distribution.

Methods. – We included *S. pneumoniae* strains isolated at our laboratory (2010–2013). The antimicrobial susceptibility was tested according to CA-SFM specifications. Serotyping was performed by agglutination of latex particles, to identify a subset of serotypes included in pneumococcal conjugate vaccines. The presence of macrolide resistance genes (*ermB, mefA, mel*) was detected by PCR.

Results. – A total of 52.8% of 140 *S. pneumoniae* isolates were macrolide-resistant: MLSB (89.2%) and M (10.8%). The MLSB phenotypes were genotypically confirmed by *ermB* gene presence. 62% had decreased susceptibility to penicillin. The serotypes were: 14, 1, 23F, and 19A. Serotype coverage by PCV7, PCV10 and PCV13 was 44.2%, 73.6%, and 75.6% respectively.

Conclusion. – 50% of *S. pneumoniae* isolates were macrolide resistant. The MLSB phenotype encoded by the *ermB* gene was the most frequent. Serotype coverage seems inadequate.

Keywords: Macrolides; *Streptococcus pneumoniae*

Résumé

Objet. – Caractéristiques phénotypiques, génotypiques et sérotypiques des pneumocoques résistants aux macrolides à Sousse.

Matériels et méthodes. – Étude des pneumocoques isolés dans notre laboratoire (2010–2013). Antibiogramme réalisé selon les recommandations du CA-SFM et sérotypage par méthode d'agglutination de particules de latex sensibilisées par des anticorps capsulaires. Les gènes de résistance aux macrolides (*ermB, mefA, mel*) ont été mis en évidence par PCR.

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Résultats. – Un total de 52,8 % des 140 souches étaient résistantes aux macrolides : MLSB (89,2 %) et M (10,8 %). Le gène *ermB* s'associait au phénotype MLSB. Soixante-deux pour cent étaient des PSDP. Les sérotypes étaient : 14, 1, 23 F et 19A. La couverture sérotypique par le PCV7, PCV10 et PCV13 était : 44,2 %, 73,6 % et 75,6 %.

Conclusion. – Dans notre région, plus de 50 % des pneumocoques sont résistants aux macrolides. Le phénotype MLSB codé par le gène *ermB* demeure le plus fréquent. La couverture sérotypique de ces souches paraît insuffisante.

Mots clés : Macrolides ; *Streptococcus pneumoniae*

1. Introduction

Streptococcus pneumoniae is a major and frequent agent of severe infections occurring at any age. The antibiotic susceptibility of this bacterium has been continuously decreasing, thus complicating the treatment of pneumococcal infections. Pneumococcal ENT infection is the most frequent and often the initial condition for other more severe infections. Macrolides are the most frequently prescribed antibiotic therapy, especially in case of beta-lactam failure, for this type of infections, and especially since the emergence of strains with decreased susceptibility to penicillins (PDSP).

Nevertheless, given the frequency of macrolide resistance, they can only be used as an empirical treatment, and only according to antibiogram data. It is thus strongly recommended to update data on pneumococcal susceptibility to the various macrolides.

We focused our study on the phenotypes and genotypes of pneumococcal resistance to macrolides and related agents in strains isolated in Sousse (Tunisia) during the previous 4 years, and on the determination of capsular serotypes of these strains.

2. Materials and methods

2.1. Population and study location

We studied non-redundant *S. pneumoniae* strains isolated in the microbiology laboratory of the Sousse Farhat Hached Teaching Hospital (2010–2013), issued from various samples.

2.2. Culture and identification of S. pneumoniae strains

The samples were seeded on fresh and cooked blood agar and incubated 18 to 24 h at 37 °C with 5% CO_2. *S. pneumoniae* was identified according to the usual methods. The internal quality control was performed with the reference strain *S. pneumoniae* ATCC 49619.

2.3. Phenotype of susceptibility to antibiotics

The antibiogram was performed and analyzed according to the recommendations issued by the French microbiology antibiogram committee (French acronym CA-SFM). The tested antibiotics were: penicillin G, amoxicillin, cefotaxime, oxacillin, erythromycin, lincomycin, pristinamycin, telithromycin, tetracycline, chloramphenicol, cotrimoxazole, levofloxacin, rifampicin, vancomycin, and teicoplanin.

2.4. Phenotype of susceptibility to macrolides

The results were analyzed according to CA-SFM recommendations. The constitutive or inducible phenotype MLSB (by modifying the target by methylation) means there is resistance to erythromycin, lincomycin, and telithromycin; pristinamycin remains active. The phenotype M (resistance by efflux pump) means there is resistance to erythromycin only.

2.5. Determination of serogroups and serotypes

S. pneumoniae serogroups and serotypes were determined by the latex particle agglutination test sensitized by the anti-capsular polysaccharide antibody (Pneumo-Test-Latex® kit, Statens Serum Institute, Copenhagen, Denmark) corresponding to the 13-valent pneumococcal vaccine, according to the manufacturer's recommendations. The quality control was performed with *S. pneumoniae* serotype 1 (Statens Serum Institute).

2.6. Genotypic detection of genes of resistance to macrolides

The studied genes of resistance to macrolide (*ermB, mefA, mel*) were identified by gene amplification (PCR). The polymerase master mix contained Taq buffer (10X), dNTPs, $Mgcl_2$. Taq polymerase, 1 μL of every primer [1], and 2 μL of DNA (extracted by thermal lysis). A negative control and a reference strain depending on the targeted gene (positive control) were included in every series. Pr. Leclercq provided the reference strains: *Enterococcus faecalis* JH2-2 (Tn 1545), *Streptococcus pyogenes* UCN64, and *Staphylococococcus aureus* RN 4220 for the genes *ermB, mefA*, and *mel*. A molecular weight marker (100pb) was used to determine the size of migrated bands. The amplified sequences were revealed with the Molecular Imager GelDoc XR (Biorad).

3. Results

One hundred and forty non-redundant *S. pneumoniae* strains were collected during the study period in our laboratory. Seventy-four strains (52.8%) were resistant to macrolides. The invasive strains (from normally sterile sites) (50%) were identified in blood cultures (23%), CSF (16.2%), puncture fluids