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Serotypes and Antibiotic Susceptibility of *Streptococcus pneumoniae* Isolates from Invasive Pneumococcal Disease in Morocco (Meningitis Cases)

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ABSTRACT

The aim of this study was to examine the antimicrobial susceptibility of Streptococcus pneumoniae isolates from invasive meningitis cases, to determine the serotypes and susceptibility of serogroups/serotypes. Pneumococcal meningitis isolates were isolated from Cerebrospinal Fluid (CSF), between 2012 and 2013. Serotypes were determined using the capsular reaction test (Quellung test). Antimicrobial Susceptibility was tested using the disk diffusion method as determined by the CA-SFM guideline. The evaluation of susceptibility to β-lactamins showed that 56% of the strains were penicillin non-susceptible strains, 31% were resistant to macrolides. The isolates were resistant to other antibiotics, 37% to trimethoprim-sulfamethoxazole, 12% to chloramphenicol, 6% was observed with glycopeptides and aminosides. Almost all strains serotyped have a power of resistance with different families of antibiotics tested. Serotypes 2, 5, 6, 16, 12, 19A, 23 had a resistance to β-lactams and macrolides. All of isolates serotypes 6 and 32 were resistant to glycopeptides and aminoglycosides. This study aims to report the resistance evolution of S. pneumoniae strains and serogroups/serotypes with penicillin and different family's antibiotics from Maroccan pneumococcal isolates.

Keywords: Pneumococcal serotypes/serogroups, Antibiotic susceptibility, Invasive disease, Pneumococcal meningitis

INTRODUCTION

Streptococcus pneumoniae (*S. pneumoniae*) is a major causative agent of severe infections, including sepsis, pneumonia, meningitis, and otitis media, and has become a major public health concern [1]. In 2005, the World Health Organization (WHO) estimated that 1.6 million deaths annually are attributable to pneumococcal disease, this estimate includes the deaths of 0.7-1 million children aged < 5 years [2,3].

S. pneumoniae is a Gram-positive encapsulated diplococcus. The polysaccharide capsule is an essential virulence factor for invasive pneumococcal disease. Based on the identification of differences in the composition of this capsule, there are about 90 distinct pneumococcal serotypes. Globally, about 20 serotypes are associated with >80% of invasive pneumococcal disease occurring in all age groups; the 13 most common serotypes cause at least 70-75% of invasive disease in children [3]. Immunization with a conjugate vaccine in children shows a reduction significant porting the included serotypes in the vaccine not only for the vaccinated patients, but also for their younger siblings, a phenomenon known as "indirect immunity" or "herd immunity" [3,4]. This herd immunity is particularly important, because the incidence and mortality rates of pneumococcal disease are high in older adults [5]. Introduction of this vaccine rapidly led to a dramatic decrease in the incidence of Invasive Pneumococcal Disease (IPD) and all so reduction of antibiotic resistance [6,7]. Pneumococcal infections pose therapeutic problems by the emergence of resistant strains antibiotics. *S. pneumoniae* is naturally sensitive.

To penicillin, but at present more and more isolation is being around the world, strains of pneumococci of Decreased Sensitivity to Penicillin (PSDP). This resistance is not limited to penicillin, but affects other ß- Lactams and other families of antibiotics [7]. Surveillance of antibiotic resistance and pneumococcal serotypes are indispensable for empirical preventive, implementation of resistance control measures, and prevention of antibiotic-resistant microorganisms [8]. This study aims to report the resistance evolution of *S. pneumoniae* strains and serogroups/serotypes with penicillin and different families antibiotics used in treatment.

MATERIALS AND METHODS

Clinical isolates collection and culture conditions'

Bacteriological exploration and serotyping were performed in the laboratory of medical bacteriology at the National Institute of Hygiene-Rabat. This study investigated 16 no repetitive isolates pneumococcal meningitis from Cerebrospinal Fluid (CSF), They were isolated between 2012 and 2013. All information concerning the identities of the patients and the results of this study were treated confidentially.

Isolates identification

These strains were re-isolated on Columbia agar (Oxoid, UK) with 5% sheep's blood supplemented with 5 ug/ml gentamicin and also in T-broth, incubated at 37°C in anaerobic jars for 24-48 h to obtain individual colonies of *Streptococcus pneumoniae*. The identification of these bacteria was based on cultural aspects of study and appeared type of hemolysis on blood agar [8]. Colonies suspected having α-hemolysis are subject to the test Catalase, the study of phenotypic characteristics has been completed by the Gram stain, the test sensitivity optochin and solubility in bile as well as the agglutination test (Dryspot Pneumo Test (Oxoid, UK). The *Streptococcus pneumoniae* strain ATCC 49619 was adopted as the reference strain for control reagents, culture media and phenotyping techniques [9].

Susceptibility testing

The antibiotic susceptibility test was performed by the medium diffusion disk method agar on Mueller Hinton enriched with 5% sheep blood and 5% CO_2 (Biofarma, Morocco) respecting the committee recommendations of antibiogram society of Microbiology, we proceeded to inoculate by friction agar surfaces with a swab impregnated with the inoculum at 0.5 McFarland turbidity forming tight tries to obtain contiguous colonies after 16-24 h of incubation at 37°C [10]. The antibiotic disks included in this study to assess their inhibitory activity, we opted for the following molecules: Penicillin G, oxacillin, amoxicillin, trimethoprim-sulfamethoxazole, cefotaxime, vancomycin, erythromycin, gentamicin, chloramphenicol, spiramycin. The detection sensitivity of pneumococci decreased to penicillin G is carried out with an oxacillin disk. If the diameter around the oxacillin disk was less than 26 mm, it is preceded thereafter to the measurement of Minimum Inhibitory Concentrations (MIC) [10].

Serotyping

Capsular typing was done by the Quellung reaction or Neufeld test, which was performed using a set of antisera obtained from Statens Serum Institute. Typing was conducted by phase-contrast microscopy according to the published procedure [11].

RESULTS

Overall in this study, resistant forms are more common with 81% against only 19% of sensitivity forms. More than half of pneumococcal strains (56%) express a resistance with penicillin G (P), 44% from erythromycin (E), 19% from spiramycin (SP), 37% from trimethoprim-sulfamethoxazole (SXT), and 12% from chloramphenicol(C). While the lowest resistance rates (6%) was observed for the three antibiotics gentamicin (G), vancomycin (VA).

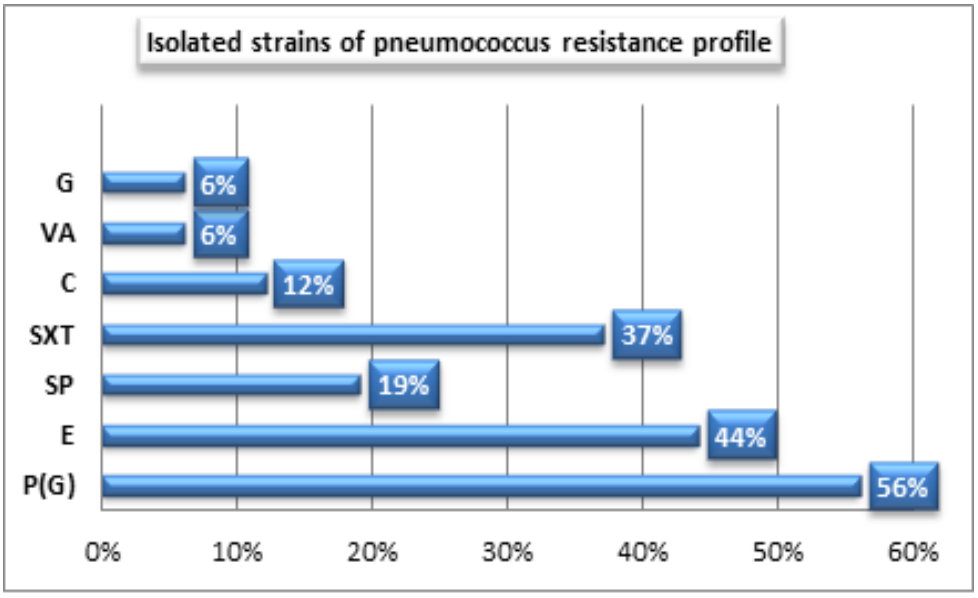

Figure 1: *Streptococcus pneumoniae* rate resistant to antibiotic discs
P: Penicillin; E: Erythromycin; SXT: Trimethoprim-sulfamethoxazole; SP: Spiramycin; C: Chloramphenicol; VA: Vancomycin; G: Gentamicin

Determining pneumococci resistance levels is performed with respect to the measurement of MIC of the antibiotic expressed in micrograms/ml (Figure 1). Thus, if the measured MIC is greater than the value of 2 ug/ml for penicillin G, the strain is described as highly resistant. And if the MIC of the same antibiotic is between 0.12 and 2 µg/ml, the bacterium is known as resistant. CMI was measured of all the sensitivity of pneumococcus strains decreased to penicillin, 44.44% expressed resistance to their highest level since CMI is equal to 4 ug/ml and 55.56% are considered resistant strains even with MICs between 0.12 and 2 ug/ml in the case of meningitis infections. All serotypes studied in the presence of molecules of antibiotics allowed to describe a particular profile of resistance.

Table 1: Profile of resistance compared to different pneumococcal serotypes

Antibiotics tested family	Serotypes *Pneumococcal* strains expressing resistance	Résistance relative fréquences
Penicillins/cephalosporins		
Penicillin G, oxacillin and amoxicillin	2, 5, 6, 16, 19A, 23	**44%**
Cefotaxime	2	
Macrolides		
Erythromycine	2, 5, 6, 23, 19A.	
Spiramycin		**31%**
Sulfamides-trimethoprim		
Trimethoprim-sulfamethoxazole	1, 6, 23, 32,	**37%**
Phenicoles		
Chloramphénicol	23	**12%**
Glycopeptides		
Vancomycin	32	**6%**
Aminosides		
Gentamicine	32	**6%**

The following Table 1 shows the distribution of serotypes depending on their position with antibiotics belonging to different families of antibiotics. Almost all strains serotyped have a power of resistance with different families of antibiotics tested.

DISCUSSION

Evolution from the Penicillin Non-Susceptibility Pneumococci (PNS) was done in the sense of a steady and statistically significant increase with 15.3% of PNSP during the period 1998-2001, 18.9% of PNSP in 2002-2005 and 23.5% in 2006-2008 [12]. A study were performed on non-invasive isolates also showed a significant increase during 2008-2009, 34.7% were reduced antibiotic susceptibility. Of these, 12.9% had a high level of resistance and 87.1% had a low level of resistance to penicillin [13]. In our study the invasive isolates of *S. pneumoniae* in 2012-2013 also shows a significant increase in resistance, More than half of pneumococcal strains (56%) expressed a resistance with penicillin G (P), 44% expressed resistance to their highest level since CMI is equal to 4 ug/ml and 55.56% are considered resistant strains even with MICs between 0.12 and 2 ug/ml from pneumococcal meningitis infections. The Same results were recorded in some studies, In Tunis 55.2% of non-invasive strains are PNSP against 50.4% invasive strains [14].

In 2005 the rate of isolated meningitis PNSP was 36% in French, and had the highest rate of beta-lactam resistance of *Streptococcus pneumoniae* than any other European country. Between July 2000 and March 2007 significant reduction of antibiotic use in the community after a nationwide campaign in French. This study observed a decline by 26.5% in the number of antibiotic prescriptions, surpassing even the national target of a 25% reduction over five years. The decrease was seen in all French regions and age groups, with the highest decrease observed in children [15-17]. In the same direction a medico-economic study was realize Through the proper use of antibiotics in the hospital, the rationalization of medical practices is required to treat as well as possible at the lowest cost [18]. The resistance mechanism is based on a modification targets beta-lactam: Protein Binding to Penicillin (PBPs). *S. pneumoniae* has 6 PBPs. The PBP 2b and 2x were mainly concerned for the action of penicillin G. Each inhibits beta-lactam several PBP which differ between antibiotic molecules. These changes follow a point mutation or genetic recombination transfer PBP genes from related species of the sphere oropharyngeal (*Streptococcus mitis, Streptococcus oralis*) which results in the formation of mosaic genes [19]. Acquired resistance to penicillin G is crossed with all other beta-lactam antibiotics but at varying levels based on antibiotics for use the most active molecules [19-24].

For no beta-lactam antibiotics resistance, we found 31% for macrolides, 37% sulfatrimethoprim, and 12%for phenicoles, 6%for glycopeptides and aminosides. In Spanish, erythromycin resistance rate for invasive isolates was 36.5%, whereas the specific rates were 47.5% and 35.5% in children and adults, respectively [20]. Similar results have been found in other studies [20,21], In Tunis, 54.3% of strains were resistant to erythromycin [14]. In French, this resistance is 70% for isolated strains of pneumococcus in children [23]. All serotypes studied in the presence of different antibiotics families allowed to describe a particular profile of resistance. In our study we found 9 serotypes of invasive isolates: 1, 2, 5, 6, 12, 16, 19A, 23, 32, resistance to penicillin have been observed in serotypes 2, 5, 6, 16, 19A, 23 with a percentage of 56%. Serotype 23 had a resistance to all antibiotics families used in this study, for other families antibiotics, expression of resistance was observed in serotypes 1, 5, 6, 23, 32. The studies showed that, penicillin resistance has been associated mainly with certain serotypes, principally serotypes 6, 9, 19, and 23 [25]. In study was realized in Spain, has been reported for serogroups 6 and 23, penicillin resistance percentage was 42.8%, 33.3%, and 64.3%, 60% resistance to erythromycin respectively in invasive cases [26]. The cases studied in Algeria between 2010-2014 showed that High level of resistance for vaccine serotypes was observed in serotypes 14, 19A, 19F, and 23F. Overall, serotypes 14, 19A, 19F, 23F, 6B, and 5 presented higher rates of resistance than the eight serotypes 6C, 9N, 20, 24F, 35B, 35F [27].

CONCLUSION

In this study more than half of the pneumococcal isolates express a resistance with penicillin and different percentages were observed with other antibiotics. All serotypes studied had a particular profile of resistance, only 6 and 23 present a power of resistant with all antibiotics families studied. Our study emphasizes on the importance of continued serotyping and surveillance of antimicrobial susceptibility of all *S. pneumoniae* clinical isolates, in order to know the percentage of vaccination coverage range and to guide the clinician in the choice of appropriate antibiotic therapy for serious pneumococcal infections.

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