THE DIVERSITY OF ANTIBIOTIC RESISTANCE PHENOTYPES AND CAPSULAR SEROTYPES IN *S. PNEUMONIAE*

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Abstract: The pneumococcus is frequently involved in pediatric infections, and the incidence of antibiotic-resistant strains is increasing. We studied 85 *S. pneumoniae* strains isolated from pediatric infections between january 2001-august 2002, and we determined the capsular serotype and the antibiotic resistance profile. Out of these, thirty-two strains were highly resistant to penicillin (P) and multiresistant to other 5 antimicrobials; 7 out of the P-highly resistant strains were also resistant to cefotaxim (CTX). Most of the multiresistant isolates belong to the serotypes 19A, 23F, 6A/6B. Every pneumococcal strain isolated from severe infections should be quantitatively tested to P and cephalosporins.

INTRODUCTION

The pneumococcus is one of the most important bacterial etiological agents of human infectious pathology, occupying one of the first places among the causes of mortality by infectious diseases in developed countries. The emergence of P-resistant pneumococcal strains in the last 25 years and the increasing prevalence of isolates multiresistant to antimicrobials (Doern, 2000), including third generation cephalosporins (Charpentier, Tuomanen, 2000), create an important issue concerning the public health.

Capsular serotypes and antibiotic-resistance phenotypes show regional variations (Jette et al., 2001). In order to reconsider the therapeutic strategy in community-acquired pediatric infections and to appreciate the efficacy of the polyvalent antipneumococcal vaccines, we ought to know the distribution of these pneumococcal capsular serotypes and resistance phenotypes in our region. The extensive use of such polyvalent vaccines could decrease the local circulation of the antibiotic-multiresistant serotypes (Appelbaum, 2002).

MATERIAL AND METHODS

We studied 85 *S. pneumoniae* strains isolated from children admitted in the „Sf. Maria" Hospital for Children in Iasi during january 2001 – august 2002. Out of the 85 strains, 30 were isolated from otitis media, 16 from ophthalmic infections, 14 from invasive infections (5 from bacteremias, 2 from meningitis, 4 from pleural effusions and 1 from urinary tract infection) and 25 from pulmonary infections. We identified the isolates by the features of the colonies on sheep blood agar and the optochin susceptibility test, followed by latex-agglutination. We determined the serotypes by the quellung-test using reference sera from the specialized center in Copenhagen. Susceptibility to penicillin (P), erythromycin (E), tetracycline (T), chloramphenicol (C), sulphametoxazol-trimethoprim (SXT) and rifampicine (RIF) was tested by the disk diffusion method, according to the NCCLS criteria for the year 2002. Strains with inhibition zone diameters to oxacillin (1μg) < 20 mm have been tested quantitatively towards P and cefotaxim (CTX) using „E-test" (AB-Biodisk, Solna, Sweden), according to the same interpretation criteria. We used *S. pneumoniae* ATCC 49619 as control strain.

The studied strains were isolated from 51 boys (60%) and 34 girls (40%), 72,9% of the patients being under the age of 2 years.

RESULTS AND DISCUSSIONS

Out of the 85 tested strains, 15 were susceptible to P, 38 were intermediate and 32 were highly resistant to P, 25 out of the last with MIC (minimal inhibitory concentration) values between 6 – 16 μg/ml. All strains with intermediate resistance to P were

susceptible to CTX, but the strains highly resistant to P (MICs of 2->32µg/ml) had a various behavior toward CTX (table 1), with 7 isolates highly resistant to CTX.

Table 1. Behavior to CTX of the P-resistant strains

MICs of CTX	**MICs of penicillin (no. of strains)**	
	I (0,1 – 2 µg/mL)	**R** (≥ 2 µg/mL)
S (≤ 1 µg/mL)	38	19
I (1,1 – 3,9 µg/mL)	-	6
R (≥ 4 µg/mL)	-	7

P-resistant strains were isolated from different sites of pneumococcal infection, but none of the strains isolated from invasive infections was resistant to CTX. Resistance to the other 5 antibiotics had different profiles, depending on the susceptibility to P: 7 out of the 15 P-susceptible strains had 1-2 resistance determinants, but 61 out of the 70 P-resistant strains had >3 resistance determinants, being considered multiresistant (table 2).

Table 2. Resistance phenotypes correlated to the susceptibility to P and CTX

Resistance phenotypes	**Susceptibility to P (no. of strains)**		**Susceptibility to CTX (no. of strains)**		
	I	R	S	I	R
P+SXT	9	-	9	-	-
P+E+SXT	2	6	3	3	2
P+SXT+T	4	-	4	-	-
P+SXT+C	1	-	1	-	-
P+SXT+RIF	2	-	2	-	-
P+E+SXT+T	18	17	28	3	4
P+E+SXT+T+C	2	1	3	-	-
P+E+SXT+T+RIF	-	7	7	-	-
P+E+SXT+T+C+RIF	-	1	-	-	1

Most CTX-resistant strains (6 intermediate resistant and 5 highly resistant) were isolated from children with otitis media. The phenotype P+E+SXT+T was the most frequently isolated, unrelated to the site of pneumococcal infection (fig. 1). Strains isolated from otitis media had a remarkable phenotypic diversity.

We found constant resistance to SXT, while only a few strains were resistant to C and RIF (5 and 10 strains respectively). Serologic identification of the strains described 15 serotypes/10 serogroups, with 6 strains that didn't match any of the available sera (we will call them non-determined). The most frequently encountered serotypes were 19A (29,4%), 23F/23B (24,7%) and 6B/6A (18,8%), other serotypes (1, 2, 9N/9V/9B, 11A, 14, 15C/15B, 20) representing less than 10% each. The distribution of the serotypes is not very different depending on the infection site, except for the isolates from conjunctival infections, 2/3 out of these belonging to the 23F/23B serogroup or being non-determined.

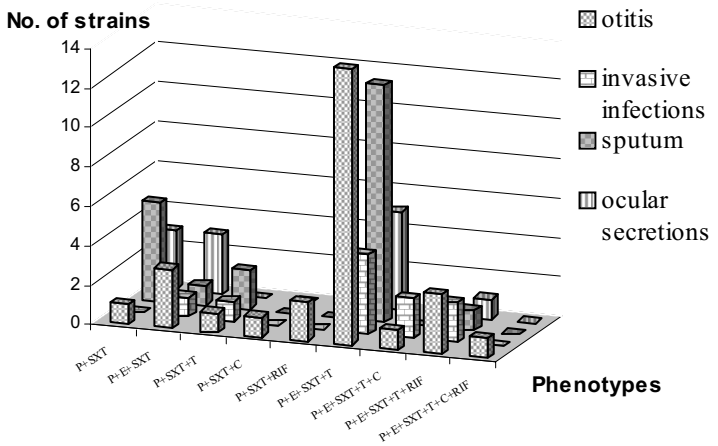

Figure 1 – Resistance phenotypes in *Streptococus pneumoniae* isolated from pediatric infections

The *S. pneumoniae* isolates from pediatric infections confirm the regional circulation of antibiotic multiresistant serotypes, especially in children (Coman et al., 2003). Strains with intermediate resistance to P have a relative uniform distribution of the capsular serotypes, covering 10 out of the 15 identified serotypes. High resistance to P and resistance to CTX were encountered only in serotypes 19A, 23F and in 3 of the 6 non-determined strains, isolated from otitis media and pulmonary infections. Multiresistant strains belonged to the serotypes 19A, 23F, 6A/6B, 14, 15 and 20. Serotypes 19A and 23F were the most frequently isolated in our study and had the most important resistance to β-lactams and other classes of antibiotics. It is difficult for us to appreciate the real prevalence of other serotypes because of the relatively small number of studied strains.

The spread of P-resistance seems to be caused by the global dissemination of several clones with altered plp genes and genes that encode resistance to other antibiotic classes, including macrolides, T, C and SXT. The incidence of P-resistant pneumococci dramatically increased in the whole world, especially in the ’90-s (Doern, 2000; Tomasz, 1997). This situation is worsened by the recent emergence of high resistance to extended spectrum cephalosporins of the third generation (Charpentier, Tuomanen 2000; Coman et al., 2002).

The use of a polyvalent antipneumococcal vaccine depends on the knowledge of the frequency of local serotypes. In our study, only 41,2% of local serotypes are represented in the heptavalent vaccine used in the US since 2000. Further studies on a higher number of local strains could indicate the most frequent serotypes that ought to be included in a polyvalent vaccine adequate for our region (Coman et al., 2003)

CONCLUSIONS

MIC testing to penicillin allows the differentiation of intermediate resistant strains from highly resistant ones. Accordingly, infections produced by intermediate resistant

strains, except for pneumococcal meningitis, could be treated with large doses of penicillin.

Detection of resistance to extended spectrum cephalosporins in isolates from pulmonary infections requires quantitative testing to CTX or ceftriaxone whenever these antibiotics are an option for the therapy.

The antibiotic resistance is correlated with the capsular serotype, most strains highly resistant to P and resistant to CTX belonging to 19A and 23F serotypes.

Identification of the capsular serotype for the circulating strains allows the evaluation of the efficacy of polyvalent vaccines commercialized by foreign companies. Before using these vaccines, we ought to know their ability to cover the local most frequent serotypes.

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