

CEFTRIAXONE RESISTANCE IN *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM PEDIATRIC INFECTIONS

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Abstract. *Streptococcus pneumoniae*- major cause of infection in children - causes serious therapeutic difficulties, because of the developed multiresistance. Extended-spectrum cephalosporins, used in empirical therapy are a therapeutic option. Recent emergence of Ceftriaxone (CRO) and Cefotaxime (CTX) resistant strains with regional differences in frequency requires their detection by quantitative determinations. The results of the first study on children from Iasi concerning CRO resistance of *S. pneumoniae*, evaluated according to NCCLS 2002 criteria, showed the presence of this phenotype in ear and lower respiratory tract infections. Authors recommend quantitative testing for CRO of high level P resistant strains, isolated from these infections, when CRO is a therapeutic option and surveillance of occurrence of this resistance phenotype in strains isolated from non respiratory infections.

Keywords: *Streptococcus pneumoniae*, ceftriaxone resistance, revision breakpoints

Rezumat. *S. pneumoniae*, cauză infecțioasă majoră la copii, creează în prezent dificultăți terapeutice prin multirezistența dezvoltată. Cefalosporinele cu spectru larg, folosite în terapia de primă intenție a unor infecții grave, reprezintă o alternativă terapeutică. Emergența recentă a unor tulpini cu rezistență la CRO și CTX, a căror frecvență variază regional, impune cunoașterea acestora prin determinări cantitative. Rezultatele primului studiu la copii din Iași privind rezistența *S. pneumoniae* față de CRO, apreciată după criteriile NCCLS 2002, au evidențiat existența acestui nou fenotip în infecții otice și respiratorii inferioare. Autorii recomandă testarea cantitativă față de CRO a tulpinilor cu rezistență înaltă la P, izolate din acest tip de infecții, atunci când acest antibiotic reprezintă o opțiune terapeutică și supravegherea apariției acestui fenotip de rezistență la tulpini izolate din infecții extrarespiratorii.

Cuvinte cheie: *Streptococcus pneumoniae*, rezistența la ceftriaxonă, puncte de ruptură revizuite

INTRODUCTION

Streptococcus pneumoniae – as a major ethiological agent of infections in children- caused a great concern during the last 25 years, by the developed multiresistance. Penicillin (P) resistant strains, widely distributed, show frequently

multiresistance to antibiotics belonging to other classes: Erythromycin (E), Clindamycin (CL), Sulphamethoxazol-trimethoprim (SXT), Tetracycline (T), Chloramphenicol (C) (1-8).

At the beginning of last decade, a new phenotype characterized by simultaneous

resistance to extended spectrum cephalosporins occurred; it caused therapeutic failure in pneumococcal meningitis treated with Cefotaxime (CTX) or Ceftriaxone (CRO) (5,9-11).

Frequency and level of resistance to third generation cephalosporins used in the empirical therapy of severe infections show large regional differences (11-14). On the other hand, the exclusive use of quantitative methods in sensitivity testing to these agents is a obstacle in routine assay. The above mentioned conditions, made the motivation of our study, performed on a certain age category of patients.

MATERIAL AND METHODS

46 clinically significant *S. pneumoniae* strains were included in the study; they were isolated as follows: otitis media (24), lower respiratory tract infections (15), bacteremia (4), meningitis (2) and arthritis (1) from children hospitalized in the Emergency pediatric hospital "Sf.Maria" from Iasi, Romania during January 2001- September 2002.

Initially, strains were tested by disk diffusion method against Oxacillin (OX), E, SXT, T, C, Rifampicine (RIF) and they were considered P resistant if the OX inhibition zone diameter was < 20 mm, according to NCCLS 2001 and 2002 (15,16). In the second stage, we determined P and CRO minimal inhibitory concentration (MIC) by E-test (AB-Biodisk- Solna Sweden) following the manufacturer instructions; interpretation

of the results was done according to NCCLS 2001 (15), and compared to NCCLS 2002 (16).

As control strain in sensitivity testing was used *S. pneumoniae* ATCC 49619.

RESULTS

Amongst the 46 P resistant strains- evaluated by disk diffusion method- 19 were intermediate resistant, with MIC 0.25- 1.5 µg/ml (fig. 1).

For this category of strains, CRO MIC varied between 0.064 and 0.5 µg/ml, being therefore considered sensitive, according to NCCLS criteria, 2001 (15) and also 2002 (table 1) (16). All the 7 strains isolated from blood, cerebrospinal fluid (CSF) and joint fluid belonged to this category.

Strains showing high P resistance MIC (2- ≥ 32 µg/ml) had a variable behavior against CRO (table 1). According to NCCLS 2001 criteria (15), 11 strains were considered sensitive (MIC: 0.25- 0.5 µg/ml), 5 with intermediate resistance (MIC: 0.75-1 µg/ml) and 11 high resistance (MIC: 2-12 µg/ml). According to NCCLS 2002 criteria (16), which indicate different breakpoints for strains isolated from meningitis and non-meningeal infections, the numbers of sensitive (MIC ≤ 1 µg/ml), intermediate (MIC: 1.5- 3 µg/ml) and resistant (MIC ≥ 4 µg/ml) strains become: 16, 7 and 4, respectively (table 1).

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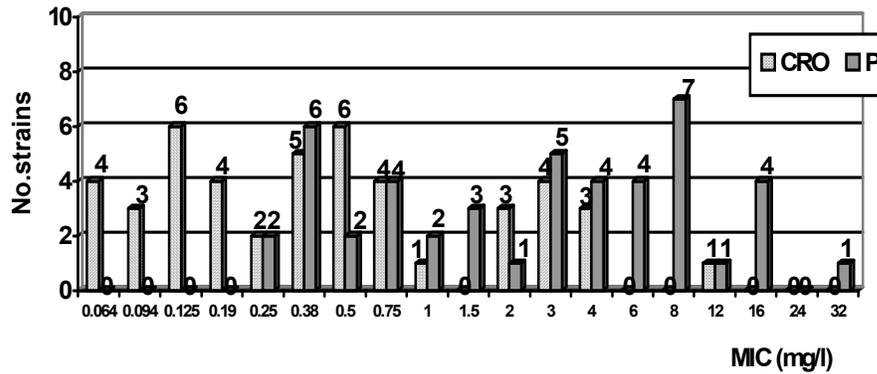


Fig. 1 MIC values for Penicillin G (P) and ceftriaxone (CRO) of the 46 *S. pneumoniae* strains

Table 1. Ceftriaxone sensitivity of *S. pneumoniae* strains intermediate or resistant to Penicillin

MIC ceftriaxone*	MIC Penicillin G	
	0.25 - 1.5 µg/ml	≥ 2 µg/ml
≤ 1 µg/ml	19	16
1.5- 3 µg/ml	0	7
≥ 4 µg/ml	0	4

* Strain classification was done according to NCCLS 2002 criteria.

Majority of strains with modified CRO sensitivity (6 with intermediate resistance and 3 with high resistance) were isolated from children with otitis media; one of these strains showed the highest resistance level to Penicillin (MIC ≥ 32 µg/ml) and CRO (MIC = 12 µg/ml). Strains with different CRO sensitivity haven't presented a different resistance pattern against 5 non β- lactam antibiotics

tested by antibiogram. Phenotype P+E+SXT+T was the most frequently encountered both in sensitive and resistant strains (table 2). The CRO hyperresistant strain (MIC = 12 µg/ml) proved to possess the biggest number of resistance determinants (the only strain multiresistant to the 7 tested antibiotics).

Table 2. Resistance phenotypes in *S. pneumoniae* strains

Patterns of resistance	CRO*		
	sensitive	intermediate	resistant
P+SXT	2	-	-
P+E+SXT	3	1	1
P+E+T	1	-	-
P+SXT+T	4	-	-
P+SXT+RIF	2	-	-
P+E+SXT+T	13	6	2
P+E+SXT+T+C	5	-	-
P+E+SXT+T+RIF	5	-	-
P+E+SXT+T+C+RIF	-	-	1

* Strain classification was done according to NCCLS 2002 criteria.

DISCUSSION

Data from literature mentioned an extremely high frequency in some countries of P resistant *S. pneumoniae*: 35% in USA (3), 40% in Spain (7), 77% in Korea (17), 67% in Hungary exclusively in pediatric cases (18). In a previous collaboration study undertaken during 1997- 1998 between our hospital and Beer-Sheva University (Israel), we found a frequency of 69% of these strains, some of them with extremely high MIC values (19). These findings proved that, in our region, there were the prerequisites for strains with cephalosporin modified resistance, the relation between increased MICs in cephalosporins and P being well known. Changes in Penicillin Binding Protein (PBP) - basis for β -lactam resistance - cause the decrease of the affinity for target of all this group of agents, but third generation cephalosporins and carbapenems are known to have a

better activity (2,5,6,20). According to data from literature, none of CRO or CTX resistant strains was P sensitive, but not all the P resistant strains show modified sensitivity to extended spectrum cephalosporins (4,13).

Disk diffusion method is not very accurate in determining the cephalosporin resistance; that is why NCCLS recommends quantitative determinations (10,11,15,16). Among these, E-test is considered a simple method and well correlated with broth dilution technique, taken as reference (21).

In 2002, NCCLS (16) changed the breakpoints for CRO and CTX for non meningial isolates, as the frequency of resistant strains decreased. Sahm et al (22) reported changes in ratio for sensitive, intermediate and resistant strains respectively, from 82.7%, 13.2% and 4.1% to 95.5%, 3.1% and 1%, compared to the new standard. In our

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study, the number of strains with high resistance level decreased from 11 to 4, these being recovered from otitis media and lower respiratory tract infections.

NCCLS annually reconsiders the inhibition zone diameters and breakpoints according to microbiological, pharmacokinetic, pharmacodynamics and clinical data, especially in species with gradually changes in antibiotic sensitivity (5,23,24). Changes in CRO and CTX breakpoints for *S. pneumoniae* were dictated by the discrepancies between *in vitro* results and good therapeutical response in infections with strains considered resistant, isolated from non meningial infections (5,10).

A similar situation is drafted for P breakpoints, at least for strains isolated from lower respiratory tract infections; there is a proposal that only strains with MIC ≥ 4 $\mu\text{g/ml}$ to be considered resistant (2,5,22,25).

Revision of P and CRO breakpoints correlated with clinical relevance will contribute in diminishing Vancomycin usage in pediatric infections. It is well known the simultaneous resistance to non β -lactam antibiotics of *S. pneumoniae*, which creates the same therapeutic difficulties as MRSA. Even if, we couldn't encounter a different behavior against these agents of CRO sensitive and resistant strains, is underlined the CRO high level resistant strain (MIC = 12 $\mu\text{g/ml}$) with simultaneous resistance to the other 6 tested antibiotics; Vancomycin can be considered only active antibiotic.

CONCLUSIONS

1. The results of the first study undertaken in Iasi concerning CRO resistance in *S. pneumoniae*, detected the new phenotype of strains isolated from otic and lower respiratory tract infections.
2. In this type of infections, when this antibiotic is an option, quantitative CRO testing of high P resistant and multiresistant strains is required. Moreover, the testing of P resistant strains from severe infections, especially in pneumococcal meningitis is required to prevent a therapeutical failure.

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