MONITORING OF ANTIMICROBIAL RESISTANCE OF NASOPHARYNGEAL *STREPTOCOCCUS PNEUMONIAE* IN CHILDREN FROM ORPHANAGES IN RUSSIA: RESULTS OF SPARS STUDY

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Revised Abstract

Children from orphanages and day-care centers are of an additional risk of colonization by pneumococci, including antibiotic-resistant isolates. It was previously shown that monitoring of antimicrobial resistance of nasopharyngeal strains is an excellent approach for prediction of resistance in clinical isolates. We are reporting the results of the very first nation-wide study involving the same group of investigators sampling children in different cities using unified methodology.

Nasopharyngeal swabs were collected from 613 children less than 5 years old in 11 orphanages in 8 cities of European (Kazan, Moscow, Saint Petersburg, Smolensk, Ufa, Volgograd) and Asian Russia (Anadyr, Khabarovsk) with immediate plating onto 5% Columbia blood agar with 5 mg/L of gentamicin. Susceptibility testing to penicillin G (PEN), amoxicillin (AMO), amoxicillin/clavulanate (AMC), cefotaxime (CTX), erythromycin A (ERY), azithromycin (AZI), clarithromycin (CLA), clindamycin (CLI), telithromycin (TEL), ciprofloxacin (CIP), levofloxacin (LEV), gemifloxacin (GEM), tetracycline (TET) and co-trimoxazole (SXT) was performed by NCCLS microdilution. Breakpoints were those of NCCLS (2002) except for TEL (0.5; 1-2; > 2 mg/L), CIP (2; 4; 8 mg/L), GEM (0.12; 0.25; 0.5 mg/L).

A total of 311 *S. pneumoniae* were isolated with carriage rate varying from 11.1% to 76.5% between different orphanages. Rate of non-susceptibility to PEN, TET and SXT in nasopharyngeal pneumococci isolated from children from orphanages was very high, 62.4%, 76.5% and 72.7%, respectively, substantially exceeding those from clinical isolates. The resistance to macrolides and lincosamides was lower being 21.9% for ERY, 22.2% for AZI, 21.2% for CLA and 15.4% for CLI. There were 14 (4.5%) strains with intermediate resistance to CTX and only 1 strain (0.3%) resistant to AMO and AMC. No resistance was found to TEL, LEV and GEM with the latter been the most active *in vitro* among all tested antimicrobials.

Introduction

Streptococcus pneumoniae is one of the leading bacterial pathogens causing a variety of community-acquired infections among different populations.

Surveillance of pneumococcal resistance in nasopharyngeal isolates from children attending day-care centers or orphanages has been found to be a practical and useful way to estimate the prevalence of resistant isolates in a community, and to be a good predictor of the development of pneumococcal resistance in clinical infections. In addition, the carriage of resistant isolates of potential respiratory pathogens (*S. pneumoniae, H. influenzae* and others) was found to be an independent risk factor for the development of infections (Syrogiannopoulos GA, Ronchetti F, Dagan R, *et al. Int J Antimicrob Agents* 2000; 16: 219–224).

All of the above provided a background for this study of antimicrobial resistance of pneumococci isolated from the nasopharynges of children from orphanages in Russia.

Materials and Methods

The protocol of this study and informed consent for parents were approved by the Independent Ethics Committee of Smolensk State Medical Academy.

The same team, including the physician and microbiologists, collected nasopharyngeal specimens using alginate calcium swabs (COPAN Diagnostics, Italy) from children under 5 years old from 11 orphanages in 8 cities of European (Kazan, Moscow, Saint Petersburg, Smolensk, Ufa, Volgograd) and Asian Russia (Anadyr, Khabarovsk) (Fig. 1).

Fig. 1. Centers where orphanages were located

Immediately after collection, swabs were plated onto 5% Columbia blood agar (bioMerieux, France) with 5 mg/L gentamicin (Sigma, USA). After that, plates were transported to the local laboratory within 2 h after collection, where they were incubated at 35⁰C and 3–5% CO_2 atmosphere for 24 h. *S. pneumoniae* was identified on the basis of colony morphology, susceptibility to optochin (bioMerieux) and a tube bile solubility test using 10% sodium desoxycholate (Sigma). Susceptibility testing to penicillin G, amoxicillin, amoxicillin/clavulanate, cefotaxime, erythromycin A, azithromycin, clarithromycin, clindamycin, telithromycin, ciprofloxacin, levofloxacin, gemifloxacin, tetracycline and co-trimoxazole was performed by a broth microdilution method. Breakpoints were those of NCCLS (2002), except for telithromycin (0.5, 1-2 and > 2 mg/L for susceptible, intermediate and resistant isolates, respectively), ciprofloxacin (2, 4 and 8 mg/L) and gemifloxacin (0.12, 0.25 and 0.5 mg/L).

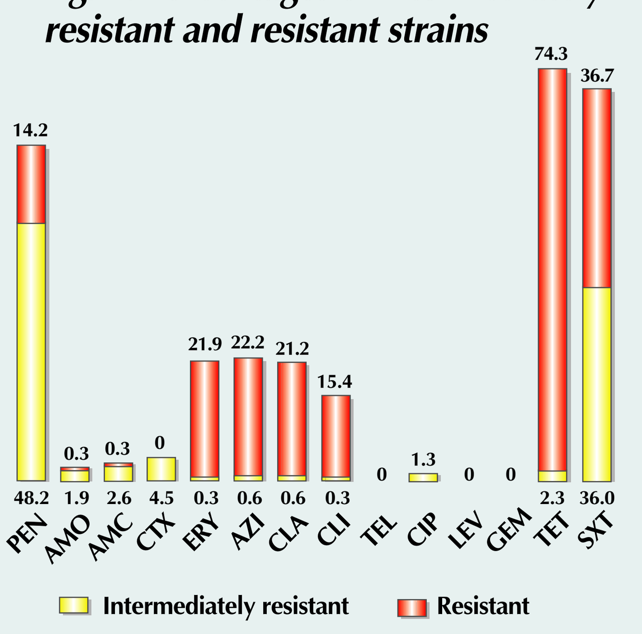
Results

Nasopharyngeal swabs were collected in 613 children under 5 years, including 497 in 8 orphanages in 6 cities of European Russia and 116 - from 3 orphanages in 2 cities of Asian Russia. The nasopharyngeal carriage rate varied from 11.1% to 76.5% with a total of 311 *S. pneumoniae* isolated. The susceptibility testing results, MIC_{50}, MIC_{90} and MIC ranges of tested antimicrobials are presented in Table 1 and Fig. 2.

Table 1. MIC_{50}, MIC_{90} and MIC ranges of tested antimicrobials

Antimicrobial	MIC_{50}, mg/L	MIC_{90}, mg/L	MIC range, mg/L
PEN	0.25	2	0.008–8
AMO	0.125	1	0.03–8
AMC	0.125	1	0.03–8
CTX	0.125	0.5	0.008–2
ERY	0.03	128	0.016–128
AZI	0.06	128	0.03–128
CLA	0.03	128	0.016–128
CLI	0.03	128	0.016–128
TEL	0.03	0.125	0.004–0.5
CIP	1	2	0.125–4
LEV	0.5	1	0.125–1
GEM	0.03	0.03	0.016–0.06
TET	16	32	0.25–64
SXT	1	8	0.06–16

Fig. 2. Percentages of intermediately resistant and resistant strains



In general, the non-susceptibility to penicillin G was 62.4% with a 14.2% of highly resistant isolates. Such percentage substantially exceeds those found in clinical isolates (10.2% of non-susceptible isolates with only 1.9% of highly resistant ones) (Kozlov R.S., Kretchikova O.I., Sivaya O.V., e.a. *Clin. Microbiol. Antimicrob. Chemother.* 2002; 4: 267-277). These strains were isolated from 6 cities, including 19 (43.2%) from Moscow, 9 (20.5%) – from Kazan, 7 (15.9%) – from Khabarovsk, 5 (11.4%) – from Ufa, 3 (6.7%) – from Saint Petersburg and 1 (2.3%) – from Smolensk. At the same time, resistance to other -lactams was lower with less than 3% of non-susceptibility to amoxicillin and amoxicillin/clavulanate and 4.5% intermediately resistant to cefotaxime isolates. MIC distributions of penicillin G and amoxicillin/clavulanate are presented on Fig. 3-4.

Fig. 3. MIC distribution of penicillin G

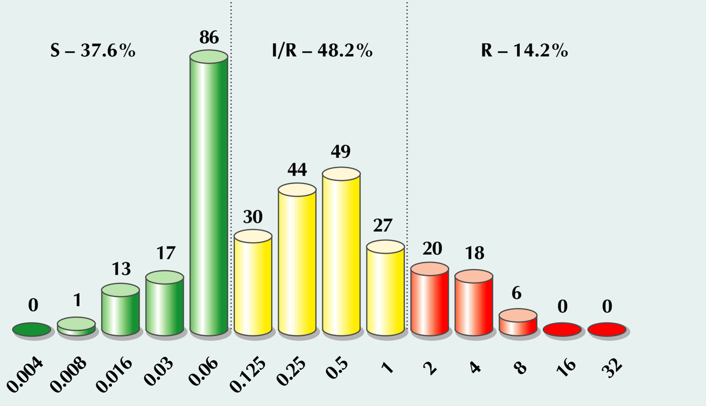
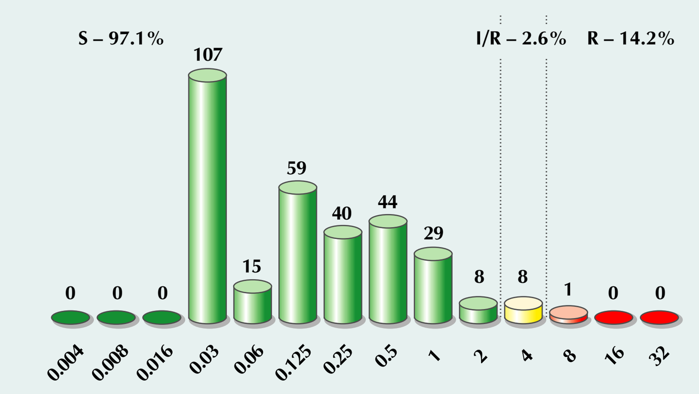


Fig. 4. MIC distribution of amoxicillin/clavulanate



Macrolides, including 14- and 15-membered representatives, also showed decreased *in vitro* activity against tested strains, with resistance being 21.9%, 22.2% and 21.2% for erythromycin, azithromycin and clarithromycin, respectively. There were less (15.4%) resistance to lincosamides (clindamycin) with no resistance to telithromycin found. MIC distributions of strains for tetracycline and co-trimoxazole are indicated on the Fig. 5 and 6, respectively.

Fig. 5. MIC distribution of erythromycin A

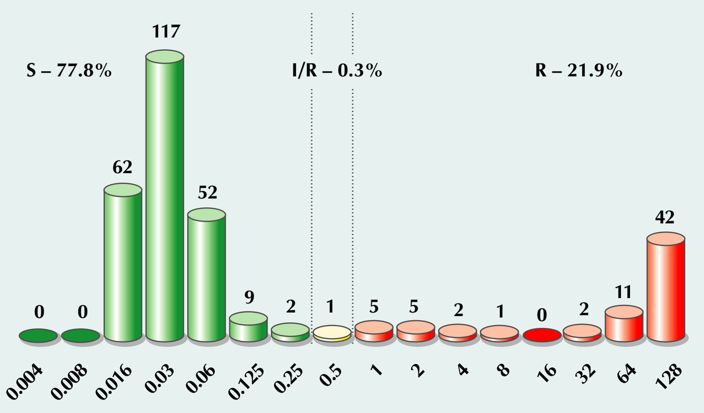
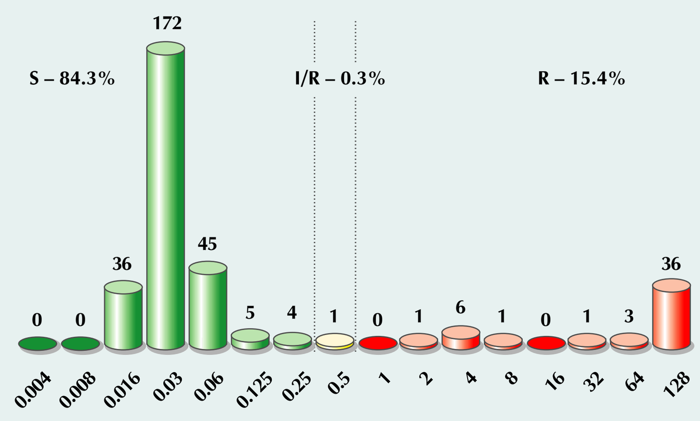


Fig. 6. MIC distribution of clindamycin



Of a particular concern, 4 strains with raised MICs to ciprofloxacin, among them 1 – in Asian Russia and 3 – in European Russia. However, these strains retained susceptibility to levofloxacin and gemifloxacin. Gemifloxacin was 4-fold more active then levofloxacin on comparison of MIC_{90}s.

The highest percentage of non-susceptible isolates was detected to tetracycline (76.5%) and co-trimoxazole (72.7%). MIC distributions of strains for tetracycline and co-trimoxazole are indicated on the Fig. 7 and 8, respectively.

Fig. 7. MIC distribution of tetracycline

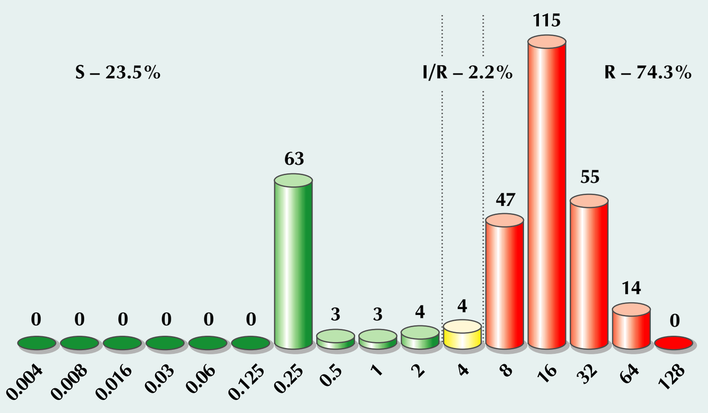
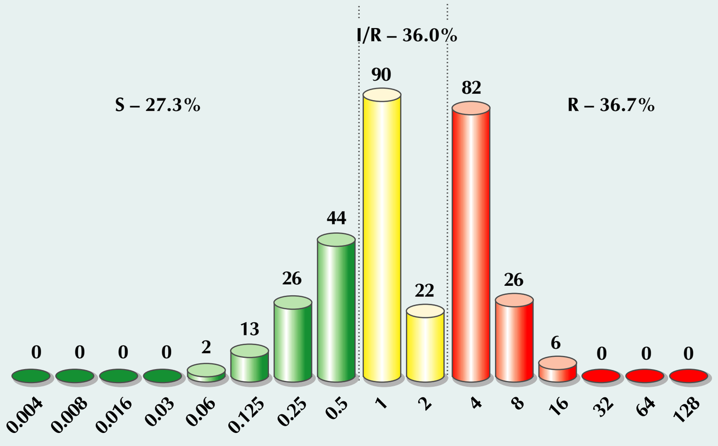


Fig. 8. MIC distribution of co-trimoxazole



Comparison of non-susceptibility rates was done for strains obtained in European and Asian Russia and the results are presented in Table 2.

Table 2. Statistical comparison of non-susceptibility rates of pneumococci from orphanages in European and Asian Russia

Antimicrobial	Asian Russia			European Russia			P
	% I/R (n)	% R (n)	% NS (n)	% I/R (n)	% R (n)	% NS (n)	
PEN	33.8 (26)	9.1 (7)	42.9 (33)	53.0 (124)	15.8 (37)	68.8 (161)	<0.05
AMO	1.3 (1)	0	1.3 (1)	2.1 (5)	0.4 (1)	2.5 (6)	ND
AMC	1.3 (1)	0	1.3 (1)	3.0 (7)	0.4 (1)	3.4 (8)	ND
CTX	2.6 (2)	0	2.6 (2)	5.1 (12)	0	5.1 (12)	ND
ERY	0	16.9 (13)	16.9 (13)	0.4 (1)	23.5 (55)	23.9 (56)	ND
AZI	1.3 (1)	16.9 (13)	18.2 (14)	0.4 (1)	23.9 (56)	24.3 (57)	ND
CLA	0	18.2 (14)	18.2 (14)	0.9 (2)	22.2 (52)	23.1 (54)	ND
CLI	1.3 (1)	6.5 (5)	7.8 (6)	0	18.4 (43)	18.4 (43)	<0.05
TEL	0	0	0	0	0	0	ND
CIP	1.3 (1)	0	1.3 (1)	1.3 (3)	0	1.3 (3)	ND
LEV	0	0	0	0	0	0	ND
GEM	0	0	0	0	0	0	ND
TET	2.6 (2)	77.9 (60)	80.5 (62)	2.1 (5)	73.1 (171)	75.2 (176)	ND
SXT	31.2 (24)	59.7 (46)	90.9 (70)	37.6 (88)	29.1 (68)	66.7 (156)	<0.001

Abbreviations: R - resistant, I/R - intermediately resistant, NS - non-susceptible (defined as resistant and intermediately resistant categories); two-sided p was calculated for NS category; ND – no difference

Non-susceptibility was found to be statistically higher to PEN and CLI in European Russia as compared to Asian Russia ($p<0.05$). Alternatively, non-susceptibility to SXT was higher in Asian Russia ($p<0.001$). There were no statistically significant differences found between AR and ER for other antimicrobials.

Discussion

The rates of antibiotic resistance in children from orphanages have been extensively used as a predictor of resistance in clinical isolates. In this study, a group of the same investigators performed collection and isolation of the strains, minimizing technical variations in this study. In addition, antimicrobial susceptibility testing was carried out at one center using NCCLS methodology.

In general, resistance in pneumococci isolated from children from orphanages in Russia substantially exceeded those published for clinical isolates. We hypothesize that it might be related to more extensive usage of antimicrobials in children in orphanages and higher rate of cross-infection with pneumococci in such closed communities. Among the -lactams, the most active *in vitro* antimicrobials were amoxicillin, amoxicillin/clavulanate and cefotaxime, which provide the basis for their usage as first choice drugs for the therapy of pneumococcal infections in these communities.

Comparatively high resistance to macrolides and lincosamides substantially limits possibility of their empiric usage for the therapy of probable pneumococcal infections in these children. Telithromycin possessed high *in vitro* activities, however it still not indicated for use in children.

The high levels of non-susceptibility to tetracycline and co-trimoxazole are consistent with those previously described in Russian clinical isolates and might be explained by their extensive out-patient use as it has been showed in Russian pharmacoepidemiological studies.

Conclusions

- Orphanages could be evaluated as a «hot spots» of antimicrobial resistance and might be considered for interventions (e.g. pneumococcal vaccination).
- High non-susceptibility (62.4%) to penicillin G was found with a 14.2% of highly resistant isolates that exceeds previously reported rates.
- Amoxicillin and amoxicillin/clavulanate retained their high activity against pneumococci from children from orphanages in both European and Asian Russia, thus might be considered as drugs of choice for the therapy of infections of probable pneumococcal etiology.
- Comparatively high macrolide-, lincosamide resistance and non-susceptibility to co-trimoxazole and tetracycline substantially compromise clinical utility of these classes of antimicrobials for the therapy of possible pneumococcal infections in orphanages.