RESISTANCE OF *STREPTOCOCCUS PNEUMONIAE* (SPN) NASOPHARYNGEAL ISOLATES FROM CHILREN IN SELECTED RUSSIAN ORPHANAGES: 2003 VS 2004

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ABSTRACT

Objectives. Orphanages have been shown to be reservoirs of resistant strains of *Streptococcus pneumoniae* (SPN) which could spread to the community. A special survey was done to monitor resistance changes of SPN nasopharyngeal isolates from children living in orphanages in 2003 and one year after - in 2004.

Methods. This survey was performed as a part of the prospective study CORPUS in 12 orphanages (##1-12) from 5 cities (Moscow, Saint-Petersburg, Smolensk, Karachev, Bryansk) located in European part of Russia. Nasopharyngeal swabs were collected from 772 children < 7 years in 2003 and from 752 children in 2004 that yielded 399 and 397 SPN isolates respectively. Susceptibility to penicillin G (PEN), amoxicillin (AMO), amoxicillin/clavulanate (AMC), cefuroxime (CEF), cefotaxime (CTX), erythromycin (ERY), clindamycin (CLI), chloramphenicol (CHL), tetracycline (TET) and co-trimoxazole (SXT) was performed by broth microdilution (NCCLS/CLSI)[and CLSI, respectively)].

Results. SPN nasopharyngeal colonization rates were quite stable during the study period - 51.7% in 2003 and 52.8% - in 2004. There were no increase in SPN non-susceptibility (NS) or[and] resistance to PEN, AMO and AMC. However our data revealed the statistically significant increase of I and R SPN isolates to II and III generation cephalosporins (CEF and CTX) - 1.3 / 39.4 vs 17.9 / 31.2 and 3.8 / 2.5 vs 9.3 / 8.3 in 2003 vs 2004 respectively. It is noteworthy that NS SPN population to CEF increased due to the higher frequency of I isolates with the stable rate of R strains, while in case of CTX NS - it was due to simultaneous (~2.4 fold and ~3.3 fold) increase of I and R isolates, respectively. At the same time no statistically significant changes in ERY, CLI and SXT susceptibility occurred and a statistically significant decrease in CHL R rates (15.0 vs 8.1%) were noted.

Conclusion. The results of the survey show stable rates of NS and R SPN nasopharyngeal isolates from children living in orphanages to penicillins, macrolides and lincosamides, tetracyclins and co-trimoxazole. Emerging resistance to II-III generation cephalosporins necessitates further evaluation of these alarming data to determine responsible risk factors and respective resistance mechanisms.

Decrease of SPN resistance to chloramphenicol can be a positive example of the possibility to diminish resistance following the restricted use of antimicrobials.

INTRODUCTION AND PURPOSE

Streptococcus pneumoniae (SPN) is the most common cause of community-acquired bacterial respiratory tract infections. Infections caused by *S.pneumoniae* have been associated with increased morbidity and mortality, especially in children under 2 years of age and elderly adults.

The control of pneumococcal diseases is threatened by the emergence of penicillin resistance, often associated with multiple antibiotic resistance. Prevalence of antibiotic resistant isolates of *S.pneumoniae* is rapidly increasing worldwide.

Surveillance of pneumococcal resistance in nasopharyngeal isolates from children has been found to be a practical and useful way of estimating the prevalence of resistant isolates in a community, and to be a good predictor of the development of pneumococcal resistance in clinical infections.

Orphanages have been shown to be reservoirs of resistant strains of pneumococci which could spread to families, day-care centers and clinical settings. Previously published data from Russian orphanages showed substantial variation in carriage rates (with the highest reaching 76.5%) and antimicrobial resistance patterns of isolated *S.pneumoniae*.

All of the above provided a background for this special survey to monitor resistance changes of SPN nasopharyngeal isolates from children living in orphanages in 2003 and one year after - in 2004.

METHODS

This survey was performed as a part of the prospective study CORPUS in 12 orphanages (## 1-12) from 5 cities (Moscow, Saint-Petersburg, Smolensk, Karachev, Bryansk) located in European part of Russia. Study protocol was approved by the Independent Ethics Committee of Smolensk State Medical Academy, Smolensk, Russia (protocol from 21.09.2003).

Nasopharyngeal specimens were collected from children under 7 years old living in the above orphanages by the same team, including the physician and microbiologists, using alginate calcium swabs (COPAN Diagnostics, Brescia, Italy). Immediately after collection, swabs were plated onto 5% Columbia blood agar (bioMerieux, Marcy l'Etoile, France) with 5 mg/L gentamicin (Sigma, St Louis, MO, USA). Plates were then transported to the laboratory, with minimal temperature variations, where they were incubated at 35^{0}C 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 (PEN), amoxicillin (AMO), amoxicillin/clavulanate (AMC), cefuroxime (CEF), cefotaxime (CTX), erythromycin (ERY), clindamycin (CLI), chloramphenicol (CHL), tetracycline (TET) and co-trimoxazole (SXT) was performed by broth microdilution method. Breakpoints were those of NCCLS (2003) and CLSI/NCCLS (2004). For comparison, intermediately resistant and resistant isolates were both described as 'non-susceptible' and combined in the same group.

RESULTS

Nasopharyngeal swabs were collected from 772 children under 7 years in 2003 and from 752 children in 2004 that yielded 399 and 397 SPN isolates respectively. SPN nasopharyngeal colonization rates were quite stable during the study period - 51.7% in 2003 and 52.8% - in 2004.

Numbers of studied children, quantities of isolated SPN strains and rates of *S.pneumoniae* nasopharyngeal carriage are presented in Table 1.

Nasopharyngeal carriage rates of pneumococci in studied orphanages are also presented in Figure 1.

However, analysis of antimicrobial resistance of nasopharyngeal SPN isolates from children living in different orphanages revealed substantial variations in antimicrobial resistance patterns and the dynamics of resistance in studied orphanages. For example, there are no PEN NS isolates in two orphanages: # 2 (St-Petersburg) and # 10 (Smolensk). At the same time high percentages of PEN NS isolates were revealed in 2003-2004 in orphanage # 4 (St-Petersburg) - 78.8% and 100%; # 6 (Moscow) - 89.9% and 93.9%; # 8 (Smolensk) - 87.6% and 90%. In orphanage # 3 (St-Petersburg) there was considerable increase of PEN NS isolates in 2004 (88.5%) vs 2003 (46,2%), while some decreases in PEN NS were noted in orphanages # 5 (Moscow) - 79.5% and 50.1%; # 7 (Moscow) - 64.3% and 52.8%; # 11 - 96.4% and 71%; # 12 - 50.0% and 16.0% in 2003 and 2004, respectively (Figure 3).

Table 1. Nasopharyngeal carriage rates (%) of SPN in studied orphanages

Orphanage	2003 N of children	2003 N of SPN isolates	2003 Carriage rate, %	2004 N of children	2004 N of SPN isolates	2004 Carriage rate, %
Orph. #1	119	43	36.1	118	75	63.6
Orph. #2	24	11	45.8	22	4	18.2
Orph. #3	38	26	55.0	32	26	81.3
Orph. #4	60	33	68.4	58	33	56.9
Orph. #5	70	30	42.9	91	48	52.7
Orph. #6	85	39	45.9	90	48	53.3
Orph. #7	86	34	39.5	75	36	48.0
Orph. #8	114	77	67.5	119	50	42.0
Orph. #9	28	12	42.9	28	17	60.7
Orph. #10	49	27	55.1	8	4	50.0
Orph. #11	70	45	64.3	72	31	43.1
Orph. #12	29	22	75.9	39	25	64.1
Total	772	399	51.7	752	397	52.8

Further studying and analysis are needed to determine factors that influence nasopharyngeal carriage rates of *S.pneumoniae* in children living in orphanages (seasonal changes, previous antimicrobial therapy, etc.

Overall susceptibility testing results are presented in Table 2 and on Figure 2. Overall percentage of non-susceptible (NS) isolates was highest for SXT (the numbers were quite stable in 2004 vs 2003 with approximately equal proportions of intermediate and resistant isolates) followed by PEN (65.4% and 61.8% of NS isolates in 2003 and 2004, respectively, with the stable proportion of I and R isolates, intermediate strains accounted for 60.6% and 68.1% of NS isolates respectively) and by TET (60.6% and 57.9% of NS isolates respectively, mostly due to R isolates - 56.6% and 55.4%, respectively).

Aminopenicillins possessed the highest *in vitro* activity comparing to other tested antimicrobials with percentages of AMO NS isolates 2.6% and 1.3% and AMC NS isolates 2.3% and 1.1% in 2003 and 2004, respectively.

CEF demonstrated poor *in vitro* activity against SPN when compared to AMO, AMC and CTX with percentages of NS isolates 40.7% and 49.1% in 2003 and 2004, respectively. According to the statistical analysis these changes were considered as significant ($p < 0.05$) and were due to the increase in the proportion of intermediate strains in 2004 vs 2003 (17.9% vs 1.3%).

CTX possessed much better *in vitro* activity against SPN than PEN and CEF, however inferior to AMO and AMC. Statistical analysis showed alarming data demonstrating statistically significant increase of I and R SPN isolates to CTX - 3.8 / 2.5 vs 9.3 / 8.3 in 2003 vs 2004 respectively ($p < 0.05$). Increasing rate of CTX NS isolates is due to simultaneous (~2.4 fold and ~3.3 fold) increase of I and R isolates, respectively. It is worth to note that CTX NS strains were isolated in 2003 only in St-Petersburg and Moscow, and in 2004 CTX intermediate isolates (4%) were found in one of the Smolensk orphanages.

At the same time there were no statistically significant changes in PEN, AMO and AMC, ERY, CLI and SXT susceptibility in 2004 vs 2003 and statistically significant decrease in percentage of CHL R isolates (15.0 vs 8.1%) was noted.

However, analysis of antimicrobial resistance of nasopharyngeal SPN isolates from children living in different orphanages revealed substantial variations in antimicrobial resistance patterns and the dynamics of resistance in studied orphanages. For example, there are no PEN NS isolates in two orphanages: # 2 (St-Petersburg) and # 10 (Smolensk). At the same time high percentages of PEN NS isolates were revealed in 2003-2004 in orphanage # 4 (St-Petersburg) - 78.8% and 100%; # 6 (Moscow) - 89.9% and 93.9%; # 8 (Smolensk) - 87.6% and 90%. In orphanage # 3 (St-Petersburg) there was considerable increase of PEN NS isolates in 2004 (88.5%) vs 2003 (46,2%), while some decreases in PEN NS were noted in orphanages # 5 (Moscow) - 79.5% and 50.1%; # 7 (Moscow) - 64.3% and 52.8%; # 11 - 96.4% and 71%; # 12 - 50.0% and 16.0% in 2003 and 2004, respectively (Figure 3).

Table 2. Percentages of intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

Drug	2003 (N = 399) I / R (%)	2003 (N = 399) MIC$_{50}$ / MIC$_{90}$, mg/L	2004 (N = 397) I / R (%)	2004 (N = 397) MIC$_{50}$ / MIC$_{90}$, mg/L
PEN	39.6 / 25.8	0.5 / 4	42.1 / 19.7	0.125 / 4
AMO	0.8 / 1.8	0.125 / 2	0.8 / 0.5	0.06 / 1
AMC	0.5 / 1.8	0.125 / 2	0.8 / 0.3	0.06 / 1
CEF	1.3 / 39.4	1 / 16	17.9 / 31.2*	0.5 / 16
CTX	3.8 / 2.5	0.25 / 1	9.3 / 8.3*	0.06 / 1
ERY	1.0 / 26.6	0.03 / 256	0.8 / 32.5	0.03 / 256
CLI	0 / 19.8	0.03 / 128	0.8 / 22.4	0.03 / 128
CHL	0 / 15.0	2 / 8	0 / 8.1*	2 / 2
TET	4.0 / 56.6	8 / 32	2.5 / 55.4	8 / 32
SXT	34.8 / 35.6	1 / 8	36.5 / 30.7	1 / 4

* $p < 0.05$

Variations in percentages of intermediate and resistant nasopharyngeal SPN isolated from children in orphanages to different antimicrobials are presented in Figures 3-10.

Alarming data concerning increase of CTX NS isolates are due to the elevation of such strains in orphanage # 3 (St-Petersburg) - 42.3% and 53.9% (23.1% of R isolates appeared in 2004); # 6 (Moscow) - 3.4% and 18.7% in 2003 and 2004, respectively and due to appearance of CTX intermediate isolates in 2004 in orphanages # 5 (Moscow) - 2.1% and # 8 (Smolensk) - 4%. However there was significant decrease in CTX non-susceptibility in orphanage # 1 (St-Petersburg) - from 15.6% to 1.3% and some decrease in orphanage # 4 (St-Petersburg) - 18.2% and 15.1% in 2003 and 2004, respectively. CTX NS strains were not revealed in 6 other orphanages (## 2, 7, 9, 10, 11, 12).

Variable percentages of SPN intermediate and resistant isolates and different trends in antimicrobial resistance rates in 2004 vs 2003 were noted in different orphanages for ERY, CLI, CHL, TET and SXT. While CHL resistance decreased in 7 orphanages ## 1, 5, 6, 7, 8, 11, 12, was absent in ## 2 and 3, stable in #4, it increased substantially in 2/3 Smolensk orphanages # 9 (from 0% to 11.8%) and # 10 (from 6.9% to 25.0%).

Taking into consideration the above variations there is a need for further investigation of potential factors influencing patterns and dynamics of antimicrobial resistance of nasopharyngeal isolates of SPN in children living in orphanages (e.g. antimicrobial usage, SPN serotypes, vaccination, etc.)

In general, resistance in pneumococci isolated from children from orphanages in Russia substantially exceeded those found in clinical isolates. For example, only 8% of clinical isolates of *S.pneumoniae* were non-susceptible to PEN; 2% - to CTX; 7% - to ERY; 4% - to CLI, compared with > 60%; 6.3-17.6; >25% and about 20% of isolates in this study, respectively. We hypothesize that it might be related to more extensive usage of antimicrobials in orphanages and higher rates of cross infection with pneumococci in such closed institutions. The rates of resistance to the majority of tested antimicrobials in orphanages were the highest ever reported in Russia, which could lead to the designation of these institutions as "hot spots" and possible reservoirs of pneumococcal resistance.

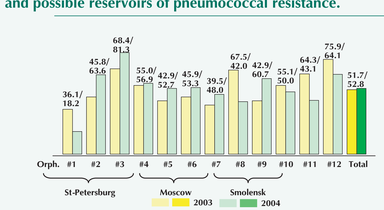

Figure 1. Nasopharyngeal carriage rates (%) of SPN in studied orphanages

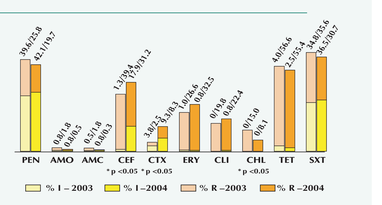

Figure 2. Percentages of intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

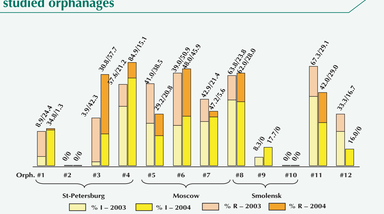

Figure 3. Variations in percentages of PEN intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

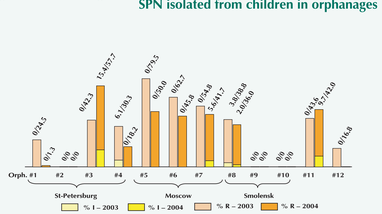

Figure 4. Variations in percentages of CEF intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

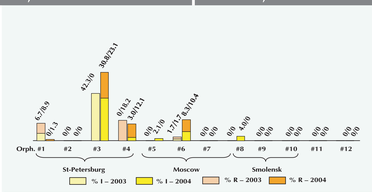

Figure 5. Variations in percentages of CTX intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

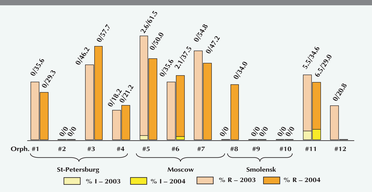

Figure 6. Variations in percentages of ERY intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

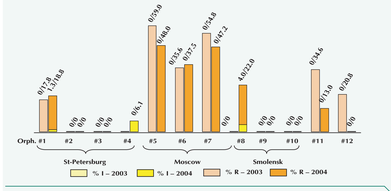

Figure 7. Variations in percentages of CLI intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

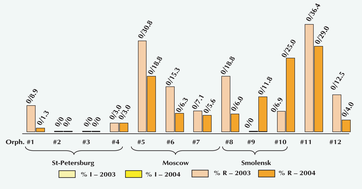

Figure 8. Variations in percentages of CHL intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

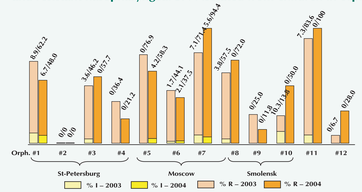

Figure 9. Variations in percentages of TET intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

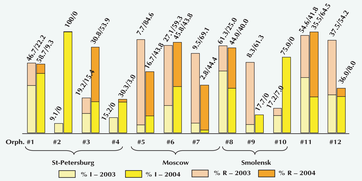

Figure 10. Variations in percentages of SXT intermediate and resistant nasopharyngeal SPN isolated from children in orphanages

Table 3. Variations in SPN antimicrobial susceptibility (%) in different orphanages, 2003 vs 2004

Drug	Year	Orphanages #1 I, %	#1 R, %	#2 I, %	#2 R, %	#3 I, %	#3 R, %	#4 I, %	#4 R, %	#5 I, %	#5 R, %	#6 I, %	#6 R, %	#7 I, %	#7 R, %	#8 I, %	#8 R, %	#9 I, %	#9 R, %	#10 I, %	#10 R, %	#11 I, %	#11 R, %	#12 I, %	#12 R, %
PEN	2003	8.9	24.4	0	0	3.9	42.3	57.6	21.2	41.0	38.5	39.0	50.9	42.9	21.4	63.8	23.8	8.3	0	0	0	67.3	29.1	33.3	16.7
	2004	34.8	1.3	0	0	30.8	57.7	84.9	15.1	29.2	20.8	48.0	45.9	47.2	5.6	62.0	28.0	17.7	0	0	0	42.0	29.0	16.0	0
AMO	2003	2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	2.5	8.8	0	0	0	0	0	0	0	0
	2004	0	1.3	0	0	0	0	3.0	0	0	0	2.1	0	0	0	2.0	2.0	0	0	0	0	0	0	0	0
AMC	2003	2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	1.3	8.8	0	0	0	0	0	0	0	0
	2004	0	1.3	0	0	0	0	3.0	0	0	0	0	0	0	0	4.0	0	0	0	0	0	0	0	0	0
CEF	2003	0	24.5	0	0	0	42.3	6.1	30.3	0	79.5	0	62.7	0	54.8	3.8	38.8	0	0	0	0	0	43.6	0	16.8
	2004	0	1.3	0	0	15.4	57.7	0	18.2	0	50.0	0	45.8	5.6	41.7	2.0	36.0	0	0	0	0	9.7	42.0	0	0
CTX	2003	6.7	8.9	0	0	42.3	0	0	18.2	0	0	1.7	1.7	0	0	0	0	0	0	0	0	0	0	0	0
	2004	0	1.3	0	0	30.8	23.1	3.0	12.1	2.1	0	8.3	10.4	2.8	0	4.0	0	0	0	0	0	0	0	0	0
ERY	2003	0	35.6	0	0	0	46.2	0	18.2	2.6	61.5	0	35.6	0	54.8	0	0	0	0	0	0	5.5	34.6	0	20.8
	2004	0	29.3	0	0	0	57.7	0	21.2	0	50.0	2.1	37.5	0	47.2	0	34.0	0	0	0	0	6.5	29.0	0	0
CLI	2003	0	17.8	0	0	0	0	0	0	0	59.0	0	35.6	0	54.8	0	0	0	0	0	0	0	34.6	0	20.8
	2004	1.3	18.8	0	0	0	0	6.1	0	0	48.0	0	37.5	0	47.2	4.0	22.0	0	0	0	0	0	13.0	0	0
CHL	2003	0	8.9	0	0	0	0	3.0	0	0	30.8	0	15.3	0	7.1	0	18.8	0	0	0	6.9	0	36.4	0	12.5
	2004	0	1.3	0	0	0	0	3.0	0	0	18.8	0	6.3	0	5.6	0	6.0	0	11.8	0	25.0	0	29.0	0	4.0
TET	2003	8.9	62.2	0	0	3.6	46.2	0	36.4	0	76.9	1.7	44.1	7.1	71.4	3.8	57.5	0	25.0	10.3	13.8	7.3	83.6	0	6.7
	2004	6.7	48.0	0	0	0	57.7	0	21.2	4.2	58.3	2.1	37.5	5.6	94.4	0	72.0	0	11.8	0	50.0	0	100	0	28.0
SXT	2003	46.7	22.2	9.1	0	19.2	15.4	15.2	0	7.7	84.6	27.1	59.3	9.5	69.1	61.3	25.0	8.3	61.3	17.2	7.0	54.6	41.8	37.5	54.2
	2004	58.7	9.3	100	0	30.8	53.9	30.3	3.0	16.7	43.8	45.8	43.8	2.8	44.4	44.0	40.0	17.7	0	75.0	0	35.5	64.5	36.0	8.0

CONCLUSIONS

The results of the survey show stable rates of NS and R SPN nasopharyngeal isolates from children living in orphanages to penicillins, macrolides and lincosamides, tetracyclins and co-trimoxazole. Aminopenicillins, and especially AMC, possessed highest *in vitro* activity against tested pneumococci, with no increase noticed in two periods in spite of the increase of consumption. Emerging resistance to II-III generation cephalosporins necessitates further evaluation of these alarming data to determine responsible risk factors and respective resistance mechanisms.

Decrease of SPN resistance to chloramphenicol can be a positive example of possibility to diminish resistance following the restricted use of antimicrobials.

ACKNOWLEDGEMENT

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