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MICROBIOLOGICAL CHARACTERISTICS OF STREPTOCOCCUS PNEUMONIAE STRAINS ISOLATED IN YAKUTSK

Pneumococcus and pneumococcal infections are still among actively discussed problems, while there is a lack of information about the population structure in the North-East of Russia.

Objective: the identification of microbiological and molecular genetic characteristics of S. pneumoniae strains found during nasopharyngeal carriage in Yakutsk.

Materials and methods: we studied *S. pneumoniae* isolates, obtained from the discharges of nasopharynx in 69 patients from the age of 6 months to 85 years living in Yakutsk and undergoing a survey for acute and chronic diseases of the ENT organs (rhinitis, sinusitis, otitis), repeated acute respiratory viral infections and nasopharyngitis. Identification of the isolated cultures was performed using the time-of-flight mass spectrometry method on a Vitek MS analyzer. For uncertain results, we used test systems to detect *S. pneumoniae* DNA on PCR-RV. Identification of sensitivity to antimicrobial agents was determined by the disk diffusion method with an interpretation according to the EUCAST recommendations and the Clinical recommendations for determining the sensitivity of microorganisms to antimicrobial agents (version 2018-03). The microbiological analyzer Vitek II Compact was used to specify the phenotype of sensitivity / resistance.

Confirmation of species identification was carried out by amplification of the autolysin gene (lytA). Identification of serological types of the isolated *S. pneumoniae* strains was carried out using multiplex PCR. We were determining presence of genetic determinants of resistance to macrolide antibiotics *erm, mef* and *msr* as well as genes associated with the pathogenicity island PPI1 *(per, nplT, FtsW)*.

Results: more than 80% of *S. pneumoniae* strains circulating among the population of Yakutsk are represented by serotypes 6A and 19F. In 50% of pneumococci 6A and 100% of serotype 19F pneumococci were detected all 3 genes associated with the pathogenicity island PPI1. Macrolide resistance was observed in all isolates of serotype 6A, while 80% of serotype 6A and 100% of serotype 19F showed the *ermB* resistance gene (MLSB phenotype) and 20% of serotype 6A pneumococci had the *mef* gene (M phenotype).

Conclusions: obtained data indicate the prevalence of virulent antibiotic resistant strains of *S. pneumoniae* among the population of Yakutsk and dictate the need for further epidemiological and microbiological studies of this problem.

Keywords: pneumococcus, nasopharyngeal carriage, virulence, resistance.

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Introduction. Despite the insertion of mass vaccination, pneumococcus (*Streptococcus pneumoniae*) is one of the main causative agents of acute bacterial infections in children, especially under the age of 5 years [2-4].

The high incidence of pneumococcal infections is combined with a steady increase of the pneumococcus resistance to the antibacterial drugs that are most widely used in clinical practice. β-lactams and macrolides are the drugs of choice in the treatment of pneumococcal infections, therefore, the increase in *S. pneumoniae* resistance to these antibiotics becomes a significant clinical problem [4].

The resistance of S. pneumoniae to penicillin and other β-lactam antibiotics is due to a change in penicillin binding proteins (PBPs), enzymes that participate in the final stages of cell wall synthesis [13].

The resistance to macrolides is mediated by two main mechanisms, which include changes in the binding target and antibiotic efflux from a bacterial cell. The first mechanism is due to the modification of the macrolide binding site with 23S-rRNA as a result of its methylation, which disrupts the interaction of the antibiotic with the target. The methylation is carried out by the methylase enzyme, which is encoded by the ***erm*** gene (*erythromycin ribosome methylation*) and causes a high level of resistance to macrolides. About 20 varieties of erm are described, however, the ***ermB*** variant plays the greatest role in the formation of resistance in pneumococcus. Most pneumococci with ***ermB*** demonstrate cross-resistance to all macrolides, as well as to lincosamides

and B streptogramin, as their targets are partially overlapped. This phenotype is called MLSB [1, 7].

The second mechanism of resistance to macrolides is concerned with their active excretion (efflux) from the bacterial cell with the help of a special pump built into the cell wall. The efflux pump is encoded by several variants of the ***mef*** gene (macrolide efflux). Mef-positive pneumococci have an M-phenotype, which is characterized by resistance to fourteen- and fifteen-membered macrolides, but maintaining sensitivity to sixteen-membered macrolides, lincosamides and B streptogramine [1, 3, 10].

Another urgent problem because of the vaccination that is carried out in the Russian Federation with conjugated pneumococcal vaccines is the possibility of genetic changes in the pathogen population under the influence of the applied vaccines. In this regard the importance belongs to the data that characterize the presence in the genomes of *S. pneumoniae* of mobile genetic elements associated with invasive potential, which include the pathogenicity island PPI1 [5].

It should be noted that despite the availability of summarizing data from large multicenter studies [2], regional data is extremely important for understanding the trends in molecular epidemiology and resistance of pneumococci both in a specific territory and in the country as a whole, and there is a lack of information about the situation in the North-East of the Russia.

Research objective: the identification of microbiological and molecular genetic characteristics of S. pneumoniae strains found during nasopharyngeal carriage in Yakutsk.

Materials and methods. We studied *S. pneumoniae* isolates, obtained from the discharges of nasopharynx in 69 patients from the age of 6 months to 85 years living in Yakutsk and undergoing a survey for acute and chronic diseases of the ENT organs (rhinitis, sinusitis, otitis), repeated acute respiratory viral infections and nasopharyngitis. Of these, 58 children (84%), 11 adults (16%), 25 inpatients (36.2%), 44 outpatients (63.8%).

The material was obtained from the nasopharynx using tampons that were placed in the transport Ames medium with coal. Chocolate agar cultures were incubated in airtight containers with gas generators to create microaerophilic conditions (bioMerieux) for 24 hours. Identification of the isolated cultures was performed using the time-of-flight mass spectrometry method on a Vitek MS analyzer. For uncertain results, we used test systems to detect *S. pneumoniae* DNA on PCR-RV. Identification of sensitivity to antimicrobial agents was determined by the disk diffusion method with an interpretation according to the EUCAST recommendations and the Clinical recommendations for determining the sensitivity of microorganisms to antimicrobial agents (version 2018-03). The microbiological analyzer Vitek II Compact was used to specify the phenotype of sensitivity / resistance.

Confirmation of species identification was carried out by amplification of the autolysin gene (lytA) [12]. Identification of serological types of the isolated *S. pneumoniae* strains was carried out using multiplex PCR [9]. The following primers were used to identify virulence genes and genetic determinants of resistance to macrolide antibiotics in the polymerase chain reaction (Table 1, 2).

Results and discussion: According to the data of the Educational and Scientific Microbiological Laboratory of the NEFU Clinic, the frequency of pneumococcal nasopharyngeal carriage in Yakutsk was 14.1% in 2010 and in 2017 was only 2.7% in the total structure of nasopharyngeal bacterial carriage. From the 69 obtained samples more than half of the confirmed *S. pneumoniae* isolates were represented by serotype 6A (53.8%), a third of the strains (30.8%) were assigned to serotype 19F, the rest 15.4% are presented by serotypes 7F and 23F.

In determining of the sensitivity to antimicrobial agents by the disk diffusion method, we paid attention to the presence of sensitivity / resistance to β-lactam antibiotics on a disk with 1 μg of oxacillin (OX) and sensitivity / resistance to macrolides on a disk with 15 μg of erythromycin (E). Thus, 42% of isolated pneumococci were resistant to β-lactams and 34.8% were resistant to macrolides, while 26.1% of the strains were resistant to both groups of drugs (Table 3).

Genetic study showed that the resistance to macrolides was noted in all isolates of serotype 6A, 80% of representatives of this type had an *ermB* resistance gene and 50% of these pneumococci revealed all 3 genes associated to the pathogenicity island PPI1. The same genes, as well as all determinants of macrolide resistance were detected in *S. pneumoniae* of serotype 19F. 6 genotypes of pneumococci isolated during nasopharyngeal bacterial carriage in Yakutsk were characterized (Table 4).

Thus, more than 80% of the *S. pneumoniae* strains studied by us are represented by serotypes 6A and 19F. In 50% of pneumococci 6A and 100% of sero-

Table 1

Primers used in the study to identify the virulence genes

Gene	Primer sequence (5'->3')	Reference genome (GenBank Acc. №)	Localization in the reference genome		Source
ftsW	ATGGCTTCCCCGTGCTTTTA	AE005672.3	1001727	1001746	actual study
	AGATACGAGCGCCAGAATGG		1001901	1001882	
pezT	CGCGCAACTCCAAAAGAACA		989035	989054	
	CCCACCTGCAACATCTCCTT		989255	989236	
nplT	GGAGACCTTTCGGGAACTGG		985812	985831	
	TGGTCCTCCAGTCAAGGCTA		986562	986543	

Table 2

Primers used to detect the genetic determinants of antibiotic resistance of the macrolide

Праймеры	Sequence 5' → 3'	Gene	Product size (nucleotide couples)	Source link
ermB-f	ATTGGAACAGGTAAAGGGC	*Erm B*	442	[8]
ermB-r	GAACATCTGTGGTATGGCG			
MEF 57-f	AGTATCATTAATCACTAGTGC	*MEF(A)*	346	[7]

Table 3

Identified phenotypes of S. pneumoniae sensitivity / resistance to β-lactam and macrolides

Phenotype	Abs (%)	Abs (%)
OX-R E-S	11 (15.9)	Ox – R 29 (42)
OX – R E - R	18 (26.1)	
OX-S E-R	6 (8.7)	E – R 24 (34.8)
OX – S E - S	34 (49.3)	

Table 4

Variety of *S. pneumoniae* genotypes isolated in Yakutsk

Macrolide resistance genes			Genes associated to the pathogenicity island PPI1			Serotype	Erythromycin
MSR	*MEF*	*ermB*	*pezT*	*nplT*	*FtsW*		
-	-	+	+	+	+	6A	R
+	+	-	+	+	+	6A	R
-	-	+	-	+	+	6A	R
-	-	+	-	-	+	6A	R
-	-	+	-	+	+	6A	R
+	+	+	+	+	+	19F	I

type 19F pneumococci were detected all 3 genes associated with the pathogenicity island PPI1. Macrolide resistance was observed in all isolates of serotype 6A, while 80% of serotype 6A and 100% of serotype 19F showed the *ermB* resistance gene (MLSB phenotype) and 20% of serotype 6A pneumococci had the *mef* gene (M phenotype).

Conclusion. The obtained data indicate the prevalence of virulent antibiotic resistant strains of *S. pneumoniae* among the children of Yakutsk and dictate the need for further epidemiological and microbiological studies in this domain. Subsequent monitoring studies should be obviously aimed at assessing the impact of mass vaccination of the population on the genetic structure of the pneumococcal population, including assessing the appearance of new strains with high pathogenic and epidemic potential.

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