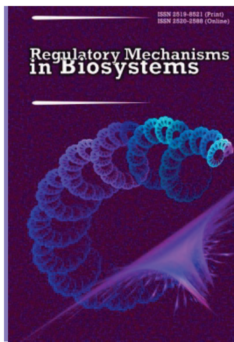

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Colonization and antimicrobial resistance of *Streptococcus pneumoniae* among pediatric patients

M. Lusta*, O. Voronkova**, T. Shevchenko**, V. Chornyi*, A. Breus*, I. Yesaulenko*, Y. Maistrenko*, Y. Shyrokykh*, O. Falko*

**Municipal Non-Commercial Enterprise "City Clinical Hospital No. 6" of the Dnipro City Council, Dnipro, Ukraine*
***Oles Honchar Dnipro National University, Dnipro, Ukraine*

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Municipal Non-Commercial Enterprise "City Clinical Hospital No. 6" of the Dnipro City Council, Manuylivskyy av., 29, Dnipro, 49023, Ukraine. Tel.: +38-066-356-61-59. E-mail: maksimlusta12@gmail.com

Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine. Tel.: +38-096-225-04-63. E-mail: voronkova.olga.04@gmail.com

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Streptococcus pneumoniae is a gram-positive microorganism that colonizes the nasopharyngeal mucosa of 30–75% of children. Samples of secretions from the nasopharynx of children suspected of having a respiratory infection were sown on Columbia agar with 5% lamb blood, incubated at a temperature of +37degrees for 18–24 hours in an atmosphere of 5% CO_2. Colonies of *S. pneumoniae* were identified using optochin, bile disk, GP-card tests on the bacteriological analyzer VITEK 2 compact 15. Susceptibility to antibacterial drugs was determined by the disk diffusion method in accordance with European Committee on Antimicrobial Susceptibility Testing recommendations. For the period of 2021–2022, 2,578 bacteriological studies of secretions from the nasopharynx of children patients were carried out, 151 strains of *S. pneumoniae* were isolated (resultancy – 5.9%). The percentage of *S. pneumoniae* strains sensitive to oxacillin is 77.5%, norfloxacin – 88.1%, erythromycin – 49.0%, clindamycin – 63.5%, tetracycline – 45.7%, levofloxacin – 97.4%. The percentage of multi-resistant *S. pneumoniae* was 40.0% and 24.0% in 2021 and 2022, respectively. In 77.5% of cases, strains of *S. pneumoniae* are sensitive to β-lactam antibiotics, in 49.0% – to macrolides, in 63.5% – to clindamycin, in 88.1% – to fluoroquinolones, which will allow doctors to prescribe empiric antibacterial therapy for pneumococcal infections among children, with further adjustment after obtaining the antibiotic pattern of a bacteriological examination. The problem of antimicrobial resistance is only gaining publicity in the system of public health and health care, which requires the results of analyzing the spread of resistant strains and indicators of the profile of sensitivity to antimicrobial drugs. The prospects for further study lie in the further dynamic monitoring of antimicrobial resistance of *S. pneumoniae* among different contingents of the population.

Keywords: pneumococcus; children; Ukraine; multi-resistance; nasopharynx; antibacterial drug; infection.

Introduction

The importance of establishing the level of colonization of *Streptococcus pneumoniae* in the nasopharynx of children and the level of sensitivity of strains to antibacterial drugs (ABD) is due to the uncontrolled and unjustified use of ABD in the treatment of various infections, in particular COVID-19, the use of ABD in veterinary medicine and the food industry, which is the cause of the development of antibacterial resistance in opportunistic bacteria.

Streptococcus pneumoniae is a gram-positive microorganism that colonizes the nasopharynx of 30–75% children, but can become a source of acute respiratory tract infections, otitis, nasosinusitis, community-acquired pneumonia, bacteremia and meningitis (Marquart, 2021; Dai et al., 2023; Li et al., 2023).

The Centers for Disease Control and Prevention (CDC) estimates that approximately 30% of cases of invasive pneumococcal disease are caused by *S. pneumoniae* isolates resistant to 1 or more clinically relevant antibiotics. Antibiotic-resistant isolates of *S. pneumoniae* caused about 900,000 infections and 3,600 deaths per year in all age groups in 2014, representing a serious threat. Worldwide, *S. pneumoniae* is the fourth leading clinically important pathogen in antimicrobial resistance-related deaths (Mohanty et al., 2023).

This pathogen accounts for about 38% of childhood deaths caused by pneumonia, which is the leading cause of death in children under 5 years of age worldwide. Detection of pneumococcal colonization of the nasopharynx is of great importance because it is a major reservoir for transmission and a prerequisite for invasive disease (Nikolaou et al., 2019; Cleary et al., 2022).

It has been established that hypopharyngeal *S. pneumoniae* carriage in childhood is associated with an increased risk of developing lower respiratory tract infections in the first 3 years of life. Conversely, respiratory tract infections can affect carriage through bacterial superinfection or changes in bacterial colonization after viral infection. Detection of bacterial carriage, monitoring of the profile of antibiotic resistance of selected strains of *S. pneumoniae*, make it possible to predict the development of infections and adjust antibiotic therapy in the treatment of pneumococcal infections (Yu et al., 2019; van Meel et al., 2020; Kielbik et al., 2023).

Nasopharyngeal colonization by *S. pneumoniae* is considered a prerequisite for invasive pneumococcal infection. Nasopharyngeal pneumococcal carriage is common in young children, particularly in low-income countries. A meta-analysis of studies conducted worldwide found that 20–93% of children in low-income countries carried nasopharyngeal pneumococci, compared with 6.5–69.8% of children in lower-middle-income countries. Another pooled study reported similar results, with carrier prevalence of 65% and 48% for children in low-income and lower-middle-income countries, respectively (Daningrat et al., 2022; Chamorro et al., 2023; Paulo et al., 2023).

Person-to-person transmission of pneumococci occurs through close contact and aerosols, and colonization is considered a prerequisite for disease, although many colonized individuals do not experience symptoms.

The ability of *S. pneumoniae* to adhere to epithelial cells of the nasopharyngeal mucosa is an important step in the process leading to pathogenesis. Pneumococcal factors have been shown to play an important role in host cell attachment and are surface proteins such as pneumococcal surface adhesin A (PsaA) and choline-binding protein A/pneumococcal surface protein C/secretory IgA-binding protein of *S. pneumoniae* (CbpA/PspC/SpsA). PsaA binds to host E-cadherin, and CbpA/PspC/SpsA binds to sialic acid, lacto-N-neotetraose, the polymeric immunoglobulin (Ig) receptor, and vitronectin. In the pathogenesis of pneumococcal infection, bacteria settle in the lungs, blood, middle ear, central nervous system or other places. The method of this establishment depends on the sensitivity of the host, the regulation of bacterial gene expression and the possibility of interaction between *S. pneumoniae* and components of the host. In the case of pneumococcal pneumonia, pneumococcal neuraminidase (NanA) is required to cleave sialic acid from glycoprotein receptors of host cells, thus promoting attachment of *S. pneumoniae* to airway epithelial cells. Pneumococcal pneumonia is characterized by inflammation of the lungs as a result of the action of bacterial factors that cause proinflammatory cytokine reactions and the recruitment of immune cells. A cholesterol-dependent cytolysin, pneumolysin, contributes to these inflammatory effects in addition to its role in pore formation in eukaryotic cell membranes (Marquart, 2021).

Empiric treatment of pneumococcal infections can cause complications due to the selection of strains with genes for resistance to multiple antibacterial drugs, which contributes to the spread of multiresistant pneumococci (El-Kholy et al., 2020; Kulkarni et al., 2023; Mohanty et al., 2023).

β-lactam antibiotics that act on the bacterial cell wall are commonly used to treat infections caused by *S. pneumoniae*, but 10% to 30% of strains are resistant to these antibiotics. Penicillin G is considered a first-line β-lactam antibiotic, after which cephalosporins of the I–V generations, carbapenems and monobactams were invented. In 2017, the World Health Organization published a list of priority pathogens for antibiotic research and development, which included penicillin-resistant *S. pneumoniae* (Narwortey et al., 2021; Dai et al., 2023; Li et al., 2023).

According to the results of a national long-term antibiotic susceptibility study of 3,017 clinical isolates of *S. pneumoniae* from 2004 to 2020 in Spain, a low level of resistance to penicillin (<6.5%), cefotaxime (<5%) was found, while 56.7% of strains were resistant to amoxicillin in 2004 and 35.7% in 2020. Antimicrobial susceptibility analysis of *S. pneumoniae* in Korea from 2012 to 2019 found a high level of penicillin resistance of 85.7% for strains causing CNS (central nervous system) infection, 13.9% for non-CNS isolates, and 18.9% for isolates of non-invasive *S. pneumoniae* infection (Li et al., 2023). Penicillin-binding proteins (PBPs) are thought to be the main cause of β-lactam resistance in *S. pneumoniae*. Six PBPs (PBP1a, 1b, 2a, 2x, 2b and 3) were found in the pneumococcal genome, while three (PBP1a, 2x and 2b) were mainly associated with antibiotic resistance (Gibson & Veening, 2023; Li et al., 2023).

Macrolides are often used as an alternative to β-lactams, but today a significant resistance of *S. pneumoniae* to this group of antibiotics is registered. A multicenter study conducted in China showed 96% resistance to erythromycin in 300 invasive *S. pneumoniae* isolates collected from 2010 to 2015. In the period 2011–2019, a high rate of erythromycin non-susceptibility (94.3%) was reported for non-encapsulated pneumococci in Japan. In Korea, for *S. pneumoniae* strains collected between 2012 and 2019, the overall erythromycin resistance rate was 79.2%, 69.4% for non-meningeal invasive infectious isolates, and 80.2% for noninvasive infectious strains. In Argentina, this indicator was 26.6% based on the results of the analysis of 2,798 isolates from 2006 to 2019 (Li et al., 2023).

The mechanism of *S. pneumoniae* resistance to macrolides is due to the erythromycin ribosomal methylase encoded by erm(B) and the macrolide efflux transporter MFS encoded by the mef gene (mefE or mefA). Erm(B) methylase is able to methylate 23 S rRNA and prevent themacrolide binding to the target, leading to antibiotic resistance through target modification. Ribosomal methylation of Erm(B) can lead to resistance to the macrolide-lincosamide-streptogramin B (MLSB phenotype) and high-level resistance (erythromycin MIC usually ≥ 256 μg/mL). Most erythromycin-resistant pneumococcal isolates carry the erm(B) gene, a minority carry the mefA gene, and some strains contain the erm(B) and mefA genes (Li et al., 2023).

Resistance to fluoroquinolones remains at a low level, which allows them to be used as alternative drugs when resistance to β-lactams and macrolides is established, however, the risk of developing arthrotoxic effects in children under 18 years of age should be remembered. The results of a national long-term study conducted in Spain from 2004 to 2020 showed a low level of resistance to levofloxacin in 3,017 analyzed clinical isolates, ranging from 6.8% in 2012 to 1.7% in 2020. In Argentina, such a study found that all strains were susceptible to levofloxacin from 2,798 pediatric invasive *S. pneumoniae* isolates from 2006 to 2019. A multicenter study conducted in 13 provinces in China also reported 100% levofloxacin susceptibility of *S. pneumoniae* causing invasive pneumococcal disease from 2010 to 2015. Today, fluoroquinolones can be used to treat *S. pneumoniae* infections caused by isolates resistant to β-lactams and macrolides. Fluoroquinolones mainly target type II (DNA gyrase encoded by genes gyrA and gyrB) and IV topoisomerase (encoded by genes parC and parE), which played an important role in DNA replication. Resistance of pneumococci to fluoroquinolones can be mediated by two main mechanisms, including target mutation in the gyrA/B and/or parC/E genes, and an efflux mechanism by reducing intracellular accumulation of the antibiotic (Amari et al., 2023; Kulkarni et al., 2023; Li et al., 2023).

Trimethoprim and sulfonamides have always been used in combination as trimethoprim-sulfamethoxazole, which targets the bacterial cell enzymes dihydrofolatereductase and dihydropteroatesynthase. The proportion of *S. pneumoniae* resistant to trimethoprim-sulfamethoxazole was 65.3% in a multicenter study conducted in 13 provinces of China in 2010–2015. Among 504 *S. pneumoniae* strains collected in Tunisia between 2012 and 2018, the rate of non-susceptibility to this antibiotic was 28.8%, but this rate among 2,798 *S. pneumoniae* strains causing pediatric invasive disease in Argentina from 2006 to 2019 was 43.4%. This antibiotic kills bacteria by inhibiting the two main enzymes in the bacterial synthesis of folic acid, with which sulfamethoxazole acts on dihydropteroatesynthetase (DHPS) encoded by the folP (sulA) gene, and trimethoprim acts on dihydrofolatereductase (DHFR) encoded by the folA gene. Substitution of the amino acid isoleu-isoleucine-100 to leucine (Ile100Leu) in DHFR can lead to trimethoprim resistance in *S. pneumoniae*. In addition to the Ile100Leu mutation, other mutations in DHFR can also increase resistance to trimethoprim and affect the substrate affinity of DHFR (Li et al., 2023; Mohanty et al., 2023).

The growth of resistance to tetracycline was established, especially when analyzing the resistance phenotypes to several drugs. Ribosomal protection mediated by the tet(M) and tet(O) genes is the main mechanism of *S. pneumoniae* resistance to tetracycline, while the tet(M) gene-dependent mechanism is more extensive. The tet(M) and tet(O) genes are located on transposons of the Tn916 family and encode ribosomal protective proteins homologous to elongation factors G. Six alleles, from tet(M)1 to tet(M)6, were identified by restriction analysis of tet(M) at high resolution, indicating genetic diversity of the tet(M) gene in tetracycline-resistant strains. Whole-genome sequencing analysis of 12,254 pneumococcal isolates from 29 countries identified a novel mosaic structure of the tet(S/M) gene carried by Tn916. Therefore, homologous recombination of resistance genes played an important role in the evolution of resistance to tetracycline (Li et al., 2023; Mohanty et al., 2023).

Multidrug resistance (MDR) (resistance to ≥ 3 classes of antibiotics) is increasing worldwide and accounts for more than 30% for pneumococci. Multidrug-resistant strains accounted for 81.2% (147/181) of all isolates causing pediatric invasive pneumococcal disease in Beijing, China, from 2012 to 2017, and the most common resistance was to erythromycin, clindamycin, and tetracycline. In Tunisia, the overall rate of such strains was 54.8% for 504 *S. pneumoniae* isolates collected between 2012 and 2018, and the most common resistance was β-lactams (98.6%), macrolides (99.6%) and tetracycline (75.4%). In Korea, 66.9% of *S. pneumoniae* showed multidrug resistance, and 2.8% of strains were resistant to all other antibiotics except vancomycin in pneumococcal antimicrobial susceptibility testing from 2012 to 2019. In Argentina, the overall rate of multiple resistance was 16.1% for pneumococcal strains isolated from a pediatric population with invasive disease from 2006 to 2019 (Li et al., 2023; Mohanty et al., 2023; Senok et al., 2023).

The purpose of this study is to establish the level of nasopharyngeal colonization and the sensitivity profile of isolated strains of *S. pneumoniae*

to ABD among children who received medical care at the Communal non-commercial enterprise "City Clinical Hospital No. 6" of the Dnipro city council.

Materials and methods

Patients gave their informed consent in accordance with the primary accounting documentation No. 003-6/o "Informed voluntary consent of the patient for diagnosis, treatment and surgery and anesthesia and for the presence or participation of participants in the educational process". This study was conducted during 2021–2022 by the bacteriological method of sowing, 2,578 samples were isolated from the nasopharynx of children with respiratory infection. Samples from the nasopharynx of children were collected on an empty stomach, before the appointment of ABD, with sterile disposable applicators and delivered to the bacteriological laboratory within 2 hours from the moment of collection.

The biomaterial was applied to Columbia agar with 5% lamb blood (Graso, Poland), incubated at a temperature of +37 ºC for 18-24 hours in an atmosphere of 5% CO_2. Colonies, the morphotype of which corresponded to *S. pneumoniae*, were further identified using tests with optochin (Erba Lachema, Czech Republic), bile disk (Pharmaktiv, Ukraine), pure culture was isolated, identification was carried out using a GP card (BioMeriuex, France) on a bacteriological analyzer VITEK 2 compact 15 (BioMeriuex, France). The sensitivity of the selected strains to ABD was determined by the disk diffusion method in accordance with the requirements of the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The following discs with ABP were used: oxacillin (1 µg) (Himedia, India), norfloxacin (10 µg) (Pharmaktiv, Ukraine), erythromycin (15 µg) (Pharmaktiv, Ukraine), clindamycin (2 µg) (Pharmaktiv, Ukraine), tetracycline (30 µg) (Pharmaktiv, Ukraine), levofloxacin (5 µg) (Pharmaktiv, Ukraine). Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (Graso, Poland) were used to determine sensitivity by the disc diffusion method according to EUCAST recommendations. The crops were incubated at a temperature of +35 ºC for 18–24 hours in an atmosphere of 5% CO_2. Calculation of the diameters of growth retardation zones around discs with ABD was measured with a calibrated ruler. The quality control of the studies was carried out in accordance with the approved intra-laboratory quality control program and EUCAST recommendations. A one-way analysis of variance (ANOVA) test was applied to diameters of growth inhibition zones (mm), considering $P < 0.05$ as statistically significant.

Results

For the period of 2021–2022, 2,578 bacteriological studies of secretions from the nasopharynx of children patients of our hospital were carried out, 151 strains of *S. pneumoniae* were isolated (result – 5.9%), including for 2021 – 1475 studies, 85 strains (5.8%), for 2022 – 1,103 studies, 66 strains (5.9%, $P < 0.05$). It was established that the level of colonization during the reporting period is at the same level, which indicates the representativeness of local data. Among pediatric outpatients, 513 bacteriological studies of secretions from the nasopharynx were conducted, 40 strains of *S. pneumoniae* were isolated (result – 7.8%), including in 2021 – 200 studies, 22 strains (11.0%), in 2022 – 313 studies, 18 strains (5.8%, $P < 0.05$). Among pediatric inpatients, 2,065 bacteriological studies of nasopharyngeal secretions were conducted, 111 strains of *S. pneumoniae* were isolated (result – 5.4%), including in 2021 – 1275 studies, 63 strains (4.9%), in 2022 – 790 studies, 48 strains (6.1%, $P > 0.05$). Carriage of *S. pneumoniae* strains among outpatients and inpatients of children in the period 2021–2022 is presented in Figure 1.

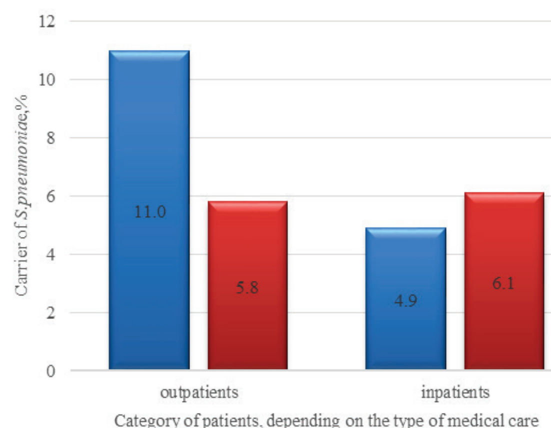

Fig. 1. Carriage of *S. pneumoniae* strains among child outpatients and inpatients in the period 2021–2022, blue columns – date for 2021, red – date for 2022

The sensitivity profile to ABD of *S. pneumoniae* strains isolated from the nasopharynx of children for 2021–2022 is presented in Table 1.

Table 1
Sensitivity level to ABD of *S. pneumoniae* strains isolated from the nasopharynx of children for 2021–2022

Antibacterial drug	General sensitivity profile (%, n = 151)			Sensitivity profile in 2021 (%, n = 85)			Sensitivity profile in 2022 (%, n = 66)		
	S	I	R	S	I	R	S	I	R
Oxacillin	77.5	–	22.5	74.1	–	25.9	81.8	–	18.2
Norfloxacin	88.1	–	11.9	91.8	–	8.2	83.6	–	16.4
Erythromycin	49.0	2.6	48.4	44.7	3.5	51.8	54.5	1.8	43.6
Clindamycin	63.5	–	36.5	54.1	–	45.9	76.3	–	23.7
Tetracycline	45.7	8.6	45.7	45.9	7.0	47.1	45.5	10.9	43.6
Levofloxacin	–	97.4	2.6	–	96.5	3.5	–	98.5	1.5

Notes: S – susceptible, standard dosing regimen; I – susceptible, increased exposure; R – resistant.

Over the period of 2021–2022, the number of strains of *S. pneumoniae* sensitive to oxacillin increased by 7.7% (correlation coefficient 1), in 2021 the indicator was 74.1%, in 2022 – 81.8% ($P < 0.05$). A tendency towards a decrease in the level of sensitivity to norfloxacin of *S. pneumoniae* by 8.2% was established (correlation coefficient –1, $P < 0.05$). An increase in the sensitivity of *S. pneumoniae* strains to erythromycin was recorded by 9.8% (correlation coefficient 1, $P < 0.05$), in 2021, the indicator was 44.7%, in 2022 – 54.5%; before clindamycin, this indicator increased by 22.2% (coefficient correlations 1, $P < 0.05$). It was found that 2 strains (1.3%) of *S. pneumoniae* showed inducible resistance to clindamycin (D-phenomenon). The level of sensitivity to tetracycline and levofloxacin of *S. pneumoniae* strains isolated from the nasopharynx of children remained at the same level during the reporting period.

Indicators in the dynamics of the level of resistance of *S. pneumoniae* strains isolated from the nasopharynx of children to each of the tested antibacterial drugs for the period 2021–2022 are presented in Figure 2.

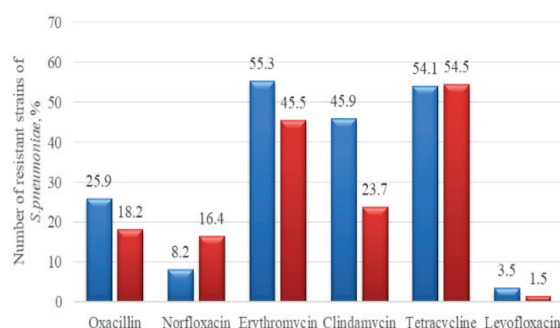

Fig. 2. Change in the level of resistance to ABD of *S. pneumoniae* strains isolated from the nasopharynx of children in dynamics for the period 2021–2022 ($P < 0.05$), blue columns – date for 2021, red – date for 2022

During the period of 2021–2022, the number of oxacillin-resistant strains of *S. pneumoniae* decreased by 7.7% (correlation coefficient –1), in 2021 the indicator was 25.9%, in 2022 – 18.2% (P < 0.05). A trend towards an increase in the level of norfloxacin resistance of *S. pneumoniae* by 8.2% was established (correlation coefficient 1). A decrease in the level of resistance to erythromycin of *S. pneumoniae* strains was recorded by 9.8% (correlation coefficient –1); in 2021, the indicator was 55.3%, in 2022 – 45.5%; before clindamycin, this indicator decreased by 22.2% (correlation coefficient –1). The level of resistance to tetracycline and levofloxacin of *S. pneumoniae* strains isolated from the nasopharynx of children remained at the same level during the reporting period.

The number of isolated oxacillin-resistant strains of *S. pneumoniae* (n = 34) from the nasopharynx of children depending on the diameter of the zone of growth inhibition around the disk with oxacillin for the years 2021–2022 is presented in Table 2.

Table 2
Distribution of isolated oxacillin-resistant strains of *S. pneumoniae* from the nasopharynx of children depending on the diameter of the growth inhibition zone around the disc with oxacillin for 2021–2022

The diameter of the zone of inhibition of growth around the disk with oxacillin, mm	Total number of strains (n = 34)	Number of strains in 2021 (n = 22)	Number of strains in 2022 (n = 12)
9–19	11/32.4%	9/41.0%	2/16.7%
<9	23/67.6%	13/59.0%	10/83.3%

It was established that the number of isolated oxacillin-resistant strains of *S. pneumoniae* from the nasopharynx of children with a growth retardation zone of 9–19 mm around the disc with oxacillin decreased by 24.3% (correlation coefficient –1, P < 0.05) and was 41.0% in 2021, in 2022 – 16.7%. The number of isolated oxacillin-resistant strains of *S. pneumoniae* from the nasopharynx of children with a zone of growth retardation < 9 mm around the disc with oxacillin increased by 24.3% (correlation coefficient 1) and was 59.0% in 2021, 83.3% in 2022, which indicates an increase in the level of resistance to β-lactam antibiotics.

The number of multidrug resistance (MDR) strains of *S. pneumoniae* isolated from the nasopharynx of children is presented in Table 3. It was established that the total number of MDR strains of *S. pneumoniae* decreesed by 16% (correlation coefficient –1, P < 0.05) and amounted to 40.0% in 2021 and 24.0% in 2022, which is a representative trend among children in the provision of outpatient medical care assistance, as well as among children who were in hospital treatment.

Table 3
Distribution of MDR strains of *S. pneumoniae* isolated from the nasopharynx of children for the period 2021–2022

Year	Total number of strains	Total number of MDR strains	Number of MDR strains isolated from outpatients	Number of MDR strains isolated from hospitalized patients
2021	85	34/40.0%	6/7.0%	28/33.0%
2022	66	16/24.0%	4/6.0%	12/18.0%

Note: MDR – multidrug resistance to ABD.

The distribution of MDR strains of *S. pneumoniae* isolated from the nasopharynx of children depending on the combinations of antibacterial drugs for the period 2021–2022 is presented in Table 4. It was established that in 2021 the number of MDR strains of *S. pneumoniae* with the ERT/CLN/TET ABD combination was 13 strains (38.2%), OXA/ERT/CLN/TET – 13 strains (38.2%), OXA/ERT/CLN – 4 strains (11.7%), OXA/NOR/TET – 1 strain (2.9%), NOR/ERT/CLN/TET – 1 strain (2.9%), OXA/NOR/ERT/CLN/LVF – 1 strain (2.9%), ERT/CLN/TET/LVF – 1 strain (2.9%). In 2022, the number of MDR strains of *S. pneumoniae* with the combination of ABP ERT/CLN/TET was 9 strains (56.3%), OXA/ERT/CLN/TET – 1 strain (6.2%), OXA/NOR/ERT/TET – 3 strains (18.7%), OXA/NOR/ERT/CLN/TET – 1 strain (6.2%), OXA/ERT/TET – 3 strains (18.7%). It was established that in 2022 the specific weight of MDR strains of *S. pneumoniae* with the combination of ERT/CLN/TET increased by 18.1% (correlation coefficient 1) (P < 0.05), the specific weight of MDR strains of *S. pneumoniae* with the combination of OXA/ERT/CLN/TET decreased by 32.0% (correlation coefficient –1, P < 0.05).

Table 4
Distribution of MDR strains of *S. pneumoniae* isolated from the nasopharynx of children depending on combinations of antibacterial drugs for the period 2021–2022

ABD combinations	Number of MDR strains in 2021/(%)	Number of MDR strains in 2022/(%)
ERT/CLN/TET	13/38.2%	9/56.3%
OXA/ERT/CLN/TET	13/38.2%	1/6.2%
OXA/ERT/CLN	4/11.7%	0
OXA/NOR/TET	1/2.9%	0
NOR/ERT/CLN/TET	1/2.9%	0
OXA/NOR/ERT/CLN/LVF	1/2.9%	0
ERT/CLN/TET/LVF	1/2.9%	0
OXA/NOR/ERT/TET	0	3/18.7%
OXA/NOR/ERT/CLN/TET	0	1/6.2%
OXA/ERT/TET	0	3/18.7%
Total	34	16

Notes: OXA – oxacillin, PEN – penicillin, CFA – ceftriaxone, NOR – norfloxacin, ERT – erythromycin, CLN – clindamycin, TET – tetracycline, LVF – levofloxacin.

Discussion

We found that the level of *S. pneumoniae* colonization of the nasopharynx of children did not change in 2021–2022, but this indicator among outpatients is 7.8%, and among inpatients – 5.4%. The carriage of *S. pneumoniae* among children in India is at the level of 8.1% (Verma et al., 2023), while this indicator among children in Singapore is 8.4% (41 strains out of 491 samples) (Daningrat et al., 2022), which correlates with our research results. The rate of carriage of *S. pneumoniae* among children in Paraguay is 34.1% (Chamorro et al., 2023), but this indicator among children in France is 57.4% (Rybak et al., 2023), which does not correlate with our research results. The indicator of the level of *S. pneumoniae* colonization of the nasopharynx of children in Hungary is 35.8% (Kovács et al., 2020), and among children in Southampton, Great Britain at the level of 32.5% (Cleary et al., 2022), which differs from the results of our study. We found that 34 (32.4%) oxacillin-resistant strains of *S. pneumoniae* with a zone of growth retardation around the disc with oxacillin of 9–19 mm are sensitive to semi-synthetic penicillins (without or with a β-lactamase inhibitor), cefepime, cefotaxime, ceftaroline, ceftobiprole, ceftriaxone, imipenem and meropenem.

The number of oxacillin-resistant *S. pneumoniae* strains with an oxacillin-disc zone of 9–19 mm decreased each year (correlation coefficient –1), but with an oxacillin-disc zone of < 9 mm increased each year (correlation correlations 1). The number of norfloxacin-resistant strains of *S. pneumoniae* is increasing every year (correlation coefficient 1). Screening for resistance to fluoroquinolones of *S. pneumoniae* is considered negative for up to 88.0% of strains, indicating sensitivity to moxifloxacin at the standard dosage regimen and levofloxacin at increased dosage. The number of strains of *S. pneumoniae* sensitive to erythromycin, azithromycin, clarithromycin, roxithromycin is 49.0%. The relevance of the obtained results of sensitivity to ABD of selected strains of *S. pneumoniae* among children, in comparison with the data obtained by researchers in other countries, is presented in Table 5.

In China, the number of MDR strains of *S. pneumoniae* with the ABD combination ERT/CLN/TET is 6.6% (153 strains out of 2326), OXA/ERT/CLN/TET – 0.9% (20 strains out of 2,326), OXA/ERT/CLN – 0.4% (10 strains out of 2,326), OXA/NOR/TET – not recorded, NOR/ERT/CLN/TET – not recorded, OXA/NOR/ERT/CLN/LVF – not recorded, ERT/CLN/TET/LVF – not recorded, which is significantly different from our studies (Dai et al., 2023).

In China, the sensitivity of non-invasive strains of *S. pneumoniae* to erythromycin is 0.1% (3 out of 2,409 strains), to clindamycin – 1.9% (46 out of 2,409 strains), to penicillin – 91.1% (2,195 out of 2,409 strains), to tetracycline – 10.1% (244 out of 2,409 strains), levofloxacin – 99.9% (2,407 of 2,409 strains), which is significantly different from the sensitivity profile to ABD obtained by us (Dai et al., 2023). In Indonesia, the number of susceptible strains of *S. pneumoniae* to erythromycin is 91.6% (1,014 of 1,107 strains), clindamycin – 95.6% (1,058 of 1,107 strains), penicillin – 95.4% (1,056 of 1,107 strains), tetracycline – 55.6% (616 of 1,107 stra-

ins), levofloxacin – 100.0% (1,107 out of 1,107 strains), which is significantly different from the results of our study (Yani et al., 2023; Safari et al., 2024). The susceptibility profile of *S. pneumoniae* strains to ABD among Brazilian children is reduced compared to the results of our study, which probably indicates the intensive use of ABD (Silva et al., 2022). Indicators of the level of sensitivity of *S. pneumoniae* strains to ABD among children in Vietnam are significantly different from the results of our study, which is probably related to the peculiarities of ABD use in this country. The susceptibility profile of *S. pneumoniae* to penicillin among children in Pittsburgh (USA) correlates with the results of our study, but the susceptibility to erythromycin, clindamycin, and tetracycline is significantly different (Lobb et al., 2023). Penicillin susceptibility among Ethiopian children correlates with the results of our study, but tetracycline susceptibility differs significantly (Mekuria et al., 2023). The susceptibility profile of *S. pneumoniae* to oxacillin among Moroccan children is significantly lower compared to our results, which is probably due to the significant use of β-lactam ABDs (Amari et al., 2023).

Table 5
Comparison of the number of isolated strains of *S. pneumoniae* sensitive to ABD among children with data obtained by researchers in other countries

Country	Number of sensitive strains of *S. pneumoniae*, %							
	OXA	PEN	CFA	NOR	ERT	CLN	TET	LVF
Vietnam (Tran-Quang et al., 2023)	–	1.1	37.1	–	1.1	11.2	–	80.9
Brazil (Silva et al., 2022)	47.5	–	–	–	33.8	86.4	67.7	–
Pittsburgh, USA (Lobb et al., 2023)	–	75.0	–	–	69.5	90.3	88.9	–
Morocco (Amari et al., 2023)	42.8	–	–	100.0	82.1	85.1	98.5	–
Ethiopia (Mekuria et al., 2023)	–	77.0	100.0	–	–	–	60.0	–
Poland (Kielbik et al., 2022)	–	65.9	–	100.0	68.3	85.4	73.2	–
Dnipro, Ukraine	77.5	77.5	84.7	88.1	49.0	63.5	45.7	97.4

Notes: OXA – oxacillin, PEN – penicillin, CFA – ceftriaxone, NOR – norfloxacin, ERT – erythromycin, CLN – clindamycin, TET – tetracycline, LVF – levofloxacin.

Penicillin susceptibility of *S. pneumoniae* among children in Poland is lower compared to the results of our study, which probably indicates the intensive use of ABD, however, this indicator for norfloxacin, erythromycin, clindamycin, and tetracycline is higher than the level of sensitivity established in our study (Kielbik et al., 2022). The rate of susceptibility of *S. pneumoniae* strains among Egyptian children to ampicillin is 32.7% (36/110), ceftriaxone – 54.5% (60/110), tetracycline – 32.7% (36/110), clindamycin – 72.7% (80/110), erythromycin – 55.5% (61/110), levofloxacin – 90.9% (100/110), which correlates according to some indicators with our research results (El-Kholy et al., 2020). In Japan in 2016, the number of *S. pneumoniae* strains sensitive to penicillin was 61.1% (33/54), erythromycin – 1.9% (1/54), levofloxacin – 100% (54/54), which correlates with our results in some respects of research (Ono et al., 2023). After analyzing our results and *S. pneumoniae* susceptibility indicators in other countries, it is possible to assert the correlation of our results with global data. Thus, we have achieved our goal and confirmed the hypothesis of our research.

Conclusions

The rate of *S. pneumoniae* colonization of the nasopharynx of children is 5.9%. In 77.5% of cases, *S. pneumoniae* strains are sensitive to β-lactam antibiotics, in 49.0% – to macrolides, in 63.5% – to clindamycin, in 88.1% – to fluoroquinolones. Among oxacillin-resistant strains of *S. pneumoniae*, 32.4% are sensitive to semisynthetic penicillins (without or with β-lactamase inhibitor), cefepime, cefotaxime, ceftaroline, ceftobiprole, ceftriaxone, imipenem, and meropenem. The number of multidrug-resistant strains of *S. pneumoniae* decreased by 16% in 2022 compared to 2021.

The results of our study established a profile of susceptibility to antibacterial drugs of strains of *S. pneumoniae*, which will allow clinicians to prescribe empiric antibacterial therapy for pneumococcal infections among children, with subsequent correction after receiving the antibioticgram of bacteriological research. The problem of antimicrobial resistance has only recently been gaining publicity in the system of public health and health care, which requires the results of analyzing the spread of resistant strains and indicators of the profile of sensitivity to antimicrobial drugs.

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