

Conference Paper

Bacterial Carriage of Pathogenic Antibiotic-resistant Staphylococci Among Conditionally Healthy Infants and Preschool-age Children

S. Kolesnikova¹, E. Tulyakova¹, A. Solovyova¹, I. Moiseeva², and N. Bystrova¹¹National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Kashirskoe shosse 31, Moscow, 115409, Russia²Faculty of General Medicine, Medical Institute, Penza State University, 440026, Penza, Russian Federation

Abstract

Staphylococci are related to opportunistic microorganisms and are part of normal human microflora. However, there are varieties that can cause pyoinflammatory diseases in organisms with weakened immune status. In the event of an inadequate therapeutic approach, they are preserved in the body, and the person becomes a bacterial carrier and a source of dissemination of pathogenic microorganisms. The formation of antibiotic resistance also plays a significant role in the emergence of bacterial carriage. The situation is especially serious in organized children's groups, where children are in close contact with one another. Thus, the study of the prevalence rate of antibiotic resistance among pathogenic strains of staphylococci in children's groups is topical and worthy of attention. The purpose of this work is to identify carriers of pathogenic and antibiotic resistant staphylococci isolated from the microflora of the oral cavity of preschool children considered healthy. The results of the research can prove useful in practical healthcare and, particularly, in pediatric practice.

Keywords: staphylococci, pathogenic cocci, bacterial carriage, antibiotic resistance, infant morbidity rate

Corresponding Author:

S. Kolesnikova
sgkolesnikova@mail.ru

Received: 17 January 2018

Accepted: 25 March 2018

Published: 17 April 2018

Publishing services provided by
Knowledge E

© S. Kolesnikova et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the PhysBioSymp17 Conference Committee.

 OPEN ACCESS

1. Introduction

Normally, the children's immune system is fairly resistant to staphylococcal infections. However, it should be kept in mind that children, especially in children's institutions, are constantly in close contact with one another: they play together, share toys, take food in one room, etc. All this increases the risk of carrier-to-healthy child infection. As a rule, the carriage of staphylococcus does not show symptoms; therefore, there is a high

probability that such a carrier may be present in a children's group. The results of the study of a group of healthy children aged 7–11 years showed the predominant amount of staphylococci possessing pathogenicity factors in salivary microorganisms [1]. Of particular interest is the relationship between the cases of carriage of pathogenic staphylococcal strains and the condition when children are prone to frequent respiratory infections. Such children are called recurrent respiratory infection (RRI) children. Among the children attending kindergartens, the RRI group is about 50% [2]. Studies confirm the leading positions of pathogenic staphylococci in the microflora of the RRI group. [3, 4].

An important role in the formation of carriage is played by open disregard of the rules of rational antibiotic therapy. For example, as the study showed, a ten-day course of medication with aminopenicillins is not sustained [5]. This leads to the development of antibiotic resistance of microorganisms. While this resistance is developed, medication with etiotropic drugs according to recommendations will not be efficient, and the risk of developing bacterial carriage will increase. There appear data already on high detection frequency of resistant strains [6, 7]. The presence of carriers of these strains among children poses an epidemiological threat.

With this background, the study of carriage of staphylococci among children acquires special significance. The study of the prevalence rate of pathogenic bacterial carriage and resistance to antibiotics is required to determine a strategy for admitting children to attend children's institutions and to conduct effective antibacterial therapy of staphylococcal infections.

2. Materials and methods

The children who took part in the study are urban residents. All the children attend the same children's institution. A total of 51 children aged 1–7 years were involved: 33 preschool-age children and 18 infants (aged 1–3 years). To detect pathogenic staphylococci, the basic principles of generally accepted microbiological diagnostics were used [8].

The object of the study was a smear from the tonsillar mucous membrane. The material was sowed on the egg yolk high salt agar culture medium. The incubation was carried out at 37 °C for 24 hours. The colonies of each type of staphylococcus were subjected to culture and microscopic analyses.

The pathogenicity of the studied staphylococci was determined using the Gross classification. According to it, a microorganism is considered to belong to the pathogen

group if it has a pronounced hemolytic activity and its plasmocoagulase coagulates the plasma for 2 hours. Staphylococcus is low-pathogenic when it forms incomplete hemolysis and its plasmocoagulase coagulates plasma for 6 hours or more. Non-pathogenic staphylococci do not have hemolytic activity and their plasmocoagulation reaction is negative [9]. Consideration was also given to the presence of pronounced lecithinase activity as an indicator of the pathogenicity of microorganisms.

To determine hemolytic activity, the colonies were resowed on 5% blood agar. An example of expressed hemolysis of Strain 41 is shown in Fig. 1. To determine the activity of the plasmocoagulase, the culture was inoculated into a sterile citrate plasma and then incubated. An example of a positive reaction is shown in Fig. 2. To determine the lecithinase activity, the zones of turbidity around the colonies on plates with egg yolk high salt agar were identified and described. In Fig. 3, Strain 25 has a pronounced lecithinase activity.

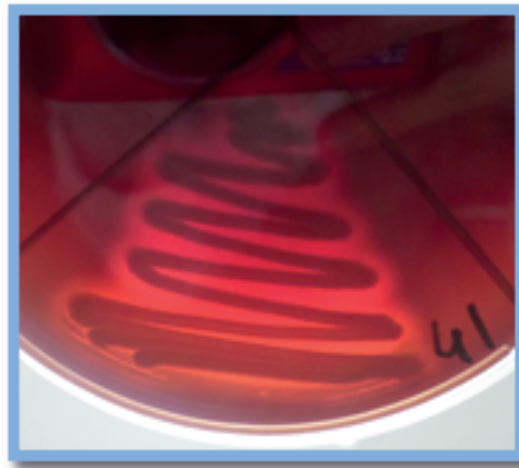


Figure 1: Determination of staphylococcus hemolytic activity.

To determine antibiotic susceptibility, a disk diffusion test was used according to the guidelines [10]. The study used commercial disks produced by the FBUN Institute of Epidemiology and Microbiology named after Pasteur and the Scientific Research Center of Pharmacotherapy (SRCP). Antibiotic susceptibility was determined to the following groups of drugs: peptidoglycan synthesis inhibitors (penicillins, cephalosporins) and protein synthesis inhibitors (aminoglycosides, macrolides). The following drugs were chosen for the study: benzylpenicillin and amoksiklav – from the penicillin series; cef-tazidime – from cephalosporins; amikacin – from aminoglycosides; and azithromycin – from macrolides. An example of an antibiogram for Strain 4 is shown in Fig. 4.



Figure 2: Staphylococcus-induced plasmocoagulation reaction.

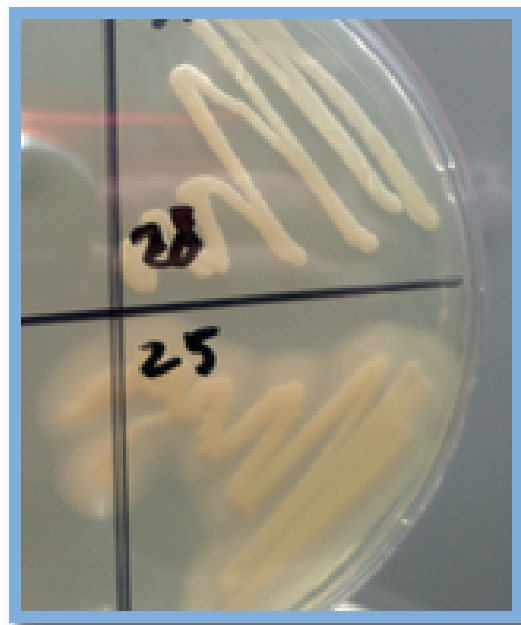


Figure 3: Determination of staphylococcus lecithinase activity.

3. Results and discussion

According to the results of questioning, at the time of sampling no child had any signs of the infectious process activity in the respiratory tract. No child took antibiotics at the time of the study. In the past, 21 children had oropharyngeal diseases.

Growth of microorganisms in culture was observed on Petri dishes 33 and 51 with egg yolk high salt agar. A microscopic analysis confirmed the presence of staphylococci. Significant lecithinase activity was detected in 14 samples. Hemolytic activity on blood

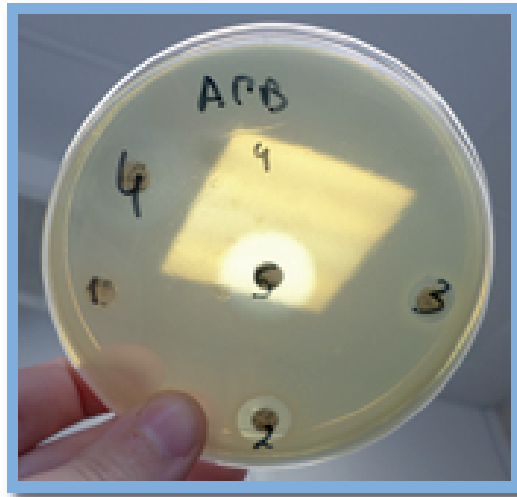


Figure 4: Determination of antibiotic susceptibility of staphylococcus isolated from child carriers: 1. Benzylpenicillin; 2. Amoksiklav; 3. Ceftazidime; 4. Azithromycin; 5. Amikacin.

agar was observed in 9 samples: 2 dishes with β -hemolysis and 7 dishes with α -hemolysis. The same strains had a positive plasmocoagulation reaction: 2 samples coagulated the plasma for 2 hours, 7 samples coagulated the plasma for 8 hours. Distribution of the results by age groups is presented in Table 1.

TABLE 1: The prevalence of staphylococcus pathogenic forms among conditionally healthy children.

Group of children	Number of children surveyed	Lecithinase activity Abs. (%)	Hemolytic activity Abs. (%)	Positive plasmocoagulation reaction
Early childhood (1-3 years)	18	2 (11%)	2 (11%)	2 (11%)
Pre-school age (4-7 years)	33	12 (36%)	7 (21%)	7 (21%)
Total:	51	14 (27%)	9 (18%)	9 (18%)

The prevalence of pathogenic strains according to the Gross classification among the examined group was 18%: 78% of them are low pathogenic and 22% are pathogenic ones. Among the children with isolated pathogenic forms, 6 (66.6%) had had oropharyngeal infections. The remaining 33.4% of the group with isolated pathogenic microorganisms did not have diseases that could be caused by Staphylococcus. There is also a tendency to increase the number of carriers of pathogenic forms among older children. This can be explained by the fact that children older than 3 years are more active in contact with other children, which means that the risk of spreading pathogenic microorganisms between them is higher.

Antibiotic susceptibility tests were carried out for 9 samples of microorganisms to confirm their pathogenicity. All the tested staphylococci showed resistance to benzylpenicillin; 3 from 9 samples were found to be susceptible to amoksiklav; 1 was susceptible to ceftazidime; 6 were susceptible to azithromycin; 3 showed an intermediate result. All the microorganisms under investigation showed susceptibility to amikacin. Polypresistance was observed in 78% (7). The data are presented in Table 2.

TABLE 2: Antibiotic resistance of pathogenic forms of staphylococci isolated from the preschool children.

Antibiotic	Susceptible strains from among the tested ones (9) Abs. (%)	Resistant strains from among the tested ones (9) Abs. (%)
Benzylpenicillin	0	9 (100%)
Amoksiklav	3 (23%)	6 (67%)
Ceftazidime	1 (11%)	8 (89%)
Azithromycin	6 (67%)	0
Amikacin	9 (100%)	0

A high resistance was identified to antibiotics which are often used in clinical practice as first-choice drugs (benzylpenicillin, amoksiklav, ceftazidime). The most effective groups of antibiotics were found to be such protein synthesis inhibitors as: macrolides (azithromycin) and aminoglycosides (amikacin).

Among the children examined, there were ones who had never previously used antibiotics. The presence of resistant forms in them indicates that these strains were obtained as a result of their spread from stable bacteria carriers.

4. Conclusions

1. In the examined group of conditionally healthy children aged 1–7 years attending kindergarten, 27% had staphylococcus with a pronounced lecithinase activity. According to the Gross classification, pathogenic forms were detected in 18% of the children: 78% of low pathogenic and 22% of pathogenic ones.
2. All the pathogenic strains were resistant to benzylpenicillin (100%), 6 strains (67%) were resistant to amoksiklav, 8 (89%) showed resistance to ceftazidime. The isolated strains of staphylococci showed high susceptibility only to azithromycin and amikacin (100%).
3. In the group with the detected pathogenic staphylococcal forms, 66.6% are RRI children. The causative agent of these diseases may be Staphylococcus. The

remaining 33.4% were not RRI children. At the time of the study, none of the 51 children had symptoms of laryngitis or pharyngitis.

Thus, within a healthy children's group, there is a persistence of pathogenic and resistant staphylococci, which is a serious problem for children's health, especially for children with weakened immune status.

Acknowledgments

This work was supported by the MEPhI Academic Excellence Project (agreement with the Ministry of Education and Science of the Russian Federation of August 27, 2013, project no. 02.a03.21.0005).

References

- [1] V.M. Chervinets, O.A. Gavrilova, and Yu.V. Chervinets, "Oral microflora of children aged 7–11 years," *Successes of Modern Natural Science*, no. 2, pp. 73–74, 2009.
- [2] G. A. Sansygina, and G. S. Koval, "Recurrent respiratory infection children: Problems of diagnosis, pathogenesis and therapy," *Lechashchiy Vrach*, no. 1, 2009 URL: <https://www.lvrach.ru/2009/01/5897153/> (Reference date: 15.04.17).
- [3] A.Yu. Deryusheva, and A.V. Deryusheva, "Composition of the microflora of the upper respiratory tract in RRI children. Prospects for modern medicine development," iss. 3, in *Proceedings of the International Scientific and Practical Conference, Voronezh, 2016*, 224 p., ITSRON, 2017.
- [4] Yu.O. Khlynina, "Characterization of microbial biocenoses of mucous membranes in RRI children: Cand. Sci. (Medicine) thesis. Volgograd, 2012.
- [5] V.N. Turchina, L.A. Dulkan, and N.A. Tempel, "Antibiotic therapy in the treatment of respiratory infections in children at the outpatient stage," *Pediatric Pharmacology*, no. 3, pp. 66–69, 2014.
- [6] Yu. Rusetsky, T. Sedykh, and V. Smirnova, "Antibiotic susceptibility of nasopharyngeal microflora in children with adenoids," *Vrach*, no. 13, pp. 34–37, 2011 (in Russian)
- [7] P. G. Bedin, S.A. Lyalikov, and T.V. Nekrashevich, "Antibiotic susceptibility of *Staphylococcus aureus* in children of the Grodno region suffering from chronic tonsillitis," *Problems of health and ecology*, no. 3, pp. 87–91, 2014.
- [8] Order of the Ministry of Health of the USSR, 22.04.1985, no. 535 "On the unification of microbiological (bacteriological) research methods used in clinical diagnostic laboratories of medical and preventive institutions," 126 p., Moscow, 1989.

- [9] A.S. Labinskaya, "Microbiology with the technique of microbiological research: Textbook," 394 p., Moscow, Meditsina, 1978.
- [10] Evaluation of Microorganisms Sensitivity to Antibacterial Medications: Methodological Guidelines MUK 4.2.1890-04. Approved and put into effect by the Chief State Sanitary Doctor of the Russian Federation G.G. Onishchenko, 04.03.2004.