

Microbiological Criteria for Assessment of Ecological Risk

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The composition of microflora from the nasal mucosa is determined in children from 4 ecologically contrasting regions, and diagnostics of the *Staphylococcus* carriership is performed studying the antilysozyme, antidefensin, and antihistone activity of microorganisms. It is established that the carriers of *St. aureus* strains with antihistone activity and the carriers of sporiferous bacilli predominated in ecologically unfavorable regions.

Key Words: *microbiological criteria; bacterial carriership; ecological risk*

As is well known, the development of an infectious process is determined by the state of the macroorganism, the external conditions, and the pathogenicity of the microorganism. Bacterial carriership is the first breach in the reactivity of the macroorganism, when the infectious process is triggered and maintained at a symptom-free, preclinical level. Therefore it seems quite likely that even minor changes in the external environment or in the ecological situation obtaining in the region will be primarily detectable at the level of bacterial carriership, the preclinical level of infection. The sensitivity of early microbiological diagnostics of ecological risk can be raised even higher if the second active principle of the infectious process - the pathogenicity of microorganisms - is involved. It has been shown that the bacterial carriership is primarily formed in the case of microorganisms with immunosuppressive properties [1,5].

The third active principle of the infectious process - the reactivity of the macroorganism - can be involved if children, who are most sensitive to environmental factors and who form the most uni-

form group with respect to age and to the absence of occupational or bad habits, are examined.

In view of the foregoing, the aim of the present study was to develop microbiological criteria for assessing ecological risk on the basis of diagnosing bacterial carriership in populations from ecologically contrasting regions.

MATERIALS AND METHODS

Bacterial carriership was diagnosed in school-age children (8-12 years old) living in regions with different incidences of infectious diseases of the respiratory tract among children. The incidence of diseases was determined for 5 years, from 1987 to 1991, according to data of the Regional Department of Health and the Regional Statistical Department. Out of 40 districts of the Orenburg region we chose 2 districts where a relatively low morbidity had been recorded over 5 years (Severnyi and Belyaevskii Districts) and 2 districts, where a high incidence of respiratory diseases in children had been sustained for 5 years (Saraktashskii and Oktyabr'skii Districts) (Table 1). In addition, the studied areas also differed with respect to another ecological parameter: the geographical situation vis-a-vis the gas-treatment plant. In each district 200 school-age children were examined. Microflora of the nasal mucosa was studied in the subjects ex-

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TABLE 1. Ecological Parameters of Examined Districts ($M \pm m$)

District	Infectious diseases of respiratory tract per 1000 pop. in 1987-1991 (individual nosological forms)					Distance from gas-treatment plant
	nasopharyn- gitis	chronic dise- ases of tonsils and adenoids	bronchitis, emphysema	bronchial asthma	pneumonia	
Severnyi	1.4 \pm 0.3	17.1 \pm 1.4	1.0 \pm 0.16	1.2 \pm 0.08	2.3 \pm 0.43	more than 100 km
Belyaevskii	0.2 \pm 0.08	6.8 \pm 0.8	0.3 \pm 0.05	1.3 \pm 0.30	20.7 \pm 7.6	
Oktyabr'skii	1.9 \pm 1.06	20.6 \pm 1.6	3.9 \pm 1.4	3.6 \pm 0.87	29.3 \pm 4.8	less than 100 km
Saraktashskii	5.3 \pm 2.26	34.7 \pm 5.7	3.5 \pm 0.93	2.6 \pm 0.26	24.2 \pm 2.1	

aminated, and the *Staphylococcus* carriership was diagnosed with respect to immunosuppressive properties of isolated microorganisms. For this purpose epitheliocytes and mucous discharge were taken from the nasal mucosa with a cotton swab. The material was placed in medium 199 and transported to the laboratory. Culturing was performed on 1.5% meat-peptone agar, and pure cultures of the microorganisms were then isolated and identified. Antilysozyme activity (ALA) [2], anti-interferon (antidefensin) activity (AIA) in response to a preparation of human leukocyte interferon (PHLI) [3], and antihistone activity (AHA) [4] were determined in *Staphylococcus* cultures. The data obtained were analyzed across ecologically different areas.

RESULTS

As is seen from Table 2, the microbiological parameters in Severnyi and Belyaevskii Districts, which were the most favorable with respect to the ecological parameters (Table 1), differed from those in the districts where the ecological parameters were worse: Saraktashskii and Oktyabr'skii Districts. The percentage of *Staphylococcus* strains was markedly (1.9-7.9-fold) higher in the Severnyi and

Belyaevskii Districts, and CNS (coagulase-negative *Staphylococcus*) strains predominated among the *Staphylococcus* strains, whereas the percentage of CPS (coagulase-positive *Staphylococcus*) strains was higher (1.4-2.2-fold) in the Saraktashskii and Oktyabr'skii Districts, although the total number of *Staphylococcus* carriers was markedly lower in these districts. All CPS, as judged by their biochemical characteristics, corresponded to the most virulent *Staphylococcus* strain - *St. aureus*. Another taxonomic parameter, namely, the number of carriers of sporiferous flora (the bacterial species *Bacillus subtilis et megatherium* being mainly encountered), also differed among the studied districts. In the ecologically favorable districts the number of carriers of sporiferous flora was 3-8.3 times lower than in the districts with less favorable ecological parameters (Table 2).

Analysis of the second microbiological criterion (the incidence of immunosuppressive *Staphylococcus* carriership) demonstrated that AHA of bacteria was the most informative index. The number of carriers of *Staphylococcus* with AHA in the regions with a low incidence of diseases was 1.6-3.1 times lower than in the regions with a high incidence of diseases. As for the other studied parameters of micro-

TABLE 2. Microbiological Criteria for Assessment of Ecological Risk in Studied Districts

District	Microflora of nasal mucosa; taxonomic criterion of bacterial carriership, %					Diagnosis of <i>Staphylococcus</i> carriership according to studied properties		
	<i>Staphylococcus</i> strains			Other strains		ALA	AIA	AHA
	total	CPS	CNS	spori- ferous	other			
Severnyi	78.7	37.1	62.9	13.6	7.7	20.0	32.1	24.3
Belyaevskii	89.5	38.5	61.5	9.0	1.5	1.1	19.0	43.6
Oktyabr'skii	41.8	55.8	44.3	41.3	16.9	9.1	28.6	67.5
Saraktashskii	11.4	71.4	28.6	74.6	14.0	19.0	23.8	76.2

Note. The parameters of *Staphylococcus* carriership with respect to immunosuppressive properties of microorganisms were assessed as the ability of CNS to inactivate lysozyme in a concentration of 5 μ g/kg and higher, defensins in PHLI in an amount of 2 arb. units and more [3], and histones in a concentration of 5 μ g/ml and higher. In CPS the assessment was performed qualitatively (the presence or absence of activity).

organisms (ALA and AIA), no considerable differences were revealed during the diagnosis of bacterial carriership across the studied regions (Table 2).

AHA of bacteria proved to be the most informative criterion in microbiological diagnosis of ecological risk, which can evidently be attributed to the fact that histones are proteins typical of eukaryotes, and, thereby, AHA of bacteria is implicated only in the case of the prokaryote-eukaryote (i.e., parasite-host) system. Meanwhile, lysozyme is encountered not only in eukaryotes, but also in prokaryotes, and bacteria with ALA may inactivate lysozyme of microorganisms at the level of the prokaryote-prokaryote system, creating an unfavorable background for diagnosing bacterial carriership.

Our findings that carriers of CPS strains predominate in ecologically unfavorable regions are entirely explicable: when environmental conditions deteriorate and the level of immunoreactivity drops, the organism primarily becomes infected with more virulent strains. On the other hand, the predominance of carriers of CNS strains among children from ecologically favorable districts may be due to the prevalence of saprophites, normal inhabitants of the skin and mucosa, among these microorganisms. The presence of sporiferous saprophitic, Gram-positive bacilli in the nasal mucosa of children from ecologically unfavorable (Saraktashskii and Oktyabr'skii) districts (Table 2) is more difficult to explain. However, if we remember that facultatively pathogenic staphylococci exhibiting immunosuppressive properties may inactivate lysozyme and histones, i.e., the factors to which sporiferous flora is sensitive [4,6], the development of sporiferous bacilli on the mucosa after the above-mentioned fac-

tors had been depleted by staphylococci with ALA and AHA becomes clear in both the pathogenetic and ecological diagnostic context. It is quite possible that the predominance of sporiferous flora in the nasal mucosa is indicative of dysbiosis and a local immunodeficiency of *Staphylococcus* ethiology.

Thus, research into the microbiological indicators of ecological risk allowed us to pinpoint the following criteria:

1. Taxonomic criteria; the higher the ecological risk, the higher the percentage of carriers of CPS (more than 50% of the total number of carriers), the percentage of carriers of saprophytic sporiferous bacilli sharply increases (attaining 40% or more).

2. Tests for immunosuppression; the carriers of genetically active microorganisms with a high level of AHA predominate among the *Staphylococcus* carriers (more than 50% of the total number of carriers).

In view of the foregoing the development and use of this microbiological approach in the early diagnostics and prophylaxis of ecological risk seem promising.

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