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CIRCULATION OF CONDITIONAL PATHOGENS IN THE NORTH-WESTERN PART OF THE BLACK SEA

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The article contains the results about circulation and isolation frequency of the representatives of the following families *Pseudomonadaceae*, *Enterobacteriaceae* and *Vibrionaceae* in 0.5 and 1.5 km zones from coastal line of north-western part of the Black Sea in October 1992. The greatest isolation frequency of investigated microbiota was revealed in 0.5 km zone along the coast with maximum in Gulf of Odessa.

Key words: Black Sea, bacterial pollution, conditional pathogenic bacteria, pathogenic bacteria.

Bacterial pollution of seas, as well as of other objects of environment, is dangerous first of all from epidemic positions. As a result of sewage, industrial and ballast waters and river carrying-out, the area of coastal water is exposed to pollution with pathogenic and conditional pathogenic bacteria. This problem is urgent for Odessa beaches in particular and for the whole coastal area between the mouth of Danube and Dnieper in general [1]. In recreation zones of seas there are shigellae, pathogenic escherichiae, yersiniae, cholera vibrions and also conditional pathogenic bacteria of other genera, such as *Citrobacter*, *Proteus*, *Aeromonas*, *Hafnia*, and *Pseudomonas* [3]. Thus, in general the area of water that is limited by a zone from 5 to 50 m from the coast is considered. The information on situation in zones farther from the coast is presented only by titres of the coliform and quantity of heterotrophs in separate sites of the Black Sea [5].

The purpose of the present research was to estimate the expansion and level of water pollution with pathogenic and conditional pathogenic microbiota. In connection with this, the problem was to characterize the sanitary condition of distant waters in the north-western part of the Black Sea.

Materials and Methods

The samples of sea water were taken in the 6-th research expedition on investigating ship "Priboy" of the Ukrainian Research Centre of Marine Ecology (UKRCME) from 17 to 20.10.1992 on 39 stations of observation in the coastal zone in the region between the mouths of Danube and Dnieper. 21 stations were in 0.5 km from the coast and 18 stations were in 1.5 km from the coast. The location of stations is submitted on fig. 1. The samples were taken with the help of Nansen's bathometer from a horizon of 0.5 m. The area of sea water between Tiligulskiy and Beresanskiy bays in the following text is called "The Northern shelf".

The sanitary-bacteriological analysis of water samples included determination of quantity of saprophytic geterotrophic microbiota with the optimum temperature growth

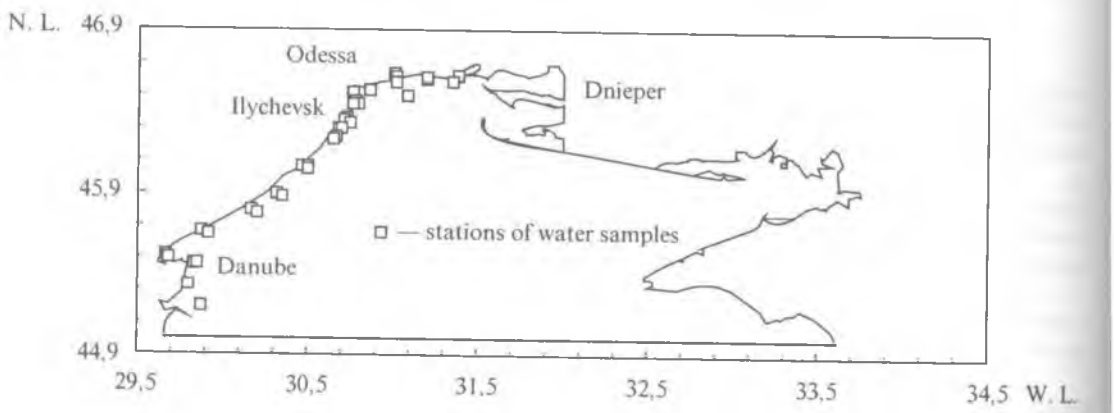

Fig. 1. Map of the studied region

37 °C, and determination of titres of the coliform and enterococcus, presence of pseudomonads, escherichiae, citrobacter, salmonellae, shigellae, klebsiellae, enterobacter, proteae, vibrions and aeromonas according to the standard technique [2, 4]. The estimation of sanitary condition of the sea water area was given according to the regulating documents worked out by Л.В. Григорьевой [3].

Results and Discussion

According to the sanitary-microbiological classification, pollution of marine area can be determined by the quantity of allochthonous bacteria with the optimum temperature 37 °C. The conducted research in 0.5 km zone has shown that only 25 % of the investigated water spaces opposite to Dnieper-Bug region could be reffered as a "clean" class (Table 1). This class corresponds to the number of microbiota less than 100 CFU/ml (colony formation unit).

The rest of the investigated water area was characterized as "polluted" or "very polluted"). The range of micobiota density was 90—10 000 CFU/ml. The same tendencies were found in distribution of saprofitic microbiota in 1.5 km zone. However, there was a decrease of the density range from 70 up to 2000 CFU/ml, that is due to dilution of contaminated waters. Nevertheless, both the previous data and these data are much higher than the data given in classification for the "clean" waters.

The basic parameter of fecal pollution is the presence of coliform. According to the data received by us, at all stations of the investigated sea water area, coliform titers were higher than 1, that corresponds to a category of "clean" waters.

Enterococcus density is also studied as a possible parameter of fecal pollution. They have greater stability to abiotic factors, and therefore their detection allows to speak about the chronization of the process of biological pollution of sea waters [2]. The application of this parameter for characteristics of the investigated regions has enabled to establish the following statement. In contrast to the characteristics of water area, that was received on the base of coliform titers, according to the density of enterococcus 57.1 % of water tests at distance of 0.5 km from the coast and 35.3 % at 1.5 km zone were characterized as "polluted" and "very polluted" (Table 2). Among all the investigated regions, the greatest quantity of stations relating to the class "very polluted" was

Table 1

Estimation of the water quality in the investigated regions according to the density of saprophytic bacteria with temperature (37 °C) optimum of growth

Investigated regions	Distance from the coast (km)	Mean CFU/ml	Number of the stations (percent) with water quality class		
			clean	polluted	very polluted
Yuzhniy port	0.5	600	0.0	100.0	0.0
	1.5	900	0.0	100.0	0.0
The Northern shelf	0.5	1000	0.0	50.0	50.0
	1.5	500	0.0	100.0	0.0
The Dnieper-Bug region	0.5	938	25.0	25.0	50.0
	1.5	900	0.0	100.0	0.0
The Gulf of Odessa	0.5	780	0.0	80.0	20.0
	1.5	1041	11.2	22.2	66.6
Ilyichevsk port	0.5	2700	0.0	0.0	100.0
	1.5	1100	0.0	0.0	100.0
The Danube-Dnister interfluve	0.5	3200	0.0	0.0	100.0
	1.5	1350	0.0	0.0	100.0
Opposite to the Danube mouth	0.5	4567	0.0	0.0	100.0
	1.5	800	0.0	66.6	33.4
All regions	0.5	1882	5.3	36.8	57.9
	1.5	967	5.2	42.2	52.6

in the Gulf of Odessa. So, for example, the highest data of enterococcus density (titer — less than 0,04) was found at the station opposite to the beach Arcadia.

Thus, there was a certain discrepancy revealed between the characteristics of the investigated water area according to the three above-stated tests. Absolutely opposite results were received during consideration of density of saprophytic microbiota with the optimum temperature growth 37 °C and coliform titers.

The characteristics of density of saprophytes and enterococcus were the most comparable, there was a positive concurrence of the water quality classes in coastal (61.9 %) and in distant zones (33.3%).

Realization of the direct indication of pathogenic and conditional pathogenic microbiota has allowed to estimate the real importance of the used above tests for sea waters. The spectrum of their allocation in the two investigated 0.5 and 1.5 km zones from the coast in the north-western part of the Black Sea is submitted in tables 3 and 4. Both pathogenic and conditional pathogenic microbiota, to which more and more attention is paid due to their potential danger for public health, were isolated in the 0.5 km zone at 66.7 % of stations and in the more distant zone at 27.8 % of stations. In the half-kilometer zone in all investigated regions, representatives of *Enterobacteriaceae* family were dominant. Thus the maximum variety (up to 4 genera) of this family representatives was observed in the water area of Odessa Gulf and Ilyichevsk port.

The bacteria of genus *Proteus* were isolated everywhere with high frequency. At the

Table 2

Determination of water quality at the investigated regions according to the enterococcus titers

Investigated regions	Distance from the coast (km)	Moda titer / %	Number of the stations (percent) with water quality class		
			clean	polluted	very polluted
Yuzhniy port	0.5	>10/100	100.0	0.0	0.0
	1.5	0.4/100	0.0	0.0	100.0
The Northern shelf	0.5	1.1; 0.4/50	0.0	50.0	50.0
	1.5	>10/100	100.0	0.0	0.0
The Dnieper-Bug region	0.5	>10/100	100.0	0.0	0.0
	1.5	0.4/100	50.0	0.0	50.0
The Gulf of Odessa	0.5	>10; >0.1/29	12.5	12.5	75.0
	1.5	0.4/44.4	33.3	16.7	50.0
Ilyichevsk port	0.5	>10; 0.1/50	50.0	0.0	50.0
	1.5	>10/100	100.0	0.0	0.0
The Danube—Dnister interfluve	0.5	>10/100	100.0	0.0	0.0
	1.5	>10/100	100.0	0.0	0.0
Opposite to the Danube mouth	0.5	>10;1.1;0.2/33	33.3	33.3	33.4
	1.5	>10/100	100.0	0.0	0.0
All regions	0.5	>10/52.6	42.9	14.2	42.9
	1.5	>10/52.6	64.7	5.9	29.4

second place according to the frequency of isolation, there were representatives of genus *Citrobacter*, at the third place there were microbiota of genera *Salmonella* and *Klebsiella*. The water of the stations, where combined isolation of representatives of genera *Salmonella*, *Klebsiella*, *Proteus* and *Citrobacter* was observed, such as the lighthouse of Bolshoy Fountain, in front of the settlment Chernomorka and in front of the Dry buy, was characterized by high density of saprophitic microbiota with the optimum temperature growth 37 °C and enterococcus. The exception was the station in front of cape Burnas, where at the similar situation high density of saprofitic bacteria and low density of enterococcus were registered. At the line of 1.5 km distance from the coast, there were isolated the representatives only of families *Citrobacter*, *Enterobacter* and *Proteus*. The greatest variety was marked in the water opposite to the Dnieper-Bug region. Isolation of bacteria of genus *Shigella* has not given a positive result.

During the study of microbiota from representatives of family *Vibrionaceae*, attention was paid not only to allochthonous species *Vibrio cholerae* and NAG (non-agglutinating) vibrions, but also to autochtonous halophilic vibrions and aeromonas. So, among them there are pathogenic species for hydrobionts, and species that cause gastroenteritis in a number of cases [3]. Autochtonous representatives of the family were isolated in five regions from the seven investigated ones. Halophilic vibrions dominated in four regions. Their frequency isolation decreased with the distance from the coast. Representatives of genus *Aeromonas* prevailed. They were isolated in regions opposite to the Danube estuarie, to the Dnieper-Bug bay and in the Gulf of Odessa. Neither *Vibrio cholerae* nor NAG vibrions were found in our investigations.

Table 3

The content of pathogenic and conditional pathogenic bacteria isolated from Danube-Dniester interfluve coastal zone (0.5 km from coast)

Investigated regions	Investigated families								
	Enterobacteriaceae						*Vibrionaceae*		*Pseudomonadaceae*
Yuzhniy port	—	—	—	—	—	*Proteus sp.*	*Vibrio sp.*	—	—
The Northern shelf	—	*Citrobacter sp.*	—	—	—	*Proteus sp.*	—	*Aeromonas sp.*	—
The Dnieper-Bug region	*E.coli (Lac)*	—	—	—	—	*Proteus sp.*	—	—	*P. aeruginosa*
The Gulf of Odessa	—	*Citrobacter sp.*	*Salmonella sp.*	—	*Enterobacter sp.*	*Proteus sp.*	*Vibrio sp.*	*Aeromonas sp.*	*P. aeruginosa*
Ilyichevsk port	*E.coli (Lac)*	*Citrobacter sp.*	—	*Klebsiella sp.*	—	*Proteus sp.*	—	—	—
Danube-Dniester interfluve	—	—	*Salmonella sp.*	*Klebsiella sp.*	—	*Proteus sp.*	*Vibrio sp.*	—	—
Oposite to the Danube mouth	—	—	—	—	*Enterobacter sp.*	*Proteus sp.*	*Vibrio sp.*	—	—

Table 4

The The content of pathogenic and conditional pathogenic bacteria isolated from Danube-Dniester interfluve coastal zone (1.5 km from coast)

Investigated regions	Investigated families								
	Enterobacteriaceae						*Vibrionaceae*		*Pseudomonadaceae*
Yuzhniy port	—	—	—	—	—	—	—	—	—
The Northern shelf	—	—	—	—	—	—	—	—	—
The Dnieper-Bug region	—	*Citrobacter sp.*	—	—	—	*Proteus sp.*	—	*Aeromonas sp.*	*P. aeruginosa*
The Gulf of Odessa	—	—	—	—	*Enterobacter sp.*	—	—	Aeromonas sp.	—
Ilyichevsk port	—	—	—	—	—	—	*Vibrio sp.*	—	—
Danube-Dniester interfluve	—	—	—	—	—	—	—	—	—
Oposite to the Danube mouth	—	—	—	—	—	*Proteus sp.*	—	*Aeromonas sp.*	—

Recently, to characterize sanitary condition, it is recommended to use *Pseudomonas aeruginosa* as the test. This representative relates to conditional pathogenic bacteria. It often is a causative agent of hospital infections, in a number of cases it causes enteric infection, and this bacteria can be isolated from soil [3]. In our studies *P. aeruginosa* was found in water area opposite to the Dnieper-Bug region both in 0.5 and in 1.5 km zones and in waters of the Gulf of Odessa at 0.5 km zone. No connection of isolation of these bacteria with other tests was shown.

The received results testify about the presence of spatial expansion of pathogenic and conditional pathogenic microbiota in the north-western part of the Black Sea. Its presence is registered even in 1.5 km from the coast. Zonality in their distribution is marked, they prevail in 0.5 km zone. In regions near the mouth that occurs due to the influence of rivers that bear terrigenic drains on large distances from the coastal line. And in water area of Odessa gulf and Ilyichevsk port it is connected with discharges.

The detection of pathogenic and conditional pathogenic microbiota at significant distance from the coast testifies to their possible adaptation to sea environment. It is known from the reference [6], that starved allochthonous bacteria, while remaining metabolically active may lose the ability to grow on the media on which they usually are cultured. Also, a genetic evolution of related pathogenic bacteria is possible in sea environment at the expense of plasmids, carrying traits of stability to the factors of external influence [1]. During eutrophy of water area, the survival of allochthonous bacteria and the risk of contamination of people can be amplified [6]. During usage of water, especially when it can get into organism, it is necessary to take into account constant water exchange between coastal regions and minimal infecting doze of a separate causative agent.

The analysis of efficiency of the number of tests, such as determination of saprophitic microbiota with a temperature optimum 37 °C, coliform and enterococcus in sea environment, has shown non-expediency of coliform titres that is proved in references [2, 3]. Periodic direct indication of pathogenic and conditional pathogenic bacteria is necessary when there is high density of saprophitic microorganisms with temperature optimum 37 °C and enterococcus.

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РОЗПОВСЮДЖЕННЯ УМОВНО-ПАТОГЕННОЇ МІКРОБІОТИ У ПІВНІЧНО-ЗАХІДНІЙ ЧАСТИНІ ЧОРНОГО МОРЯ

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Резюме

Представлено дані про розповсюдження і частоту вилучення представників родин Pseudomonadaceae, Enterobacteriaceae і Vibrionaceae у зонах 0,5 та 1,5 км від берегової лінії північно-західної частини Чорного моря в жовтні 1992 р.. Найбільша частота вилученої досліджуваної мікробіоти виявлена у зоні, віддаленій від берега на 0,5 км, з максимумом в Одеській затоці.

Ключові слова: Чорне море, мікробне забруднення, умовно-патогенні бактерії, патогенні бактерії.

РАСПРОСТРАНЕНИЕ УСЛОВНО-ПАТОГЕННОЙ МИКРОБИОТЫ В СЕВЕРО-ЗАПАДНОЙ ЧАСТИ ЧЕРНОГО МОРЯ

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Резюме

Представлены результаты о распространение и частоте выделения представителей семейств Pseudomonadaceae, Enterobacteriaceae и Vibrionaceae в зонах 0,5 и 1,5 км от береговой линии северо-западной части Черного моря в октябре 1992 г. Наибольшая частота выделенной исследуемой микробиоты выявлена в зоне, удаленной от берега на 0,5 км, с максимумом в Одесском заливе.

Ключевые слова: Черное море, микробное загрязнение, условно-патогенные бактерии, патогенные бактерии.