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Comparative characteristics of methods for determining of the microbial Paysage at Phlegmons of the maxillofacial area in children

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Abstract
Comprehensive analysis of the discharge from the purulent wounds was carried out by bacteriological, PCR and GLC of methods. It was found that the greatest numbers of strains were presented obligate non-spore-forming anaerobic bacteria (34.6%), which prevailed over the facultative-anaerobic, aerobic forms and fundus (22.7%) for bacteriological examination. In normergic type of inflammatory reaction microbial associations were often presented by 4-6 species of microorganisms (61.3%), in hypergic type till 6-8 species (90.3%) and in hyperergic type with 3-4 species.
PCR study was found higher percentages of microbial associations (Prevotella intermedia 79, 2%, Porph. Gingivalis 87, 5%) in comparison with bacteriological study (2.5% and 0.8%, respectively). The high correlation observed between microbiological and GLC and makes the detection all occurring markers of microorganisms - more than 0.9.

Keywords: phlegmons of the maxillofacial area, microbial associations, PCR, GLC

1. Introduction
Patients with acute nonspecific purulent-inflammatory diseases of maxillofacial area ranged from 40 to 60% of the total number of patients referred for surgical dental aid [2, 6]. This is due to the fact that the incidence of low-intensity, hyporeactive forms of the inflammatory response, against a background which often develop local and general complications, such as the spread of process to the surrounding tissues, sepsis, cavernous sinus thrombosis, and so on has been steadily increasing in recent years [2, 11, 12]. The problem of perfecting the methods and means of prophylaxes, diagnosis for treatment of such patients remains one of the most actual problems of modern dentistry. Applicable as of today in clinical diagnostic methods of infection have certain limitations and defects. A major defect of the classical bacteriological study, in addition to the high price and duration (7-10 days), is the inability to evaluate the role of uncultivated microorganisms in infectious and inflammatory process, first of all - anaerobes [5, 6, 8]. In connection with this perspective is to determine the part of specific microbial agents and their associations in the etiology of inflammatory diseases of the maxillofacial area depending on the form and type of inflammatory reaction, and depending on the volume and nature of the lesion [6, 8, 12].
Timely determination of the type of microflora is important when choosing a tactic of treatment. Until recently, the traditional methods of bacteriological diagnosis of anaerobic and mixed infections, including nosocomial, presented informative insufficiently. Of certain interest in this plan is the study of the spectrum of pathogens discharging from the purulent wound by the polymerase chain reaction (PCR) which allows to identify the unit molecules of specific microbial DNA in a few hours [4, 6, 14].
Today, there is more informative alternative method for determining the microbial associations by gas-liquid chromatography (GLC). The method is based on high-precision determination of specific marker molecules that make up cellular lipids of microorganisms.
The method is the identification of microbial associations on specific fatty acids [1, 3, 7, 8, 10, 13]. Currently, it was worked out the technique of assessing the state of infection of purulent wounds on the markers specific to a certain type of microorganism, which allows to carry out accelerated indication (less than two hours) of microorganisms [1, 3, 7, 8, 10, 13]. In spite of this improvement and development of new methods of separation and identification of markers of microbial associations and assessment of their informational content in chronic inflammatory diseases of MFA is actual.

2. The Aim
The aim of the research was the microbiological monitoring of purulent wounds in phlegmons of the maxillofacial area in children with various diagnostic methods.

3. Materials and Methods
Research were involved 138 sick children (48 girls, 90 boys) with phlegmons of the maxillofacial area of different locations who treated in the department of maxillofacial surgery of regional multi-disciplinary children's medical center of Samarkand city.
As a material used discharge from the wound after opening phlegmons of MFA. Compilation of material was carried out under aseptic conditions. The complex analysis was carried out by bacteriological, PCR and GLC of methods.
Microbiological testing was performed according to standard procedures [1]. Determination of the DNA of potential

pathogens was performed by polymerase chain reaction (PCR).

Markers of microorganisms - fatty acids in the form of their methyl esters were determined by gas liquid chromatography. Motionless phase is 15% lestosil on chromaton NA-W with particle size of 0,150-0,250 mm, glass column with size of 0,04h1,00m; carrier gas flow - nitrogen - 32 ml / min; detector - flame ionization, the ratio of nitrogen: hydrogen: air = 1: 1: 10, volume of introduced test- 3-2 mkl, hexane extract of methyl esters of fatty acids.

Identification of fatty acids - markers in the microorganism was performed by "witnesses" and based on the method of structural-group components [3, 7, 10], but a quantitative analysis with a method of absolute calibration [8, 9]. Statistical data of findings was conducted with use of package of applied programs Statistica 6.0.

4. Results

In bacteriological study of purulent wounds in children with phlegmons of MFA was sown following microflora: - Bacteroides in 13.2% peptostreptococci - in 9.58%, fusiformis - at 5.01%, staphylococci - 8.27% of obligate anaerobic bacteria - 34.64%, microaerophilic streptococci - 6.53%, propionibacteria - 2.83%, enterobacteria - 1,74%, Neisseriaspp - 0,43%, facultative anaerobic and aerobic bacteria - 17.64% (see Table. 1).

Table 1: Microbiocenosis of purulent exudate at phlegmons of MFA during bacteriological examination

Species and type of agents	Number of strains	%
Bacteroides (total)	61 (14; 45; 2)	13,2 (3,02; 9,73; 0,43)
Prevotella melanino genica	22 (8; 12; 2)	4,76 (1,73; 2,6; 0,43)
P. capillosus	18 (3; 15; 0)	3.89 (0,64; 3,24; 0)
P. oralis	13 (2; 11; 0)	2,81 (0,43; 2,38; 0)
B. fragilys	3 (0; 3; 0)	0,64 (0; 0,64; 0)
B. imiformis	5 (1; 4; 0)	1,08 (022; 0,86; 0)
Peptostreptococci (total)	44(13;26;5)	9,58(2,83;5,66;1,09)
P.anaerobius	21(7;10;4)	4,58(1,53;2,18;0,89)
S.intermedius	16(4;11;1)	3,48(0,87;2,39;0,22)
P.micros	7(2;5;0)	1,52(0,43;1,09;0)
Fusiformis (total)	23 (12; 9; 2)	5,01 (2,61; 1,97;0,43)
F. nucleatum	13 (4; 8; 1)	2,83 (0,88; 1,74; 0,21)
F. necroforum	10 (8; 1; 1)	2,18 (1,74; 0,22; 0,22)
Staphylococci (total)	38(10;26;2)	8,27(2,18;5,66;0,43)
Staphylococcus aureus	17(4;12;1)	3,7(0,87;2,61,8;0,22)
S.epidermidis	21(6;14;1)	4,57(1,3;3,04;0,22)
Obligate anaerobic bacteria (total)	159(41;112;6)	34,64(8,93;24,4;1,3)
Microaerophilic streptococci (total)	30(14;16;0)	6,53(3,05;3,48;0)
Streptococcus,sangius	7(4;3;0)	1,52(0,87;0,65;0)
S.milled	15(5;10;0)	3,27(1,09;2,18;0)
S.mitis	8(5;3;0)	1,74(1,09;0,65;0)
propionibacteria	13(3;10;0)	2,83(0,65;2,18;0)
Enterobacteriaceae (total)	8 (2;6;0)	1, 74(0,44;1,3;0)
Escherichiacoli	4(1;3;0)	0, 87(0,22;0,65;0)
Enterobacterspp.	3(1;2;0	0,65(0,22;0,43;0)
Proteusspp.	1(0;1;0)	0,22 (0;0,22;0)
Neisseriaspp. (total)	2(0;2;0)	0, 43(0;0,43;0)
Facultative anaerobic and aerobic bacteria (total)	81(28;47;6)	17,64 (6,09;10,24;1,4)
Total strains	459	100 %

Note: The figures written in each bracket denote the value of this indicator at normergic hypergic or hyperergic types of inflammatory reactions

The greatest number of strains was represented by obligate non-spore-forming anaerobic microrganisms (34.64%), which together with close to them in their properties microaerophilic staphylococci (6.53%) were the absolute predominance on facultative-anaerobic, aerobic forms and fungus (22.65%). Obligate non-spore -forming anaerobes were isolated from all bacteriological examined patients (100%). They were both in the associations between them (27 inoculations- 22.3%), but more often in association with facultative-anaerobic and aerobic forms (81 inoculations - 66.9%). Quantity isolated species of microorganisms was differentiated in patients with different types of course of the inflammatory reaction. Thus, in normergic type of inflammatory reaction of microbial associations were often represented by 4-6 speceis of microorganisms (61.3%). Among the obligate non-spore-forming anaerobes dominated peptostreptococci and Bacteroides. It was often recovered fusiformis (F. necroforum and F. nucleatum), microaerophilic streptococci (S. milleri, S. mitis). Less distinguished staphylococci and propionebacteria. In general, if normergic type of inflammatory reaction produced less amount of microbial species (3-4 species), they were mostly presented with associations of obligate non-spore -forming anaerobic bacteria that is the most pathogenic forms.

In hypergic type of inflammatory reaction the number of selected microbial species in the associations increased compared to normergic till 6-8 species (90.3%). In a few cases was found out 3-5 species (9.7%). Among these bacteria dominated Bacteroides and peptostreptococci. It was often discharged B. capillosus, B. melaninogenicus, P. lanceolatus, S. intermedius. More often than the normergic type of inflammation was discharged anaerobic

actinomycetes in pathogenic forms. The increase of bacteria in the associations occured mainly due to the frequent isolation of aerobic and facultative-anaerobic species, including Enterobacter spp., Eschrichia coli, Proteus spp., Pseudomonas aeruginosae, Enterococcus faecalis and others.

In hyperergic type of inflammatory reaction the microbial paysage in the three cases was represented by three and in two cases by four species. Half of the strains were obligate non-spore-forming anaerobic bacteria, which are dominated by peptostreptococci and fusiformis, and the other half - staphylococci, mainly S. aureus and S. epidermidis, in associations which have a considerable degree of pathogenicity.

It was studied the spectrum of pathogens discharge from the purulent wound by the polymerase chain reaction (PCR), the results of which are presented in table 2.

Table 2: Microbiocenosis of purulent exudate at phlegmons of MFA in the diagnostic of PCR

Spectrum of agents	Bac. testing (n=121)		PCR (n=24)		Consilience of results	
	Abs.unit	%	Abs.unit	%	Abs.unit	%
Prevotella intermedia	3	2,5	19	79,2	2	8,3
Porph. gingivalis	1	0,8	21	87,5	1	4,2
B.forsythus	0	0	10	41,7	0	0
Treponema denticola	0	0	5	20,8	0	0

During the PCR diagnostics investigated the discharge from purulent wound in 24 patients with odontogenic phlegmons characterizing hypergic type of inflammatory reaction. Performing PCR diagnosis in patients with hypergic inflammation is most important, as during the work with them in the clinic there is the greatest number of difficulties associated with the selection of antibacterial preparations, as well as the increased the risk of severe complications, which is partly due to the inefficiency of antibacterial treatment. For PCR and reverse hybridization used DNA probes for genetic markers of the most virulent anaerobic bacteria: Prevotella intermedia, Porphyromonas gingivalis, B. forsythus, Actinobacillus actinomycetem commitans, T.denticola.

Thus, Prevotella intermedia by the PCR method followed by reverse hybridization was observed in 14 patients from 19 patients (73.7%), while, as a bacteriological method conducted in 121 patients, allowed to reveal given microorganism only in 3men (0.9%). This agreement between the results of bacteriological and PCR diagnostics observed only in one case (5.3%). In addition, it should be noted that bacteriological accurately identify Prevotella intermedia is not always possible, as Prevotella intermedia and Prevotella melaninogenica is difficult to differentiate in bacteriological analysis. This fact further increases the informative value of the method of PCR diagnostics. Porphyromonas gingivalis was determined in 84.2% of the studies, especially in patients with accompanying periodontitis, in use of PCR with inverse hybridization. Given microorganism was isolated in one patient (0.3%) by bacteriological study. Bacteriological Porphyromonas gingivalis is difficult to identify with Porphyromonas asaccharolyticus. The percentage of agreement of the results of the two methods of diagnosis was also not great. In addition, using PCR was able to reveal the markers of B.forsythus (36, 8%), Actinobacillus actinomyce temcommitans (15, 7%), T. denticola (21, 1%), which in bacteriological study in the inflammatory foci of patients with phlegmons weren't determined. In addition, it should be noted that B. forsythus T. denticola and cultural study did not previously reveal but Actinobacillus actinomycetemcommitans only in a few cases were determined directly in the foci of chronic periodontitis. Usually one patient was revealed the markers of one or two species of periodontal bacteria; predominantly Prevotella intermedia and Porph. gingivalis, at least three species. Two patients were revealed four species of markers, and in one - all five species. Despite such low resistance of these microbes cannot be disregarded their etiopathogenic role during antibacterial treatment.

Abovementioned facts, it can be stated that many microorganisms can be determined by cultural methods, but this method occupies 48-72 hours and consists of the steps of cultivation on the medium enrichment, identification in character of fermentation of certain substrates - all this is the defect of this method. PCR method in our research determined the causative agents which cultivate difficulty with bacteriological methods. The reliability of the research reaches 100%. PCR diagnostics discloses the presence of agents of infectious diseases, even in cases where other methods (immunological, bacteriological and microscopic) cannot do this, even it allows to disclose unit cells of bacteria or viruses.

The presence of microflora is also determined on improved method by the method of GLC. Given qualitative and quantitative results are presented in table 3.

Table 3: The results of determining the markers of microorganisms from the contents of purulent wounds with method of GLC

S. No.	Type of microorgamisms	Marker	content*
1	Lactobacillus,Streptococcus, Clostridium	Myristic(14:0)	$Y=4,73*10^{5}*x$
2	Staphylococcus, Bacillus	Anteizohodecanoic acid (a19)	$Y=1,38*10^{5}*x$
		Anteizo tridecanoic	$Y=1,52*10^{5}*x$
3	Pseudomonas stutzeri	Pentadecanoic(15:0)	$Y=2,61*10^{5}*x$
4	Corynebacterium, Bacteroides, Nocardiopsis, Nocardia	Anteizo heptadecanoic (a17:0)	$Y=2,51*10^{5}*x$
5	Actinomyces	Eicosenic(20:0)	$Y=4,21*10^{5}*x$
6	Propionibacterium jensenii, Streptococcus thermophilus, St. salivarius, St. mutans, Actinomyces	Eicosenic (20:1)	$Y=1,58*10^{5}*x$
7	Francisella	Behenic(21:0)	$Y=0,17*10^{5}*x$

8	Mycobacterium	Heptadecenic acid($C_{17:1}$)	$Y=0,37*10^{5}*x$
9	микроэукариоты	Tetracozanoic (24:0)	$Y=0,58*10^{5}*x$
10	Enbacteriwn, Clostridium	Octadecenoic(18:1a)	$Y=0,44*10^{5}*x$
11	Fundus type of Candida	Heptadecenic acid ($C_{17:1}$)	$Y=0,37*10^{5}*x$
12	Enterococcus faecalis	Cyclononyl decanoic acid (19cyc)	$Y=8,23*10^{5}*x$

Note: * Y - The content of the marker in a microorganism;
x - Height of the peak in the chromatogram mm.

As can be seen from Table 3 that for each microorganism is characteristically its marker in the form of a specific fatty acids which content is determined by analytical dependence shown in table.

After studying various methods of diagnosis of microflora at phlegmons of MFA in children we found the relationship between the procedures determining the microorganisms microbiologically and GLC PCR the results of which are shown in table 4.

Table 4: The value of the correlation coefficient of microorganisms by different methods

S. No.	Type of microorganisms	Correlation coefficient		
		M+PCR	M+GLC	PCR+ GLC
1	Lactobacillus	0,726	0,932	0,761
2	E. coli	0,885	0,957	0,783
3	Staphylococcus	0,789	0,965	0,771
4	Streptococcus	0,780	0,942	0,737
5	Fundus of type Candida	0,714	0,913	0,718

Table 4 shows that the correlation coefficient containing of microorganisms between the microbiological and PCR; microbiological and GLC; PCR and GLC in all cases is high, particularly high correlation observed between microbiological and GLC and makes the detection of all occurring markers of microorganisms - more than 0.9.

5. Conclusions

1. It was found that the microbiological, PCR and GLC methods for determining the type of microorganisms at phlegmons of MFA in children give identical results with misdiagnosing till 5-10%.
2. The method of GLC for determination of microorganisms based on recover of their markers in wound exudation in children with phlegmons of MFA is informative, for rapidity predominates microbiologically, and in terms of efficiency - PCR diagnostics.

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