

9. ANTIBIOTIC RESISTANCE OF MICROORGANISMS: CURRENT ISSUES OF DIAGNOSIS AND WAYS TO OVERCOME

9.1

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THE EFFECT OF SUB-BACTERICIDAL DOSES OF ANTISEPTICS ON DNA AND PHENOTYPIC MARKERS OF VIRULENCE OF MICROORGANISMS

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Local antiseptics in sub-bactericidal concentrations are active against antibiotic-resistant microorganisms, including Gram-negative bacteria. The studies were performed *in vitro* and *in vivo*, on the culture of fibroblast cells of human embryonic skin. Our study shows anti-adhesive activity of QATs against *S. aureus*, their ability to suppress hyaluronidase and staphylococcal plasminogen activator. Poviargol (silver nanoclusters) is able to suppress protein A of staphylococcus, prevents the formation of microbial biofilms on biotic and abiotic surfaces. Polyhexanide shows antiadhesive properties against Gram-positive and Gram-negative bacteria, enhances the effect of antibiotics against resistant microbes due to increased permeability of the cell wall, affects plasminogen activator, collagenase. The effect of sodium hypochlorite on microbial DNA was assessed by UV spectroscopy and electrophoresis. For the first time, a dose-dependent effect of sodium hypochlorite on individual nucleotides and polynucleotides was obtained, and complete destruction of the plasmid DNA of *Escherichia coli* DH5-Alpha strain was demonstrated. It has been established that the interaction with sodium hypochlorite involves the destruction of the secondary structure of DNA (denaturation) and the chemical modification of nitrogenous bases, presumably chlorination. The presence of a secondary structure slows down the chemical reaction of sodium hypochlorite with nitrogenous DNA bases. The ability of sodium hypochlorite to destroy formed (48 h) microbial biofilms of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* has been studied. Various antiseptics in non-bactericidal concentrations complexly affect the antibiotic-resistant microbial cell: increase the permeability of the cell membrane, inhibit the enzyme-inactivators of antibiotics, suppress the epidemic factors of the transfer of antibiotic resistance markers by transduction and conjugation.

9.2

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METABOLIC ACTIVITY OF PLANKTON IN COMPARISON WITH BIOFILM PHENOTYPE SOME MICROORGANISMS OF HUMAN MICROBIOTA

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An important interest for the detection of serious metabolic disorders of macroorganism, and its microbiota, are tricarboxylic acids common to bacteria and mitochondria

of eukaryotic cells, and phenylcarboxylic acids. They are called “sepsis-associated metabolites”, since the imbalance of the profile of phenylcarboxylic acids in the blood is most noticeable in septic states.

The purpose of our study is to carry out a comparative assessment of production sepsis-associated exometabolites by clinically-significant bacteria in biofilms and planktonic form.

Strains were used: *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and clinical isolates of these species, isolated from the blood of ICU patients. Biofilms of these microorganisms were grown according to the method developed by us. The determination of exometabolites was carried out using gas chromatography-mass spectrometry (GC-MS).

S. aureus and *S. epidermidis*. Lactate was produced by the planktonic form in a substantially greater concentration than by biofilms. As for the biodegradation products of aromatic amino acids (phenyllactic and p-hydroxyphenyllactic acid), a more intense production by the *S. aureus* biofilm was demonstrated as compared to plankton.

K. pneumoniae and *E. coli*. The metabolism of the investigated *Klebsiella* strains to a large extent coincides with the metabolism of *E. coli*. The *K. pneumoniae* biofilm produced phenyllactic and para-hydroxyphenyllactic acids significantly more actively than the planktonic form.

Conclusions: 1. processes associated with the hydrolysis of carbohydrates in biofilms are less intense than in the planktonic form of the same microorganisms. This is indicated by a significant difference in the concentrations of lactate between plankton and biofilm; 2. the processes associated with hydrolysis of proteins take place in biofilms much more intensively.

9.3

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STUDY OF ANTIMICROBIAL RESISTANCE IN MEDICAL INSTITUTIONS IN CONAKRY (REPUBLIC OF GUINEA)

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Extensive apparition of resistance of pathogenic microbes to many antibiotics causes a serious preoccupation of public health agencies in developing countries. WHO considers the surveillance on this resistance development as one of the important task of public health system of every country.

The aim was to study of problem of antibiotics resistance in Republic of Guinea.

We studied 875 bacterial strains having medical importance, from some medical institutions of Conakry city. The identification was accomplished on commercial mediums or on nutritive mediums prepared in IRBAG. For each strain one made an antibiogram with agar-agar precipitation method on Muller–Hinton medium. The antibiotic discs of more than 20 antibiotics were test-

ed. The interpretation of results we accomplished according to criteria of Committee on antibiograms of French Microbiological Society.

There were identified the following pathogen bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* sp., *g. Shigella* sp., *Staphylococcus aureus*, *Streptococcus* sp., *Streptococcus β -hemolyticus*. It was established that resistance to different antibiotics isn't the same for different bacteria. Occurring everywhere resistance to antibiotics in medical institutions of Conakry is conditioned by following causes: anarchy use of these drugs by population and by unreasonable physician recommendations. One uses antibiotics often without physician prescriptions, its sale is not controlled, the storage conditions are not kept, one use expired medicines. It increases the bacteria resistance. As an example the sharp down of susceptibility to tetracycline is conditioned by its wide distribution on the Guinean medicine market on obtainable prices. One uses this drug for self treatment.

Our study showed the wide development of resistance of pathogenic bacteria to antibiotics in Guinea by reason of its easy accessibility on medicine market. It's necessary the monitoring of antibiotics use with permanent control of the susceptibility of pathogenic bacteria to them. One must control the quality of antibiotics incoming on the market. These drugs should be used only by physician prescription or of other medical personnel having rights to do it. It is necessary the wide national, regional and international collaboration for this problem resolution.

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MICROBIOLOGICAL ASPECTS OF PURULENT SEPTIC INFECTIONS BREAKOUT INVESTIGATION IN Khabarovsk CITY

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The aim of the study — to conduct an etiological interpretation of purulent-septic infection cases registered in August-September of 2017 among newborns in the maternity hospital of Khabarovsk city. A total of 8 cases of different diseases were registered (nosocomial pneumonia, urinary tract infection, omphalitis, subcutaneous whitlow, conjunctivitis).

A microbiological examination of newborns and medical staff of the maternity hospital followed by phenotypic and molecular-biologic evaluation of the isolated strains was performed. Cultures of *Staphylococcus aureus* and *Klebsiella pneumoniae* were isolated from different sites of infection from children and personnel. Vitek-2 Compact and MALDI-TOF Biotyper analyzers were used to identify the bacterial strains. The antibiotic sensitivity of the isolated strains was evaluated by disc diffusion method to 22 drugs.

S. aureus isolated from 3 children (oral pharynx, whitlow) and 13 employees did not belong to the multiresistant MRSA strains. Two groups of *K. pneumoniae* isolated from 3 newborns (oral pharynx, sputum, omphalitis) and from 7 staff members (urine, oral pharynx) differed from each other by antibiotic sensitivity. The strains isolated from newborns were producers of β -lactamase (typical feature for nosocomial strains) and were susceptible to 4 antibacterial drugs out of 22 evaluated. All strains isolated from

the personnel were susceptible to the most of the antibiotic drugs. The RAPD-PCR confirmed the difference between two groups of *K. pneumoniae* strains. All strains isolated from the newborns were typed as genovariant A when those isolated from personnel belonged to different types (B, C, D, E).

The phenotypical and molecular-biological survey of *K. pneumoniae* strains isolated during the period of purulent-septic infections breakout in the maternity hospital of the Khabarovsk city indicates on the difference between two identified bacterial strains. It highlights the fact that during the breakout there were two independent epidemic foci of *K. pneumoniae* infection among newborns and medical staff.

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SEROVAR SPECIFIC ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA

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Antimicrobial susceptibility of 564 *Salmonella* strains isolated in 2014–2017 in St. Petersburg from the patients with gastroenteritis was studied. Three leading serovars — *S. Enteritidis*, *S. Typhimurium* and *S. Infantis* accounted for 89.4% of strains (78.9; 5.9 and 4.6%, respectively). Other serovars were represented by single strains. 79.4% of *Salmonella* were resistant to at least 1 antimicrobial group: *S. Enteritidis* — 86.3; *S. Typhimurium* — 63.6; *S. Infantis* — 88.5%.

S. Enteritidis is characterized by high level of resistance to fluoroquinolones (75.0%) and nitrofurans (70.0%). Beta-lactam resistance was noted to ampicillin (2.7%) and extended spectrum cephalosporins (1.8%). Resistance to other antimicrobial groups ranged from 0.4 (aminoglycosides) to 8.8% (tetracyclines). Multidrug resistance (3 and more groups) was unusual for this serovar and was found in 10.8%.

In *S. Typhimurium* resistance to many antimicrobial groups was noted: tetracycline (45.5%), ampicillin (39.4), aminoglycosides (21.2), trimethoprim/sulfamethoxazole (21.2), fluoroquinolones (18.2). This serovar is characterized by the highest proportion of beta-lactam resistance: ampicillin (39.4) and extended spectrum cephalosporins (9.1). Multidrug resistance was found in every fourth strain of this serovar.

S. Infantis were characterized by very high proportion of the resistance to fluoroquinolones, nitrofurans, tetracyclines and trimethoprim/sulfamethoxazole (88.5; 80.8; 80.8 and 61.5%, respectively). This serovar was characterized by multidrug resistance: almost 8 out of 10 strains. At the same time, resistance to extended spectrum cephalosporins and chloramphenicol has not been noted.

Rarely isolated serovars were characterized by lower proportion of antimicrobial resistance (31.7%): resistance did not exceed 20.0% (quinolones) and ranged from 1.7 (extended spectrum cephalosporins and aminoglycosides) to 15.0 (nitrofurans). Multidrug resistance was observed in 10.0%.

So, in *Salmonella* isolated in St. Petersburg in 2014–2017, the resistance to drugs of choice for the treatment of light and medium-severe salmonellosis (nitrofurans), as well as complicated and severe forms of salmonellosis (fluoroquinolones) is very high. This is typical for the leading serovar *S. Enteritidis*, causing up to 80% of cases of salmonellosis. The use of these antimicrobials should be accompanied by a mandatory antimicrobial susceptibility testing for timely correction of the treatment.