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CHARACTERISTICS OF BIOLOGICAL PROPERTIES OF ETIOLOGICAL AGENTS OF ACUTE BACTERIAL CONJUNCTIVITIS

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ABSTRACT
During the study, algorithms for microbiological and mycological analysis of bacterial conjunctivitis in adults were developed based on the obtained microbiological test results. Their advantage lies in the ability to plan microbiological and mycological tests, implement the process step by step, and provide timely and reliable reporting of test results. The proposed algorithms for microbiological and mycological analysis are recommended for the first time for microbiological confirmation of the diagnosis of bacterial conjunctivitis and are convenient for laboratory testing.

The conjunctiva, or the mucous membrane of the eye, is a thin mucous layer that connects the eyeball and the eyelids. It lines the posterior surface of the eyelids and then reflects onto the eyeball. The main physiological function of the conjunctiva is to protect the eye [1, 4, 5]. When a foreign particle enters the eye, tear secretion and blinking increase, resulting in the removal of the foreign body from the conjunctival sac [8, 13]. In addition, the mucus (secretion) of the conjunctival sac continuously moistens the surface of the eyeball, reduces friction during eye movements, and helps maintain the transparency of the hydrated cornea [16].

It has been established that the mucus of the conjunctival sac is rich in non-specific protective factors of the body, including β-lysin, lysozyme, immunoglobulins, particularly secretory immunoglobulin A (sIgA), and other similar defense factors [11, 12].

According to data reported in recent scientific literature, even under normal conditions in healthy individuals, the conjunctiva consistently harbors small quantities of saprophytic microorganisms such as *Staphylococcus epidermidis, Corynebacterium xerosis, Corynebacterium pseudodiphtheriticum, Neisseria* spp., and *Sarcina* spp., which are generally incapable of causing infectious-inflammatory processes in humans [14, 15].

Aim of the study. The aim of the study was to analyze the biological characteristics of the causative agents of acute bacterial conjunctivitis in children and adults and to assess their variability.

Тадқиқот материали ва усуллари. The study included children aged 12–18 years and adults aged 19–60 years with a clinically confirmed diagnosis of acute conjunctivitis (n=62). For comparative analysis, bacteriological examination of ocular discharge samples was also performed in apparently healthy individuals (n=21) who were representative of the patient group in terms of age, sex, place of residence, and lifestyle. Patients with occupational eye diseases associated with adverse effects on the eyes, as well as those with ocular infections caused by specific pathogens such as gonococcal or tuberculous agents and others, were excluded from the examined patient group (the main group).

The healthy group (comparison group) included individuals without ocular infections and with no history of complaints characteristic of such diseases during the previous two years.

Among the pediatric patients, girls accounted for 52.6±11.5% (n=10) and boys for 47.4±11.5% (n=9). Among the adult patients, women constituted 46.5±7.6% (n=20) and men 53.5±7.6% (n=23). The bacteriological study was conducted at the teaching and research bacteriological laboratory of the Department of Microbiology, Virology, and Immunology of Bukhara State Medical Institute.

Conjunctival discharge (biological material) was collected during the active stage of the purulent-inflammatory process in compliance with aseptic principles. For bacteriological examination, all medications and medical procedures were discontinued 5–6 hours before sample collection. The biological material was obtained in accordance with technical and biosafety requirements and was then sent to the bacteriological laboratory for further analysis [7].

According to the researchers, the possibilities for identification are broadened by conducting the study in a stepwise manner: first, preliminary data are obtained using a limited number of characteristics, and then, based on a more extensive assessment of biological properties, the pathogen is identified up to the genus level and, in some cases, up to the species level. Such an approach to investigation is useful; however, the prolonged duration of the study should also be taken into account.

Bacteriological examination was performed for cultured pathogens at a concentration of >10^5 CFU/mL and inoculated according to Gold. Identification and differentiation of microorganisms were carried out in accordance with ***Bergey's Manual of Systematic Bacteriology*** [10]. For the identification of *Escherichia* spp., *Citrobacter* spp., *Enterobacter* spp., and *Staphylococcus* spp., the recommendations of Sasova V.A. and Zalesskikh N.V. [9], as well as Iskhakova Kh.I. et al. [2], were used; for the identification of *Enterococcus* spp., the recommendations of Iskhakova Kh.I. et al. [3] were followed.

For identification of members of the ***Enterobacteriaceae*** family (*Escherichia* spp., *Citrobacter* spp., *Enterobacter* spp.) up to the genus level, not only their morphological, tinctorial, and cultural characteristics (on Endo medium) were used, but also their biochemical properties. Nine tests ensuring identification of these microorganisms to the genus level were employed: presence of lysine decarboxylase, presence of urease, hydrogen sulfide production, indole production, glucose fermentation, lactose fermentation, sodium citrate utilization, sodium malonate utilization, and bacterial motility.

Identification of *Staphylococcus* spp. to the genus level was carried out on the basis of their morphological, cultural, and biochemical characteristics.

Enterococcus spp. were identified on the basis of the following differential characteristics: Gram-positive staining, chain-like arrangement within the microscopic field, confluent S-type black colonies on bile esculin agar, fermentation of lactose, glucose, and mannitol to acid without gas production, and growth in a medium containing 6.5% NaCl.

For bacteriological examinations, culture media manufactured by **HiMedia** (India) were used.

Variation statistics methods were used for statistical processing of the obtained results. All analyses were performed on a personal computer equipped with a **Pentium-4** processor using the specialized **Excel** software for biomedical research. The organization and conduct of the study were carried out in accordance with the principles of evidence-based medicine.

Results and Discussion. During the study, 16 strains of *Staphylococcus aureus* were identified. All strains were isolated as monocultures, and no microbial associations were detected. One of the cultured strains exhibited hemolytic activity and was therefore classified as a hemolytic strain. In terms of morphological characteristics, *Staphylococcus aureus* demonstrated the taxonomic features typical of the genus: Gram-positive, spherical cells arranged in grape-like clusters within the microscopic field (100%, n=16); no atypical strains were observed. With regard to cultural characteristics, the following taxonomic features of *Staphylococcus aureus* were identified: raised, opaque, S-type colonies grew on the surface of the culture medium (egg-yolk salt agar) (100%, n=16); all colonies of the pathogen produced a golden pigment (100%, n=16); and the ability to grow on a culture medium containing 10% NaCl was demonstrated in 15 strains (93.8±6.0%).

At the next stage, the results of extended bacteriological examinations aimed at identification of cultured *Staphylococcus* spp. representatives to the species level were as follows: coagulase activity testing revealed coagulase-positive strains (100%, n=16); growth in nutrient broth with diffuse turbidity was observed in 93.8±6.0% of cases (n=15); the ability to ferment mannitol under anaerobic conditions (100%, n=16) and sensitivity to novobiocin (87.5±8.3%, n=14) were demonstrated; the Voges–Proskauer reaction was positive in 93.8±6.0% of strains (n=15); lecithovitellase activity, or the ability to hydrolyze lecithin with formation of a “rainbow halo” around the colonies, was detected in 68.8±11.6% of cases (n=11); fermentation of glucose was observed in 100% (n=16), lactose in 100% (n=16), mannose in 100% (n=16), and sucrose in 93.8±6.0% (n=15) of strains.

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Thus, on the basis of their morphological, tinctorial, cultural, and enzymatic characteristics, all 16 strains of staphylococci isolated from the pathological material of patients with bacterial conjunctivitis were identified as *Staphylococcus aureus*. The pathogenic nature of these cultured strains was thereby confirmed.

Twenty-one strains of *Staphylococcus epidermidis* were cultured from the pathological material of patients with a confirmed diagnosis, all of which were isolated as monocultures. This microorganism was regarded as an etiological agent when its concentration exceeded 1×10^8 CFU/mL. Its morphological and tinctorial characteristics did not present any difficulty for genus-level identification, and these features were fully expressed in all strains (100%, n=21).

In the species-level identification of *Staphylococcus epidermidis*, a positive Voges–Proskauer reaction and the fermentation of glucose, lactose, maltose, sucrose, and mannitol were used. The distinguishing features separating *Staphylococcus epidermidis* from *Staphylococcus aureus* were the absence of a golden pigment, as well as the lack of coagulase activity and hemolytic properties. At the same time, *Staphylococcus epidermidis* showed lower levels of plasmacoagulase activity (6.3±6.1%) and hyaluronidase activity (12.5±8.3%), while lecithinase activity was absent (0%), all of which were lower than the corresponding parameters observed in *Staphylococcus aureus* strains.

Thus, the *Staphylococcus epidermidis* strains isolated as etiological agents from the pathological material of patients with bacterial conjunctivitis were similar to *Staphylococcus aureus* strains in their morphological, tinctorial, cultural, and enzymatic characteristics, but differed from them in several biological properties. These differences included the absence of a golden pigment in the colonies, lack of coagulase activity, absence of hemolytic properties, relatively lower detection rates of plasmacoagulase and hyaluronidase, and the absence of lecithinase activity.

During the study, 16 strains belonging to *Streptococcus* spp. were identified in monoculture. Of these, 14 strains were classified as *Streptococcus haemolyticus*, and 2 strains as *Streptococcus pyogenes* (group A streptococci, or β-hemolytic streptococci).

These strains exhibited genus-specific morphological characteristics in 100% of cases. Their biological features with respect to cultural characteristics were explained by the absence of catalase activity (catalase-negative) and the presence of hemolytic activity. Both biological characteristics were observed in all strains (100%). In differentiation from *Staphylococcus* spp., attention was paid not only to their morphological features but also to the two above-mentioned biological properties.

It is well known that representatives of the ***Enterobacteriaceae*** family include not only beneficial human symbionts and saprophytes, but also pathogenic microorganisms capable of causing disease in humans, such as *Salmonella* spp., *Shigella* spp., and others. In recent decades, diseases caused by opportunistic microorganisms belonging to this family have also remained highly relevant; examples include *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., and *Providencia* spp. To date, 10 genera, 115 species, and 19 subspecies of the ***Enterobacteriaceae*** family have been described, while the taxonomic position of some of them in the international classification has not yet been fully resolved. This creates difficulties in strain identification. The large number of genera and species within the ***Enterobacteriaceae*** family also complicates their differentiation. As a result, identification requires more time and financial resources, increases the demand for highly qualified specialists, and reduces the guarantee of accurate bacteriological diagnosis.

During bacteriological investigations, *Escherichia* spp. and *Proteus* spp. were isolated among representatives of the *Enterobacteriaceae* family. *Escherichia coli* was identified to the species level (n=3). The *Proteus* spp. isolates (n=2) were not identified to the species level, since no pathogenic differences of significance were observed between the species. They were cultured and identified on differential-diagnostic media characteristic of this taxonomic group (Endo medium). All *Escherichia coli* strains did not differ from one another in their morphological and tinctorial characteristics (P>0.05). In the microscopic field, they appeared as medium-sized, short rod-shaped cells (100%), arranged irregularly (100%), and were Gram-negative (100%). On Endo medium, their colonies were raised, with smooth edges and a smooth surface, circular in shape (S-form), predominantly translucent (100%), with a metallic sheen and red coloration (100%).

At present, according to ***Bergey's Manual*** [10], 43 biochemical characteristics are recommended for use in the identification and differentiation of the main groups of enterobacteria, whereas for *Staphylococcus* spp. the number of such biochemical characteristics amounts to 48.

In addition to glucose and lactose fermentation to acid and gas, five further differential tests are recommended for members of the ***Enterobacteriaceae*** family: hydrogen sulfide production, phenylalanine deaminase activity, presence of urease, sodium citrate utilization, and sodium malonate utilization. However, with the aid of this set of tests, only the following genera of the ***Enterobacteriaceae*** family can be differentiated: *Escherichia* spp., *Shigella* spp., *Hafnia* spp., *Salmonella* spp., *Citrobacter* spp., *Klebsiella* spp., and *Enterobacter* spp.

Differentiation of bacteria on the basis of a limited set of 4–8 characteristics is of only approximate value. In bacteriological practice, however, there is a need for a set of tests that ensures reliable identification of microorganisms of major diagnostic importance at least to the genus level. In this regard, a panel incorporating the following 9–10 characteristics has broad potential: lysine decarboxylase, urease detection, hydrogen sulfide production, indole production, glucose and lactose fermentation, sodium citrate and sodium malonate utilization, and motility. Using this panel, identification can be achieved up to the genus level in 95.7% of cases and up to the species level in 63.2% of cases.

In practice, it is convenient to use a one-step identification approach for determining enterobacteria to the genus level and, in some cases, to the species level, since this reduces the time required for examination; in such cases, 11–14 tests are employed. Adding four additional taxonomic characteristics to the above-mentioned panel of nine tests—namely, inositol utilization, sorbitol utilization, ornithine utilization, and the Voges–Proskauer reaction—increases the rate of identification up to 100.0% at the genus level and up to 88.9% at the species level [9].

At the same time, it should not be ruled out that cultures isolated from natural biological material may be encountered whose phenotype differs from the expected pattern with respect to one or another taxonomic characteristic. For this reason, it is advisable to add a further seven tests to the panel consisting of 13 tests. These include assessment of the bacteria's ability, in the presence of glucose, to utilize sodium citrate (an analogue of Christensen's medium), as well as sucrose, mannitol, maltose, arabinose, lactose, and arginine. The inclusion of these tests increases the accuracy of identification of the studied culture up to the species level to 98.6% [6].

In the present study, the biochemical characteristics of the ***Enterobacteriaceae*** family were investigated using seven tests. On this basis, the isolates were differentiated from other enterobacteria by their ability to ferment lactose (100%). Species-level identification was further carried out using additional reactions, including indole production (100%), the phenylalanine deaminase test (100%), and the Voges–Proskauer reaction (100%).

No strains lacking the full range of biological characteristics (atypical strains) were identified; therefore, this pathogen was classified as *Escherichia coli*. Hemolytic activity was not detected among these strains.

Two strains of *Proteus* spp. were isolated from the pathological material of patients, and both were recovered as monocultures; no microbial associations were observed. The main biological characteristics of *Proteus* spp.—including morphological (100%), tinctorial (100%), and cultural (100%) features—were similar to those of other enterobacteria. The principal differentiating characteristics included "swarming growth" according to Shukevich (100%), lactose fermentation (100%), lysine decarboxylase activity (100%), sorbitol utilization (100%), indole production (100%), and malonate activity (100%), all of which yielded positive results in additional bacteriological tests. No factors indicative of pathogenicity were identified in these strains (0%). As can be seen, no atypical strains were identified.

Thus, the members of the ***Enterobacteriaceae*** family isolated from the biological material of patients with bacterial conjunctivitis—*Escherichia coli* (3 strains) and *Proteus* spp. (2 strains)—were found to be typical strains exhibiting the principal biological characteristics specific to their genus and species.

A total of three cultures of yeast-like fungi belonging to the genus *Candida* (*Candida* spp.) were isolated as monocultures. Colonies grown on Sabouraud medium were identified to the genus level on the basis of their morphological and tinctorial characteristics. Species-level identification was not performed, since no pathogenetic differences were considered significant among the pathogenic species of this genus (*Candida albicans* and non-*albicans Candida*). In differentiation from saccharomycetes, attention was paid to the absence of ascospores in *Candida* spp., as well as to their ability to form pseudomycelium, verticils, and chlamydospores; negative lactose fermentation; positive glucose, maltose, and sucrose fermentation; and terminal budding.

Another important biological characteristic of the causative agents of bacterial conjunctivitis is their response to various antibacterial drugs, namely susceptibility (S) and resistance (R). The importance of this biological property lies in the fact that it determines the rational selection of empirical therapy (antibiotic therapy) for the treatment of diseases caused by these pathogens.

Staphylococcus aureus strains (n=16) demonstrated high susceptibility to antibiotics such as cefazolin (S = 81.3±10.8%), ciprofloxacin (S = 75.0±12.5%), ceftriaxone (S = 75.0±12.5%), cefoperazone (S = 75.0±12.5%), and amoxiclav (S = 75.0±12.5%). In contrast to the above-mentioned agents, the isolated strains of this pathogen showed low susceptibility to tetracycline (S = 12.5±8.3%), gentamicin (S = 18.8±9.8%), and chloramphenicol (S = 18.8±9.8%). Antibiotics to which low susceptibility was also observed included doxacillin (S = 31.3±11.6%), ampiox (S = 31.3±11.6%), erythromycin (S = 43.8±12.4%), and kanamycin (S = 43.8±12.4%).

Thus, *Staphylococcus aureus* strains, which were the principal causative agents of acute bacterial conjunctivitis, demonstrated high resistance to tetracycline, gentamicin, and chloramphenicol, while lower levels of resistance were also observed to doxacillin, ampiox, erythromycin, and kanamycin.

In terms of antibiotic resistance, the indices of *Staphylococcus epidermidis* strains (n=21) were similar to those of *Staphylococcus aureus*, although the degree of resistance was lower (P<0.05). The obtained results showed that the cultured *Staphylococcus epidermidis* strains demonstrated high susceptibility to cefazolin (S=100%), ciprofloxacin (S=95.2±4.7%), ceftriaxone (S=95.2±4.7%), cefoperazone (S=90.5±6.4%), and amoxiclav (S=76.2±9.3%). The resistance level (R) of *Staphylococcus epidermidis* strains was lower than that of *Staphylococcus aureus*: for tetracycline, this parameter was R=71.4±9.9%; for gentamicin, R=66.7±10.3%; for chloramphenicol, R=66.7±10.3%; for doxacillin, R=57.1±10.8%; for ampiox, R=57.1±10.8%; for erythromycin, R=52.4±10.9%; and for canamycin, R=52.4±10.9%.

Therefore, in bacterial conjunctivitis caused by *Staphylococcus epidermidis*, cefazolin, ciprofloxacin, ceftriaxone, cefoperazone, and amoxiclav may be used as part of the treatment regimen.

The antibiotic susceptibility profile of *Streptococcus* spp. (n=16) was similar to that of *Staphylococcus aureus* and *Staphylococcus epidermidis*, with no statistically significant differences observed (P>0.05); therefore, the antibiotics and their corresponding susceptibility percentages are not repeated here.

A noteworthy finding was that the antibiotic resistance profile of the isolated ***Enterobacteriaceae*** strains (n=5) differed significantly from that of Gram-positive cocci (P<0.05). The highest susceptibility was observed to ceftriaxone, cefoperazone, and amoxiclav (all S=100%), followed by ciprofloxacin and cefazolin (80.0±8.9% each). The percentages of antibiotic susceptibility (S) were significantly lower (P<0.05) than the corresponding values for *Staphylococcus aureus* and *Staphylococcus epidermidis*. In cases of bacterial conjunctivitis in which *Escherichia coli* and *Proteus* spp. were recognized as causative agents, ceftriaxone, cefoperazone, amoxiclav, cefazolin, and ciprofloxacin were recommended for inclusion in the treatment regimen.

As a result of all microbiological investigations carried out during the study—including bacteriological, bacterioscopic, and mycological examinations—an algorithm for the microbiological analysis and an algorithm for the mycological analysis of acute bacterial conjunctivitis were developed. Their main advantages consisted in enabling the planning of microbiological and mycological investigations, their step-by-step implementation, and the timely as well as high-quality delivery of analytical results. These microbiological and mycological analysis algorithms are being recommended for the first time for the purpose of microbiological confirmation of the diagnosis of bacterial conjunctivitis and are convenient for use in microbiological investigations.

Conclusions. 1. It was established that all 16 staphylococcal strains isolated from the conjunctival discharge of patients with acute bacterial conjunctivitis belonged to *Staphylococcus aureus*, based on their morphological, tinctorial, cultural, and enzymatic characteristics. The pathogenicity of these cultured strains was confirmed. *Staphylococcus epidermidis* differed from *Staphylococcus aureus* strains by the absence of a golden pigment, catalase activity, and hemolytic properties, as well as by the lower frequency of detection of plasmacoagulase and hyaluronidase, and the absence of lecithinase activity.

2. Representatives of the ***Enterobacteriaceae*** family isolated from the biological material of patients with this pathology—*Escherichia coli* (3 strains) and *Proteus* spp. (2 strains)—were found to be typical strains exhibiting the principal biological characteristics specific to their genus and species.

3. *Candida* spp. were isolated in the form of monocultures, and the colonies grown on Sabouraud medium were identified to the genus level on the basis of their morphological and tinctorial characteristics. In differentiation from

saccharomycetes, attention was paid to the absence of ascospores in *Candida* spp., as well as to their ability to form pseudomycelium, verticils, and chlamydospores; negative lactose fermentation; positive glucose, maltose, and sucrose fermentation; and terminal budding.

4. Candida spp. were isolated as monocultures, and the colonies grown on Sabouraud medium were identified to the genus level based on their morphological and tinctorial characteristics. For differentiation from saccharomycetes, attention was paid to the absence of ascospores in *Candida* spp., as well as to their ability to form pseudomycelium, verticils, and chlamydospores, negative lactose fermentation, positive glucose, maltose, and sucrose fermentation, and terminal budding.

5. As a result of microbiological investigations, an algorithm for the microbiological analysis and an algorithm for the mycological analysis of acute bacterial conjunctivitis were developed for the first time. Their main advantages include facilitating the planning of microbiological and mycological studies, enabling their step-by-step implementation, ensuring the acquisition of reliable results and well-founded conclusions, and allowing the analyses to be performed in a timely manner.

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