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Antibiotic sensitivity analysis of pathogenic strains of microorganisms of the mucous membrane of the palatine tonsils in tonsillitis

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Abstract. Upper respiratory tract infections are among the most common reasons for seeking primary medical care. Although the majority of these infections are of viral origin, approximately 40% of annual antibiotic prescriptions are associated with acute respiratory infections.

This study presents the results of an investigation into the microbial landscape of the mucous membrane of the palatine tonsils and the antibiotic susceptibility of the isolated strains. The material for the study consisted of swabs taken from the surface of the palatine tonsils of 24 patients with acute tonsillitis. The oropharyngeal samples were collected, transported, and analyzed using standardized bacteriological methods and the Kirby-Bauer method.

A total of 84 microbial strains were identified, representing 10 bacterial genera and one genus of yeast (*Candida* spp.). The most frequently isolated microorganisms included β -, α -, and γ -hemolytic *Streptococcus* spp., *Staphylococcus aureus*, coagulase-negative staphylococci, *Haemophilus* spp., *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Antibiotic susceptibility varied, with high sensitivity observed for cephalosporins (85,4%) and lincosamides (100%), and lower sensitivity to semisynthetic ampicillins (64,7-90,0%). Cephalosporins and lincosamides were the most effective treatments for bacterial tonsillitis, particularly for isolates of β -hemolytic *Streptococcus* and *Staph. aureus*. A significant proportion of isolates showed sensitivity to cephalosporins and lincosamides, while fewer were sensitive to semisynthetic ampicillins.

Key words: antibiotics, bacteria, β -hemolytic *Streptococcus* group A, antibiotic resistance, tonsillitis, microbiota, pharynx

Аналіз антибіотикочутливості патогенних штамів мікрофлори слизової оболонки піднебінних мигдаликів при тонзиліті

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Резюме. Інфекції верхніх дихальних шляхів є однією з найпоширеніших причин звернення за первинною медичною допомогою. Хоча більшість цих інфекцій мають вірусне походження, приблизно 40% щорічних призначень антибіотиків пов'язані з гострими респіраторними інфекціями. У роботі представлені результати дослідження мікробного пейзажу слизової оболонки піднебінних мигдаликів та визначення чутливості виділених штамів до антибактеріальних засобів. Матеріалом для дослідження послужили мазки з поверхні піднебінних мигдаликів у 24 пацієнтів з гострим тонзилітом. Зразки з ротоглотки збирали, транспортували та аналізували стандартизованими бактеріологічними методами та методом Кірбі-Бауера. Загалом було ідентифіковано 84 штами мікробів, що представляють 10 родів бактерій і один рід дріжджів (*Candida* spp.). Найбільш частими виділеннями були β -, α - та γ -гемолітичні *Streptococcus* spp., *Staphylococcus aureus*, коагулазонегативні стафілококи, *Haemophilus* spp., *Klebsiella pneumoniae* та *Pseudomonas aeruginosa*. Чутливість до антибіотиків різна, висока чутливість до цефалоспоринів (85,4 %) і

лінкозамідів (100 %) і менша чутливість до напівсинтетичних ампіцилінів (64,7-90,0 %). Цефалоспорины та лінкозаміди були найбільш ефективними при бактеріальному тонзиліті, особливо щодо ізолятів β -гемолітичного стрептококу та *Staph. aureus*. Значна кількість ізолятів була чутлива до цефалоспоринів та лінкозамідів, менше до напівсинтетичних ампіцилінів.

Ключові слова: антибіотики, бактерії, β -гемолітичний стрептокок групи А, антибіотикорезистентність, тонзиліт, мікробіота, глотка.

INTRODUCTION

Upper respiratory tract infections (URTIs) are the most common reason for urgent visits to primary care physicians [1;2]. Although the majority of URTIs have a viral etiology [3], approximately 40 % of annual antibiotic prescriptions are associated with acute respiratory infections. The treatment of tonsillitis caused by Group A β -hemolytic *Streptococcus* is recommended with antibiotics [4;5] to reduce both purulent and non-purulent complications of tonsillitis. Acute tonsillitis is defined as inflammation of the tonsils, typically of infectious origin. The condition is more frequently observed in school-aged children but can affect patients of any age. As in European countries, the USA, and Ukraine, first-line antibiotics for acute tonsillitis caused by *Streptococcus* species are penicillins. Due to the increasing prevalence of bacteria producing beta-lactamase, treatment is often supplemented with aminopenicillins [6;7]. However, the treatment of palatine tonsil inflammation should be comprehensive and based on the results of bacteriological studies and the determination of the sensitivity of pathogenic microorganisms to antibiotics.

The problem of bacterial antibiotic resistance has become widespread globally. The natural resistance of microorganisms to antibacterial drugs is aimed at ensuring their survival. Excessive use, especially unsystematic administration without adherence to proper dosages and treatment regimens, is not only ineffective but can also lead to the development of bacterial resistance to these drugs, thereby reducing the available treatment options. Irrational use of antibiotics is one of the causes of dysbiosis in the oropharyngeal microbiota, which, in turn, leads to sensitization of the body and a decrease in immune resistance. This contributes to the activation of microbiota in infections caused by viruses capable of inducing immunodeficiency and localized immune reactions [8].

The aim of the study is to investigate the microbial landscape of the mucous membrane of the palatine tonsils and determine the sensitivity of the isolated strains to antibacterial agents.

MATERIAL AND METHODS

The study included 24 patients with acute tonsillitis, treated by primary care physicians, were examined. The mean age of the patients was (12,29 $\pm$ 1,48) years (ranging from 10 to 15 years); 13 patients (52,1 %) were female, and 11 (47,9 %) were male. Inclusion criteria for the study were: sore throat, high fever, sneezing, and runny nose. The diagnosis was established according to the unified clinical protocol for primary, secondary (specialized),

and tertiary (highly specialized) medical care for "Tonsillitis". The study adhered to the recommendations outlined in the Council of Europe's Convention on Human Rights and Biomedicine, the ethical principles of the Declaration of Helsinki by the World Medical Association, and Order No. 690 of the Ministry of Health of Ukraine, dated September 23, 2009. Compliance with the requirements of the Bioethics Committee of I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine was also ensured. All patients provided informed consent to participate in the study. The collection and transportation of the material were carried out in accordance with the requirements for the delivery of materials for bacteriological studies. The time frame for delivering clinical material to the laboratory was minimized. The collected material was transported to the laboratory within no more than 2 hours after collection, properly packaged and documented in compliance with the State Sanitary Rules, "Rules for Organization and Safety in Microbiology Laboratories (Departments, Units)" [9]. The material for bacteriological research was smears from the oropharynx, the collection was carried out with a sterile swab. The material was applied to the surface of elective media: blood agar – streptococci, corynebacteria, neisseria, moraxella, rothia, yolk-salt agar- staphylococci, Endo agar - enterobacteria group, Sabouraud agar for the isolation of *Candida* species [10;11;12]. The identification of the isolated microorganisms (morphological, tinctorial, cultural, and biochemical properties) and the assessment of their clinical significance were performed using commonly accepted methods [13;14;15]. For this purpose, an 18-24 h culture was used, from which an inoculum was prepared at a bacterial concentration of 1-2 x 10⁸ CFU/ml, which was prepared to an optical density of 0.5 according to the McFarland turbidity standard. The tests utilized Mueller-Hinton agar and standard antibiotic-impregnated disks. The evaluation and analysis of results were conducted by measuring the diameters of the inhibition zones, according to EUCAST standards. Strains demonstrating intermediate sensitivity were classified as resistant, in line with the recommendations of the European Committee (version 12.0) [16]. Data were collected and tabulated using MS Excel 2016, with quantitative data presented as percentages. The constancy index - C (%), was calculated using the formula:

$$C = p \times 100 / P,$$

where C – the constancy index expressed as a percentage, p -the number of samples in which the species under study was detected, and P - the total number of samples analysed.

The following scale was used to interpret the results: species with a persistence index greater than 50% were considered to be constant, common species - from 20 % to 50 %, rare species - 1-19 % and uncommon species - less than 1 % [17]. The Shapiro-Wilk test was used to test whether the distribution of clinical trial data conformed to the law of normal distribution. The arithmetic mean and standard error ($M \pm m$) were used to describe the data. When testing statistical hypotheses, the null hypothesis was rejected at a statistical significance level (p) of less than 0.05[18].

RESULTS AND DISCUSSION

The bacteriological analysis of the tonsil surfaces revealed and identified the following microorganisms:

β -hemolytic *Streptococcus* Group A, α -hemolytic *Streptococcus* spp., γ -hemolytic *Streptococcus* spp., *Rothia* spp., *Neisseria* spp., *Haemophilus* spp., *Staphylococcus aureus*, coagulase-negative *Staphylococcus* spp., *Moraxella* spp., *Corynebacterium* spp., *Enterococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and fungi of the genus *Candida*.

A total of 84 microbial strains were isolated, belonging to 10 bacterial genera and 1 yeast genus. The results of the bacteriological examination of the material samples obtained from the examined patients showed that 50.0% were representatives of gram-positive microorganisms belonging to 5 genera and 50.0% (5 genera) were gram-negative bacteria. Among the identified strains, cocci predominated, specifically α - and γ -hemolytic *Streptococcus* spp., which accounted for 79,16 % and 58,33 %, respectively (fig.1).

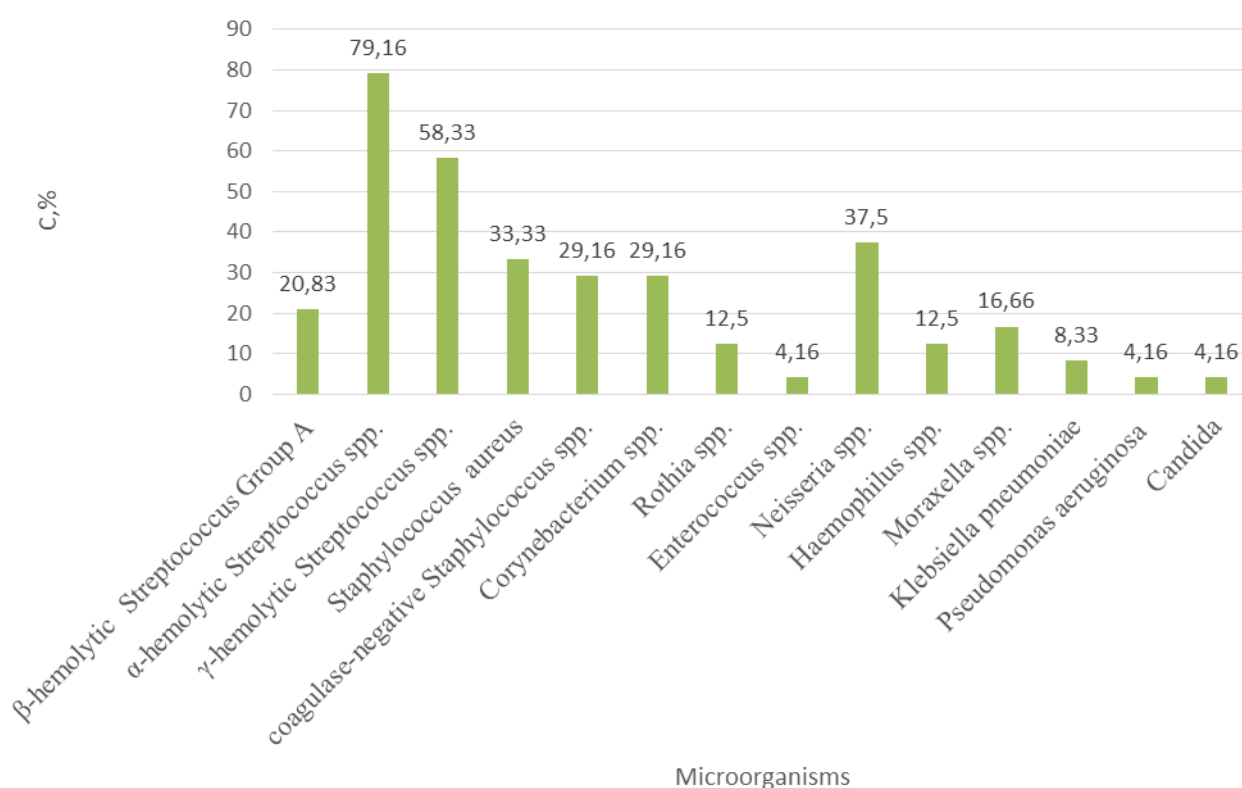


Figure 1. The dominance index for microorganisms isolated from the throat

The detection rate of β -hemolytic *Streptococcus* Group A in the analyzed samples was 20,83 %. However, the frequency of *Enterococcus* spp. among gram-positive cocci was only 4,16 %. The prevalence of staphylococcal groups in the studied samples was 62,50 %, including *S. aureus* (33,33 %) and coagulase-negative *Staphylococcus* spp. (29,16 %). Populations of *Neisseria* spp. (37,5 %) and *Corynebacterium* spp. (29,16 %) were frequently observed in the microbial communities analyzed. Populations of *Rothia* spp. and *Haemophilus* spp. were infrequently detected in the communities, with a prevalence of 12,50 % each, while *Moraxella* spp. had a detection rate of 16,66 %. Among gram-negative rod-shaped bacteria, populations of

K. pneumoniae (8,33 %) and *P. aeruginosa* (4,16 %) were rarely isolated. Similarly, yeast-like fungi of the genus *Candida* were infrequently identified in the studied microbiocenoses, with a detection rate of 4,16 %. Antibiotic susceptibility testing was performed for strains of opportunistic bacteria isolated using bacteriological methods from the tonsil surfaces of patients with acute tonsillitis. The dominance level of these bacterial populations in the microbiocenosis ranged from 20 % to 50 % of the detected bacterial populations. The obtained antibiotic susceptibility results demonstrated variability in the sensitivity of the isolated bacteria. Clinical isolates of *S. aureus* and β -hemolytic *Streptococcus* Group A exhibited differing

levels of susceptibility to all tested antibiotics. The most effective antibiotics were cephalosporins and lincosamides, commonly used for the treatment of bacterial acute tonsillitis caused by gram-positive bacteria. The susceptibility of these microorganisms to cephalosporins and lincosamides was 85,40% and 100,0 %, respectively. For semisynthetic ampicillins, the susceptibility ranged from 64,7 % to 90,0 %. Macrolides showed significantly lower efficacy: 42,71 % for *S. aureus* and 10,41 % for β -hemolytic *Streptococcus* Group A. Antibiotic susceptibility testing was also conducted for gram-negative bacteria, *K. pneumoniae* and *P. aeruginosa*, which were resistant to most antibiotics included in the study. However, it was determined that these bacteria demonstrated susceptibility to ceftriaxone at a rate of 73,5 %.

The results of the study of the species composition of the microbiocenosis of the mucous membrane of the palatine tonsils in all children with acute tonsillitis examined do not contradict the results of other researchers, the microbiota, which was represented by resident and transient microorganisms. *S. aureus*, β -hemolytic *Streptococcus* group A, *K. pneumoniae*, *P. aeruginosa* - opportunistic microorganisms, which with high colonisation activity can cause severe inflammatory processes [19;20]. Our results on the antibiotic susceptibility of the strains tested are consistent with the results of a meta-analysis of 35 clinical trials involving 7125 patients conducted by Casey J. R. co-author, which demonstrated the superiority of cephalosporins over penicillin in achieving a positive treatment outcome and susceptibility testing (OR: 2.31; 95% CI: 1.39-3.85) [21]. It should be noted that in the treatment of acute tonsillitis, the choice between penicillins and cephalosporins often depends on factors such as efficacy, cost and bacterial resistance. Although some studies suggest that cephalosporins may provide higher bacteriological cure rates, the clinical significance of this advantage is controversial, and penicillin remains a cost-effective and reliable first-line treatment. [22]. The study of susceptibility to macrolides revealed a low sensitivity of the strains examined. Azithromycin is considered an alternative drug for allergic reactions to penicillins. Studies of the efficiency of azithromycin and penicillin showed a 93% success rate with azithromycin compared to penicillin (89%); however, when comparing the

duration of treatment with penicillin (10 days), the efficacy is better compared to the use of azithromycin (3 days) [23]. On the other hand, the analysis carried out by the research group suggests that there are probably no clinically significant differences in the disappearance of symptoms when cephalosporins, macrolides and penicillin are used [24;25]. Another drug of choice is clindamycin from the lincosamides, to which our study revealed high susceptibility (85.4-100%) in gram-positive cocci. In clinical trials, the use of clindamycin in combination with penicillin has been shown to be effective in the treatment of severe streptococcal infections [26]. The results of antibiotic susceptibility we obtained for enterobacteria indicate resistance to macrolides, lincosamides. Enterobacteria are characterised by natural resistance to beta-lactam drugs, and in the absence of throat culture, the use of antibiotics may lead to ineffective treatment [27]. The use of antibacterial drugs leads to improvement in most patients, but inappropriate antibiotic therapy for acute tonsillitis may play a significant role in the development of bacterial resistance in the future.

CONCLUSION

According to the results of bacteriological investigations, 10 genera of bacteria and *Candida* fungi were found to form microbial associations on the surface of the tonsils of patients with acute tonsillitis. Among the bacteria by cell wall type, 50.0% belong to gram-positive microorganisms of 5 genera and 50.0 % (5 genera) are gram-negative bacteria. It was established that according to the index of constancy, bacteria from the genera *Streptococcus* of groups β and α are constant (more than 50 %), the group of microorganisms that occur frequently (from 20-50 %) in the microcenosis includes *Corynebacterium*, γ -hemolytic *Streptococcus* spp., *S. aureus*, coagulase-negative *Staphylococcus* spp., *Neisseria* spp., as well as rarely occurring (1-19 %) representatives of the genera *Enterococcus*, *Moraxella*, *Haemophilus*, *Candida*. The results of the study of antibiotic susceptibility of *Streptococcus* strains of group β , *S. aureus* showed sensitivity to cephalosporins (85.4 %), lincosamides (100 %), semi-synthetic ampicillins (64.7-90.0 %). Strains of gram-negative bacteria *K. pneumoniae* and *P. aeruginosa* were susceptible to ceftriaxone (73.5 %).

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