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Microbiocenosis in patients with acute tonsillitis influenced by smoking

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Abstract. Acute tonsillitis is a widespread disease, the course of which can be affected by the patient's smoking status, which leads to changes in the oropharyngeal microbiota. The purpose of the study was to evaluate the effect of smoking on the development of palatine tonsil microbiocenosis in patients with acute tonsillitis. The study included 54 patients who were divided into two groups: smokers (n = 26) and non-smokers (n = 28). The microbiological study included inoculating samples on various nutrient media, followed by identification of microorganisms by phenotypic methods. Microbiota density was estimated by counting colony-forming units. The Mann-Whitney U-test, Fisher's exact test, and principal component analysis were used for statistical analysis. Principal component analysis did not reveal a clear clustering of the samples, which indicated that there were no global changes in the tonsil microbiota depending on smoking status. However, selective shifts were found, and these included a significantly reduced colonisation density of *Corynebacterium* spp. ($p = 0.04$) and the exclusive presence of fungi of the genus *Candida* among smokers. Clinically, the groups did not differ in the severity of the disease on the Centor scale, but smokers were more likely to receive antibiotic therapy (30.8% and 10.7%) and had a higher incidence of respiratory infections (42.3% and 27.3%). The results showed that smoking does not change the overall structure of the tonsil microbiota in acute tonsillitis, but causes selective dysbiosis. This substantiated the need to consider the patient's smoking status to assess the risk of recurrent infection and develop more personalised approaches to prescribing antibiotic therapy

Keywords: bacteria; bacteriological analysis; diagnostic tests; viruses; microbiota; oropharynx

INTRODUCTION

Acute tonsillitis remains one of the most common respiratory tract infections, and the palatine tonsils, which are part of the Waldeyer-Pirogov ring, play a key role in shaping the immune response and serve as a barrier to pathogens [1]. Researchers are increasingly paying attention to the role of the mucosal microbiota as a critical factor in maintaining health and developing pathological conditions. As noted by J.L. Pathak *et al.* [2], the oral microbiome is in close interaction with the respiratory system, and its dysbiosis is an important factor in the development of respiratory diseases, which confirms the relevance of studying the tonsil microbiota in acute tonsillitis. The structure of the microbiota is significantly influenced by external factors, including smoking as one of the important stress factors [3]. It is important to note that the problem is not only traditional tobacco smoking, but also the impact of electronic cigarettes. Thus, the study by M. Al-Alawneh *et al.* [4] found an increased incidence of tonsillectomy among children exposed to e-cigarette aerosol. The researchers noted that even indirect exposure of children to aerosols of electronic devices is associated with pathological changes in lymphoid tissue, which increases the likelihood of the need for surgical treatment. The data obtained are particularly relevant in the context of the growing popularity of vaping among young people.

According to S. Cicchinelli *et al.* [5], exposure to tobacco smoke leads to changes that are manifested by a loss of bacterial diversity, a decrease in the number of commensal species (for example, genera *Corynebacterium* and *Streptococcus*) and the growth of opportunistic microorganisms. This effect was confirmed by studies of the oral microbiota. For example, the paper by G. D'Angiolella *et al.* [6] presented

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data according to which tobacco smoking significantly reduces the number of Gram-positive bacterial populations in saliva. Specifically, in the review analysed, a decrease in the diversity of Gram-positive bacterial species was recorded from 18 in non-smokers to 7 in smokers, indicating suppression of key species responsible for colonisation resistance. Based on the results of the literature review conducted by S. Cicchinelli *et al.* [5], the authors pointed out that these changes are systemic in nature, but they are most pronounced in places of direct contact with smoke. Research by X. Wang *et al.* [7] showed that both traditional cigarettes and electronic devices cause significant shifts in the microbial community of saliva, with e-cigarettes showing a unique exposure profile different from tobacco, but also leading to dysbiosis. These data are confirmed and detailed in the metagenomic study by S. Chattopadhyay *et al.* [8], which revealed clear signs of oral microbiota dysbiosis in smokers, characterised by increased colonisation of pathogens against the background of a decrease in the number of beneficial commensal species. These changes disrupt colonisation resistance and immune homeostasis of the mucous membranes, which can contribute to the chronisation of infectious processes.

According to O.I. Lemko *et al*. [9], any form of tobacco smoking, including alternative smoking, significantly increases the risk of developing respiratory infections. In their review, C. Jiang *et al.* [10] systematised data confirming the association between smoking and an increased risk of infectious diseases of various localisation, including respiratory infections, tuberculosis, and pneumonia. The paper described in detail the pathophysiological mechanisms of this phenomenon, which, in addition to dysbiosis, include damage to the ciliated epithelium, impaired mucociliary clearance, alveolar macrophage dysfunction, and weakened adaptive immunity. In particular, M. Hilty *et al.* [11] emphasised that smoking not only increases the likelihood of infection, but also worsens the course and results of treatment of infectious diseases. Experimental studies in mouse models have confirmed that exposure to cigarette smoke alters the composition of the oropharyngeal microbiota and reduces its diversity. T. Wüthrich *et al.* [12] in a comprehensive study demonstrated that exposure to cigarette smoke causes disorganisation of the microbiota, which, in turn, increases the severity of influenza A virus infection. An important aspect is that changes in the microbiota caused by smoking are long-lasting and can maintain a pathological condition even after stopping exposure to smoke. Dysbiosis caused by smoking leads to a violation of the functional state of the respiratory mucosa and increases the inflammatory response, creating favourable conditions for the development of a viral infection. The researchers provided direct evidence that changes in the microbiota caused by smoking are not only a concomitant phenomenon, but also an active participant in the pathogenesis of respiratory diseases, increasing their destructive potential.

However, despite the general recognition of the effects of smoking on the body, the question of what changes in the microbiota of the palatine tonsils occur during an acute inflammatory process under the influence of tobacco smoke remains poorly understood. Most existing studies focus on oropharyngeal microbiocoenosis in general or in health. The purpose of the study was to conduct a comparative analysis of the structure and density of the palatine tonsil microbiota in patients with acute tonsillitis, depending on their smoking status.

MATERIALS AND METHODS

The study included 54 patients with clinical signs of acute tonsillitis. The study included patients who visited family doctors at primary care centres in Ternopil with complaints of sore throat and high temperature. Verification of the diagnosis of acute tonsillitis was made according to the clinical protocol of primary, secondary (specialised), and tertiary (highly specialised) medical care for tonsillitis [13]. Exclusion criteria: chronic or recurrent tonsillitis, immunodeficiency conditions, cancer, autoimmune or severe concomitant pathology, pregnancy and lactation, recent surgical interventions, systemic glucocorticoid therapy, and refusal to participate in the study. Anamnestic data were collected through a survey, namely on antibiotic use (yes/no) and respiratory infections in 3 months (yes/no), smoking (yes/no). Based on the questionnaire, patients were divided into two groups: the smoking group (n = 26); the non-smoking group (n = 28). Clinical assessment was carried out according to the following criteria: pain intensity was assessed on a visual-analogue scale (VAS, 0-10 cm) in two states: rest and swallowing. The clinical severity of tonsillitis was determined on the Centor scale and its modification Centor/McIsaac (1-4 points).

Detection of pathogens was carried out using immunochromatographic tests (on the *Streptococcus* group A (Ecotest, China), influenza A/B viruses, adenoviruses, respiratory syncytial viruses (RSV), SARS-CoV-2 (Medbioalliance, Ukraine). Rapid immunochromatographic tests were purchased by patients and performed by a doctor. The positive result was evaluated in accordance with the manufacturer's instructions. Sterile swabs with Amis transport medium (manufactured by VOLES, Ukraine) were used for bacteriological research. The material was delivered at a temperature of +18...22 °C for two hours to the laboratory. The material was cultured on nutrient media: blood agar with 5% sheep erythrocytes "Sanimed" (Ukraine) streptococci, staphylococci and corynebacteria; mannitol salt agar "Farmaktiv" (Kyiv) as a selective medium for staphylococci; endo agar "Farmaktiv" (Kyiv) for the detection of Gram-negative representatives of the family Enterobacteriaceae; Sabouraud agar "Farmaktiv" (Kyiv) for the isolation of yeast-like fungi of the genus *Candida*. Identification of microorganisms was carried out using a complex of phenotypic methods, including Gram staining, biochemical tests (catalase, coagulase, lecithinase for Gram-positive cocci; Simmons citrate, indole, mobility for Gram-negative rods, "Farmaktiv", Kyiv), hemolysis results (blood agar with 5% sheep erythrocytes, "Sanimed-M", Kharkiv), and sensitivity to novobiocin, bacitracin and optoquine (LLC "Ukrmediasnab", Dnipro). The cultures were incubated under aerobic conditions at 37°C for 24 hours. Identification was performed according to the classical bacteriological protocols described by M. Goodfellow *et al.* [14], national guidelines and manuals on microbiological diagnostics by V.V. Minukhin *et al.* [15] and S.I. Klymnyuk *et al*. [16]. The number of colony-forming units (CFU) per 1 ml of an oropharyngeal smear sample suspended in a sterile transport medium was

determined to quantify the oropharyngeal microbiota. The results were expressed as the decimal logarithm (lg CFU/ml). Statistical data processing was performed using Python 3.11 software (Python Software Foundation, USA) using the scikit-learn, pandas, and numpy libraries in the Google Colaboratory environment. Initial data collection and structuring was performed in MS Excel 2010 (Microsoft Office 2010, USA). In addition, online services from Social Science Statistics (Social Science Statistics, Washington, Virginia, USA) were used for certain statistical calculations. Quantitative data were presented as the arithmetic mean ± standard deviation (m ± SD). The normality of the distribution was checked using the Shapiro-Wilk test. To compare quantitative indicators between groups, the Mann-Whitney U-test was used for data whose distribution deviated from the normal one. Fisher's exact test (at expected frequencies <5) was used to analyse categorical variables. The level of statistical significance was set at $p < 0.05$. Principal Component Analysis (PCA) was used to visualise and analyse multidimensional microbiological data. PCA is a method of reducing the dimension of data that allows identifying the main areas of maximum variability in a data set. Each primary component (PC) reflects a certain percentage of explained variance, which characterises the degree of influence of this factor on the overall data structure. The explained variance of the principal components was interpreted on the following scale: values <30% indicated a low influence of the factor on the data structure, 30-60% – on moderate influence, 60-80% – on significant influence, and >80% – on dominant influence. Heat maps were constructed to visualise the intensity of microbial colonisation across all samples. The study was conducted in accordance with the recommendations set out in the Convention on Human Rights and Biomedicine [17], considering the ethical principles set out in the Declaration of Helsinki [18], and in accordance with Order of the Ministry of Health of Ukraine No. 690 [19], as well as the requirements of the Bioethics Committee of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 81 dated 03.04.2025).

All patients were familiar with the study design and signed informed consent to participate in the study. The study is part of the research work of the Department of Microbiology, Virology and Immunology at the I. Ya. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine "Comprehensive study of microbiota, immune system, antibacterial resistance, and clinical and laboratory indicators for the diagnosis, prognosis, and development of therapy for human diseases" (state registration number 0125U000121). This study is a continuation of the previous research on the influence of smoking on oropharyngeal microcoenosis and evaluates differences (at the time of consideration of this paper in the journal, the study is not yet available in the public domain). A limitation of this study is the single-centre design and sample size, which may affect the generalisability of models.

RESULTS AND DISCUSSION

A comprehensive comparative analysis of clinical and anamnestic parameters was performed between two study groups: patients who smoke (Group 1, n = 26) and patients who do not smoke (Group 2, n = 28), with an established diagnosis of acute tonsillitis (Table 1).

Table 1. Comparison of basic clinical parameters between a group of smokers and non-smokers

Parameters	Group of smokers (n = 26)	Group of non-smokers (n = 28)	Statistical significance (p-value)
Body mass index (BMI)	24.76 ± 2.9	25.93 ± 3.5	0.2221, (p (x ≤ Z) = 0.111
VAS scale (sore throat at rest) (cm)	5.71 ± 1.09	5.71 ± 1.4	0.9011, (p (x ≤ Z) = 0.4506
VAS scale (pain when swallowing) (cm)	6.76 ± 1.22	6.82 ± 1.4	0.6939, (p (x ≤ Z) = 0.6531
Centor scale (score)	3.23 ± 0.68	3.21 ± 0.7	0.9157, (p (x ≤ Z) = 0.4579
Use of antibiotics throughout the month	30.76%	10.71	0.0675
Previous respiratory infections in the last 3 months	42.30%	27.27%	0.6455

Source: compiled by the author

The Centor/McIsaak clinical scale was used to objectively assess the severity of the disease. Statistical analysis of the results obtained did not reveal a significant difference between the groups ($p > 0.05$). The results showed the uniformity and comparability of the groups according to the key clinical criteria included in the scale, namely: the presence of high body temperature (above 38°C), the detection of exudate on the surface of the tonsils, the absence of coughing, and palpatory painful cervical lymph nodes. Anthropometric indicators also showed no statistically significant differences. In particular, the mean BMI values were similar in both groups, which was confirmed by the corresponding statistical criterion ($p = 0.222$). To quantify pain, a survey of patients was conducted using VAS. Analysis of pain intensity at rest and during the act of swallowing revealed no significant differences between patients who smoke and those who do not smoke ($p > 0.05$ for both types of pain). When analysing anamnestic data, it was found that the incidence of respiratory infections during the last three months was higher in the group of smokers (42.30%) compared to the group of non-smokers (27.27%). However, this difference did not reach the level of statistical significance ($p = 0.645$). An analysis of antibiotic treatment over the past month revealed a trend: antibiotic use was more frequent among smokers (30.76%) compared to 10.71% in non-smokers, with the p-value approaching the significance level ($p = 0.067$). In the group of smokers (n = 26), the vast majority of patients (22 people) used traditional cigarettes. The smoking intensity was 10.47 ± 4.0 cigarettes per day (range: 4-22 cigarettes). The remaining 4 patients in the group used e-cigarettes (vapes). According to the results of rapid diagnostics, the most common pathogen in the study

cohort was influenza (types A/B), which was diagnosed in 22% of patients. The detection rate of adenoviruses (18%) was only slightly lower, while Group A streptococcus was detected almost 2.5 times less frequently (9%) (Fig. 1).

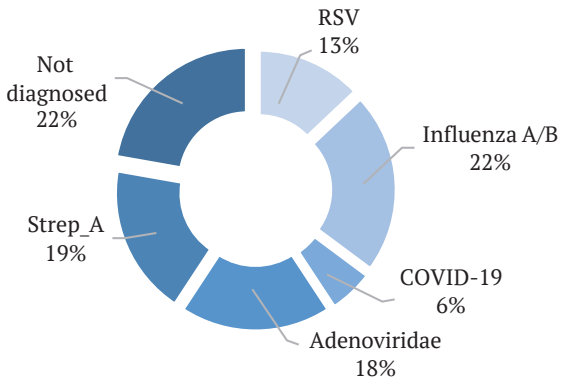

Figure 1. Results of immunochromatographic tests of the study cohort of patients with symptoms of acute tonsillitis
Source: compiled by the author

The remaining pathogens were found with a noticeably lower frequency: RSV – in 13% of cases (about half as often as influenza), and SARS-CoV-2 – only in 6% (almost 4 times less often than influenza). It is important to note that in 22% of those surveyed, the aetiology of the disease remained unknown, since the results of testing for all target pathogens were negative. Bacteriological examination of oropharyngeal smears confirmed the bacterial aetiology of acute tonsillitis in 10 of the 54 examined patients (18.51%). The detection rate of β-haemolytic Streptococcus spp.,, the main bacterial pathogen, was higher in the group of non-smokers (6 patients, or 21.4%) compared to the group of smokers (4 patients, 15.4%), but this difference did not reach statistical significance ($p > 0.05$). In addition to the target pathogen, routine microbiological analysis revealed a wide range of microorganisms that colonise the tonsil biotope. To systematise the detected microbiota, microorganisms were classified according to the type of metabolism and cell morphology. The vast majority of the identified species were aerobic and facultative anaerobic bacteria. Among them, Gram-positive cocci dominated, in particular, various species of Streptococcus (α-, β - and γ-haemolytic), *Staphylococcus aureus*, and coagulase-negative Staphylococcus spp. Gram-positive rods represented by genera *Corynebacterium* and *Rothia* were also present. Of the Gram-negative bacteria, they were identified as cocci (*Neisseria* spp., *Moraxella* spp.), and rods (*Klebsiella pneumoniae*, *Haemophilus* spp., *Enterobacter* spp.). Additionally, representatives of fungi of the genus *Candida* were found in the microbiota. PCA was used to assess the overall effect of smoking status on the structure of the tonsil microbiota. As shown in Figure 2, graphical representation of PCA results did not reveal a clear cluster distribution of patients' microbial profiles depending on smoking status.

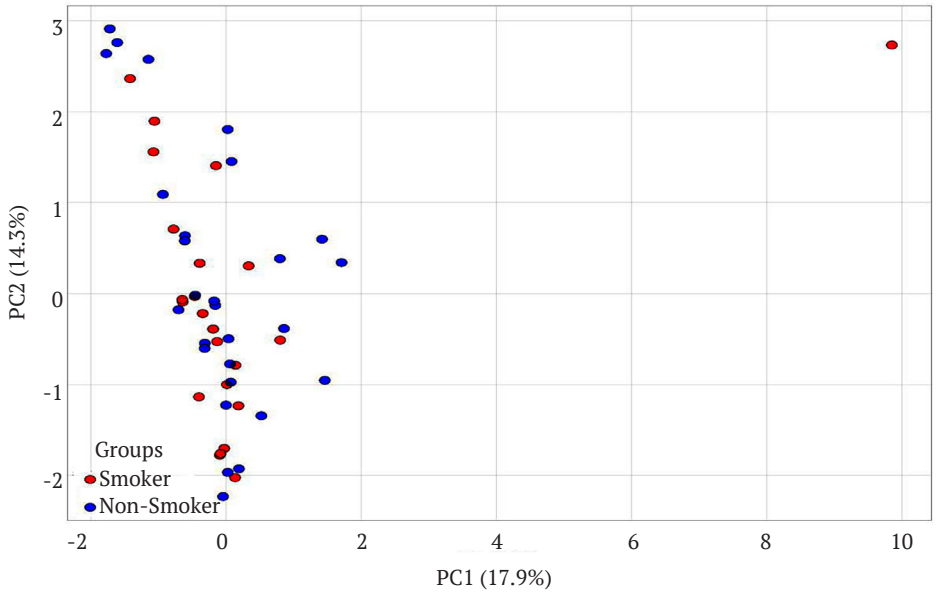

Figure 2. PCA analysis of the microbiome of patients with tonsillitis symptoms in smokers and non-smokers
Source: compiled by the author

The groups of smokers and non-smokers overlap significantly, which indicates that there is no global rearrangement of the microbiota under the influence of tobacco. It is important to note that the first two main components (PC1 and PC2) account for only 32.2% of the total variance (17.95% and 14.3%, respectively), which indicated that the smoking factor is not dominant in the development of microbiota composition, inferior to other individual factors. This conclusion was confirmed by the results visualised using a heat map (Fig. 3), which reflected the intensity of colonisation of individual microorganisms in each patient. The map did not show the development of separate clusters corresponding to the study groups. Individual microbial load profiles showed high variability among both smokers and non-smokers. A detailed comparative analysis of the colonisation density (CFU/mL) of individual microorganisms between the groups presented in Table 2 also found no statistically significant differences.

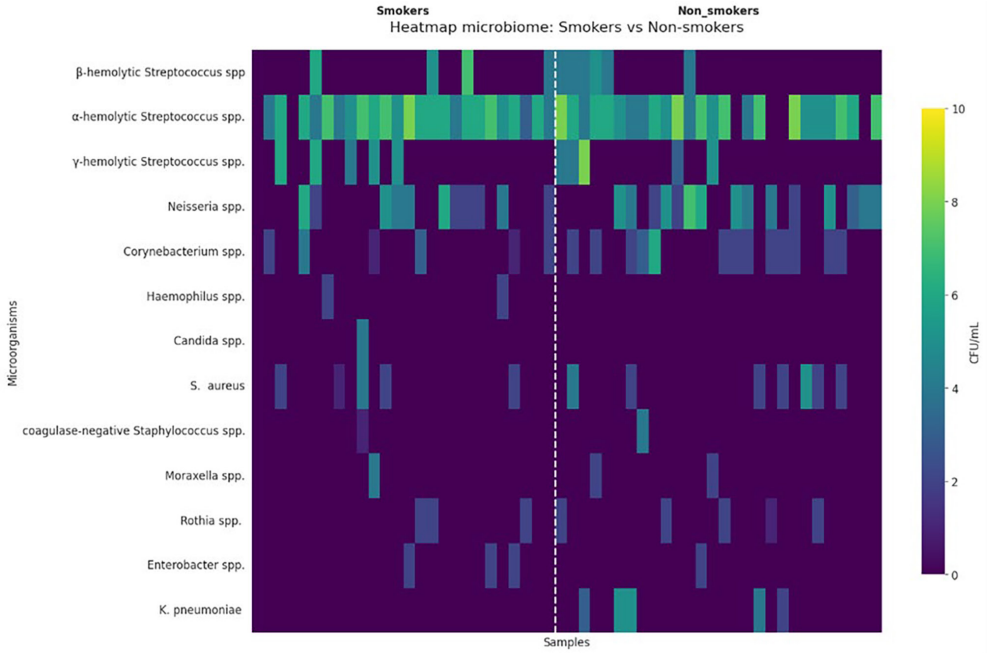

Figure 3. Heat map of the intensity of colonisation of isolated microbial isolates in the examined patients of the smoking and non-smoking groups

Source: compiled by the author

Table 2. Estimation of microbial colonisation density (CFU/mL) between a group of smokers and non-smokers

Microorganisms / Patient groups	Group of smokers (n = 26)	Group of non-smokers (n = 28)	Mann-Whitney U-test
α-haemolytic *Streptococcus* spp.	10^4-10^8	10^5-10^8	0.95
β-haemolytic *Streptococcus* spp.	10^7-10^4	10^4-10^5	0.48
γ-haemolytic *Streptococcus* spp.	10^6-10^4	10^5-10^8	0.82
Corynebacterium spp.	10^1-10^4	10^2-10^6	0.04
Neisseria spp.	10^2-10^6	10^2-10^7	0.28
S. aureus	10^1-10^4	10^2-10^5	0.55
Moraxella spp.	$<10^4$	$<10^2$	0.64
Haemophilus spp.	$<10^2$	10^3	0.50
Rothia spp.	$<10^2$	10^1-10^2	0.56
K. pneumoniae	-	10^2-10^5	-
Enterobacter spp.	$<10^2$	$<10^2$	0.28
Coagulase negative *Staphylococcus* spp.	$<10^1$	10^4	0.96
Candida spp.	10^4	-	-

Source: compiled by the author

Comparison of CFU/mL levels of individual isolates showed that the average densities of α-haemolytic Streptococcus spp. and *Neisseria* spp. were close between the groups ($p > 0.05$). A significant increase in colonisation with *Corynebacterium* spp. was observed among non-smokers ($p = 0.04$). In turn, *Candida* spp. were found only among smokers, which may indicate a local decrease in mucosal colonisation resistance in this group. Overall, the results show that smoking does not alter the global taxonomic profile of the tonsil microbiota, but may contribute to selective changes in the colonisation density of individual representatives, potentially associated with an increased risk of secondary infections or dysbiosis.

The results obtained in the study helped to deepen our understanding of the effect of smoking on the oropharyngeal microbiota in acute tonsillitis. Comparison of the data with the results of other studies revealed both similar trends and important differences, which may indicate the specific effects of smoking during acute infection. The key conclusion of the study was the lack of a global rearrangement of the tonsil microbiota under the influence of smoking. PCA did not reveal a clear cluster distribution between the groups, suggesting that smoking status is not the dominant factor determining the overall taxonomic composition of the microbiota at the time of acute illness. This result is partially consistent with a large-scale study by J. Wu *et al*. [20], which also found no global changes in the composition of the oral microbiota in smokers under health conditions. However, the author's study, conducted in an active infection setting, demonstrated that exposure

to smoking is not a decisive factor in determining the initial clinical severity. This result may be conditioned by the fact that severe inflammation serves as a powerful factor that masks and unifies the microbial environment, levelling the individual effects of smoking. Importantly, a weak explanation of the overall variance (only 32.2% was explained by PC1 and PC2) confirmed the multivariate aetiology and the presence of numerous, as yet unidentified, determinants that form the patient's microbial profile.

The lack of clear separation of microbial profiles by smoking status can be explained by the high individual variability of the oropharyngeal microbiota. As noted by J.T. Nearing *et al.* [21], this variability is a fundamental characteristic of a healthy microbiome. Separate analysis by bioinformatic approaches performed by J.H. Moon & J.H. Lee [22] also confirmed a significant level of diversity in the composition of the healthy oral microbiota. The study by L.L. Bach *et al.* [23] found that the oropharynx microbiota, despite the presence of a stable nucleus, is characterised by significant interpersonal differences and certain temporal dynamics. This finding of temporal variability in the oral microbiota was confirmed by E. Vogtmann *et al.* [24]. Thus, the significant contribution of individual non-communicable factors (such as genetic characteristics, diet, hygiene, and a history of concomitant diseases) can be so significant in the development of the microbial profile that the influence of an individual factor, even such a significant one as smoking, is levelled against the background of general variability. This is especially pronounced in the context of acute infection, which itself is a powerful stress and unifying factor for the microbiota [25].

Although the overall structure of the microbiome did not undergo major statistically significant changes, the selective shifts identified by the researcher are potentially clinically significant. Specifically, the significantly lower microbial colonisation of *Corynebacterium* species in smokers ($p = 0.04$) confirmed its known role as an indicator of a healthy microbiome and may indirectly indicate the dysbiotic effect of tobacco smoke. An even more revealing finding was the discovery of yeast fungi of the genus *Candida* spp. only in the group of patients who smoke. These results showed that smoking, without radically changing the overall structure, selectively modifies the niche, creating a favourable environment for colonisation of specific, potentially pathogenic microorganisms. This is consistent with the findings of L. Bach *et al*. [26], which showed that smoking reduces the stability of the pharyngeal microbiota and promotes selective growth of individual taxa. Similarly, a systematic review by N.L.M. Senaratne *et al*. [27] noted that various forms of tobacco can lead to specific shifts, in particular, promote the growth of yeast fungi of the genus *Candida*. A possible mechanism is that tobacco smoke damages the mucosal epithelium and suppresses local immune mechanisms, reducing colonisation resistance and opening niches for such microorganisms.

An important aspect of this study was the use of the Centor/McIsaac scale for an objective assessment of the severity of the disease. The results showed that, despite the identified microbiological and clinical differences, the groups of smokers and non-smokers were homogeneous according to the key clinical criteria included in this scale. Statistical analysis revealed no significant differences in Centor scores between the groups ($p > 0.05$), and in parameters such as the presence of exudate on the tonsils, body temperature >38°C, no cough, and soreness of the cervical lymph nodes. This showed that the increased risk of complications and more frequent administration of antibiotics to smokers is not associated with a more severe clinical picture at the time of treatment, but is a consequence of other mechanisms. These data were confirmed by T.E. Klug *et al*. [28], who proved that smoking is an independent risk factor for paratonsillar abscess, and this effect was not associated with changes in the microbial spectrum. Thus, it can be assumed that the effect of smoking is realised not because of the deterioration of clinical manifestations of pharyngitis, but because of the suppression of local immune mechanisms and violation of the barrier function of the mucous membrane, which creates conditions for the development of complications even with a standard clinical picture.

The clinical correlation of the identified microbiological trends may be the difference in antibiotic use observed in this study. Although it did not reach a strict level of significance ($p = 0.067$), the frequency of their use among smokers was almost three times higher (30.76% vs 10.71%). The study by E.A. Saliba-Gustafsson *et al.* [29] indicated that clinical factors such as the presence of exudate on the tonsils are key to deciding whether to prescribe antibiotics for respiratory infections. The large-scale study by M.B. Steinberg *et al.* [30] demonstrated that smoking is an independent risk factor, as smoking patients are 20-30% more likely to receive antibiotics. This suggests that in addition to objective clinical signs, smoking status may influence drug decision, possibly due to the expectation of a more severe or prolonged course of infection. In particular, K. Ahmadi *et al*. [31] noted that the mechanisms underlying this may be impaired mucociliary clearance, stimulation of biofilm formation, and suppression of local immunity under the influence of tobacco smoke.

Moreover, the long-term impact of such treatment should be considered: a recent meta-analysis by I. Adamu *et al*. [32] showed that prescribing antibiotics for respiratory infections increases the risk of future consultations. Thus, it can be assumed that there is a cyclicity in which smoking contributes to infections that lead to more frequent use of antibiotics, which, in turn, can increase the tendency to future diseases. This cycle may partly explain the higher incidence of respiratory infections among smokers that was observed in the study (42.30% vs 27.27%), and which was confirmed in global estimates in the paper by F. Sitas *et al*. [33], 22.5% of deaths from respiratory infections were associated with smoking.

For the key pathogen, β-haemolytic Streptococcus spp.,, there was no statistically significant difference in the frequency of its detection between the study cohorts of patients, although there was a numerical advantage among non-smokers (21.4% vs 15.4%). This trend correlated with data by I. Brook & A.E. Gober [34], who observed microbiological changes after smoking cessation. It can be hypothetically assumed that in a healthy state, smoking suppresses competitive microflora, potentially creating a niche for colonisation by pathogens. However, in the context of an acute inflammatory process, this primary dysbiotic effect can be eliminated due to the intense immuno-inflammatory load that dominates the local mechanisms of microbial competition.

Thus, the study concluded that in acute tonsillitis, smoking does not lead to a global change in the tonsil microbiota, which is confirmed by the lack of clear clustering of samples by smoking status when analysing the main components. However, it manifests itself at a more subtle, selective level, contributing to dysbiotic shifts such as a decrease in the level of *Corynebacterium* spp. and colonisation with *Candida* spp. These selective shifts are likely associated with a decrease in local immune defences and changes in mucosal properties under the influence of tobacco smoke, which, in turn, may lead to clinical trends towards more frequent prescribing of antibiotics and respiratory infections in this category of patients.

CONCLUSIONS

The study demonstrated the complex and multi-level nature of the effect of smoking on the oropharyngeal microbiota in acute tonsillitis. Although the smoking factor does not lead to a global rearrangement of the microbiota, which was confirmed by the lack of a clear cluster division on the PCA graph and a low percentage of explained variance (32.2%), it causes selective dysbiotic shifts of clinical significance. There was a decrease in colonisation of *Corynebacterium spp.* ($p = 0.04$) – commensals associated with a healthy microbiota, and the exclusive presence of fungi of the genus *Candida* in smokers. This indicates a violation of the colonisation resistance of the mucous membrane, probably due to the suppression of local immune mechanisms under the influence of tobacco smoke.

The key fact was that despite the absence of significant differences in the clinical picture on the Centor and VAS scale between the groups, smokers showed clear trends towards more frequent use of antibiotics (30.8% and 10.7%; $p = 0.067$) and a higher incidence of respiratory infections in the anamnesis (42.3% and 27.3%). Therefore, smoking does not worsen the severity of clinical manifestations of tonsillitis, but creates a "hidden" dysbiosis, which probably underlies an increased tendency to relapse of infections and the need for antibiotic therapy. This indicated that tobacco exposure is realised mainly by inhibiting local immunity and disrupting microbial homeostasis, rather than by directly enhancing the inflammatory response. The data obtained highlighted the importance of taking smoking status into consideration when assessing the risks of complications and recurrent oropharyngeal diseases. Further studies with larger samples are needed to investigate the mechanisms by which selective shifts in the microbiota translate into clinical consequences.

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CONFLICT OF INTEREST

None.

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Мікробіоценоз у пацієнтів з гострим тонзилітом під впливом фактору куріння

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Анотація. Гострий тонзиліт є широко поширеним захворюванням, на перебіг якого може впливати статус куріння пацієнта, що призводить до змін у мікробіоті ротоглотки. Метою роботи було оцінити вплив куріння на формування мікробіоценозу піднебінних мигдаликів у пацієнтів із гострим тонзилітом. Дослідження охопило 54 пацієнти, які були розподілені на дві групи: курці (n = 26) та некурці (n = 28). Мікробіологічне дослідження включало культивування зразків на різних поживних середовищах з подальшою ідентифікацією мікроорганізмів за фенотиповими методами. Щільність мікробіоти оцінювали шляхом підрахунку колонієутворюючих одиниць. Для статистичного аналізу використовували U-критерій Манна-Уітні, точний тест Фішера та аналіз головних компонент. Аналіз методом головних компонент не виявив чіткої кластеризації зразків, що свідчило про відсутність глобальних змін у мікробіоті мигдаликів залежно від статусу куріння. Однак були виявлені вибіркові зміни, серед яких значно знижена щільність колонізації *Corynebacterium* spp. (p = 0,04) та виключно наявність грибків роду *Candida* серед курців. Клінічно, групи не відрізнялись за тяжкістю перебігу захворювання за шкалою Centor, але курці частіше отримували антибіотикотерапію (30,8 % та 10,7 %) та мали більшу частоту респіраторних інфекцій в анамнезі (42,3 % та 27,3 %). Отримані дані свідчили, що куріння не змінює загальну структуру мікробіоти мигдаликів при гострому тонзиліті, але викликає селективний дисбіоз. Це обгрунтовало необхідність врахування статусу куріння пацієнта для оцінки ризику рецидивуючого перебігу інфекції та розробки більш персоналізованих підходів призначення антибіотикотерапії

Ключові слова: бактерії; бактеріологічний аналіз; діагностичні тести; віруси; мікробіота; ротоглотка