

## SPECIES COMPOSITION AND SUSCEPTIBILITY TO ANTIBIOTICS OF MICROORGANISMS ISOLATED FROM TOOTH SOCKETS OF EXTRACTED TEETH IN CASES OF ALVEOLAR OSTEITIS

V.O. Malanchuk<sup>1</sup>, A. Javadias<sup>1</sup>, A.V. Rybachuk<sup>1</sup>,  
M.V. Oblap<sup>1</sup>, V.V. Potochilova<sup>2</sup>

<sup>1</sup>Bogomolets national medical university,  
1 Zoologichna str, 03057, Ukraine

<sup>2</sup>Kyiv Regional Clinical Hospital,  
1 Baggovutivska Str., Kyiv, 04106, Ukraine  
e-mail: [afshin.javadi@gmail.com](mailto:afshin.javadi@gmail.com)

Alveolar osteitis (AO) is one of the most common infectious complications after dental extractions. The data on the species composition of AO pathogens and their susceptibility to antimicrobial drugs can be the basis for their empirical use in case of inflammatory process aggravation. **Objective.** To determine the species composition and susceptibility to the antimicrobial agents of microorganisms, which were detected in patients with AO, who sought medical help in the oral surgery department of the dental medical center of Bogomolets National Medical University. **Methods.** Throughout 2018–2021, microbiological examination of tooth sockets from 30 patients with AO and 20 patients without AO was performed. The studied biological material was plated on appropriate nutrient media for isolation of aerobic, facultative and obligate-anaerobic microorganisms. Anaerobic conditions were achieved in GENbox 7.0 L and GENbox 2.5 L aerostats using GENbox anaerobic packages (“Biomerieux”, France). The genus and species identity of the bacteria were determined according to Bergey. Antibiotic susceptibility of the isolated strains was determined by disk diffusion method. **Results.** It was found that most commonly microorganisms from tooth sockets in case of AO are: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Escherichia coli*, *Bacteroides* spp., *Clostridium* spp., and *Candida* species, as well as their mixed cultures of 3–5 species of microorganisms. These aerobic and facultative anaerobic bacteria were susceptible to amoxicillin, ceftriaxone and ciprofloxacin in 92.6–100 % of cases. The growth of anaerobic bacteria in 100 % of cases was inhibited by colistin and meropenem. **Conclusions.** AO developing is caused by pathological colonization of socket of the extracted tooth by representatives of endogenous microbiota, namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Escherichia coli*, which are present mainly in the mixed cultures with *Candida albicans*. For empirical antibiotic therapy of complicated forms of AO, amoxicillin or ceftriaxone or ciprofloxacin in complex with colistin or meropenem should be used, since these drugs suppress the growth of 92.6–100 % of strains of aerobic, facultative and obligate anaerobic microorganisms, which are potential pathogens of the purulent forms of AO.

**Keywords:** alveolar osteitis, alveolar osteitis pathogens, complication after tooth extraction, susceptibility to antimicrobial drugs.

Among the inflammatory complications after tooth extraction one of the leading places is occupied by alveolar osteitis (inflammation of tooth socket) (AO), which divides into purulent and serous types. According to various authors, they develop in 15–45 % of patients [1, 2, 3, 4]. It has been proved that endogenous and exogenous microorganisms that colonize the mucous membrane of the oral cavity play an essential

role in the development of AO [5]. According to various authors, these include *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Clostridium*, *Bacteroides* as well as *Escherichia coli* [6]. Moreover, the above-mentioned microorganisms were seen, typically, in alliances, especially with *Candida* species.

It should be noted that the species composition of AO pathogen and their associations depends on the

immunological resistance of patients, the presence of concomitant diseases of the nasopharynx, as well as periodontal diseases. Treatment of AO is based on the extensive use of local various antiseptic agents, broad spectrum antibiotics, biogenic materials with antimicrobial effect, etc. Thus, in oral and maxillofacial surgery, chlorhexidine, hexetidine, triclosan, antiseptics based on biologically active components of medicinal herbs, etc. are widely used for mouthwash in the complex of hygienic procedures of oral cavity.

Despite the significant number of scientific works devoted to the search of approaches to AO treatment, this problem is still relevant due to the different susceptibility to antiseptics and antibiotics of microorganisms and their associations, which colonize the alveolus and cause its ignition, in different patients.

Based on the above, the **objective of work** was to determine the species composition and susceptibility to antimicrobial agents of microorganisms that were isolated from patients with AO who obtained medical care in the surgical department of the dental medical center of Bogomolets National Medical University.

**Materials and methods.** Throughout 2018–2021, microbiological examination was performed on alveolar extractions of 50 patients between 18 and 40 years of age who obtained medical care at the Department of oral and maxillofacial surgery of dental medical center of Bogomolets National Medical University. The treatment included 30 patients who were diagnosed with AO two to three days after extraction of the tooth. 20 patients without AO formed a control group. The examined patients did not have diabetes. There were no pregnant women or patients undergoing steroid therapy. All surgical interventions were performed using anaesthetics containing vasoconstrictors.

The material for microbiological investigations was the socket substance, which was sterilely taken with a syringe and put into tubes with the thioglycolic medium, this medium was first heated in a water bath (regenerated) for 20–30 seconds at 100 °C, and then sharply cooled in a crystalline crust in order to prevent the medium from being enriched with atomic oxygen from the air. To reduce the diffusion of atmospheric oxygen, after adding biologic material the medium was covered with sterile Vaseline oil (ball thickness 1–1.5 cm) and incubated at 37 °C for 48–72–96 hours until visible growth of microorganisms in the sample. For the isolation of obligate anaerobic

microorganisms, the suspension was incubated in 5 % blood agar, chocolate agar with Poly ViteX, Schiedler's broth with vitamin K3, Schiedler's agar with 5 % sheep's blood. The biological material was plated by the sector method according to Gold and cultured in anaerobic conditions at 37 °C for 48–96 h until the visible growth appeared on the agar surface. Identification of anaerobic organisms was performed according to the method [7]. Anaerobic conditions were achieved in GENbox 7.0 L and GENbox 2.5 L anaerostats using GENbox anaeropackages ("Biomérieux", France). Anaerobiosis control was performed using Anaer Indikator ("Biomérieux", France).

Selective nutrient medium was used to identify facultative anaerobic and aerobic microorganisms: 5 % blood agar, chocolate agar, selective salt agar ("Biolife", Italy), heterococcus agar ("Biolife", Italy), Endo agar ("Biolife", Italy), Saburo agar ("Biolife", Italy).

For bacteria bioluminescence accumulation the material was added to the sucrose broth and from it every 24 h, transferred according to the gold method, repeatedly cultured in the thermostat at 37 °C in aerobic conditions for 24 h. Some media, i.e., chocolate agar, blood agar and modified medium for isolation of anaerobic bacteria, were prepared according to the appropriate prescriptions. The genus and species identity of the bacteria were determined according to Bergey [8].

The susceptibility of the isolated strains to antibiotics was determined by disk diffusion method according to Bauer-Kirby [9] using commercial disks with antibiotics on Müller-Hinton medium – for uninhibited microorganisms, on Müller-Hinton medium supplemented with 5 % blood and 20 mg/L NAD. When evaluating the activity of antibiotics, the criteria of the disk manufacturer were used. Depending on the diameters of the zones of microorganism growth inhibition near the discs with antibiotics the strains were classified as susceptible, moderately resistant or resistant. Antibiotic disks and media were monitored using test cultures of reference strains of *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, which were received from the museum of living cultures of microorganisms of Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.

**Results.** It was found that from the socket of patients with AO mainly contain *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus*

spp., *Escherichia coli*, *Bacteroides* spp., *Clostridium* spp. in 90.0±5.5; 60.0±8.9; 53.3±9.1; 46.7±9.1; 60.0±8.9; 56.7±9.0 % of cases respectively (Table 1).

*Candida* species were isolated in 53.3±9.1 % of patients with AO, indicating a violation of their colonization resistance, as a result, it can be the development of an infectious process in the socket of the extracted teeth, the etiological factor of which is represented by the normal oral cavity microbiota.

The socket of the control group of patients in 45–75 % of cases had representatives of endogenous microbiota – bacteria of the genus *Staphylococcus*, namely *S. aureus* and *S. epidermidis*, *Streptococcus* spp., especially *S. salivarius*, as well as *Clostridium* spp. and *Veillonella* spp. Other microorganisms including *C. albicans* were isolated only from 5.0 to 15.0 % of patients.

Thus, in case of AO after tooth extractions the sockets are colonized mainly by facultative anaerobic microorganisms, which may indicate a possible etiological role of these microorganisms

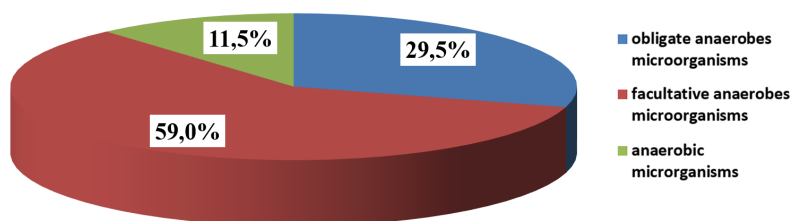
in the development of AO (Fig. 1).

It should be noted that these microorganisms in 100 % of cases were isolated in association of 3–5 species. The most frequent were the associations of *C. albicans* with *Streptococcus* spp. and *E. coli* (16.7±6.8 %), *S. aureus* + *Streptococcus* spp. + *C. albicans* (13.3±6.2 %), *S. aureus* + *Bacteroides* spp. + *C. albicans* (10.0±5.5 %), *S. epidermidis* + *S. aureus* + *Streptococcus* spp. + *C. albicans* (10.0±5.5 %), *C. albicans* + *Clostridium* spp. + *S. aureus* (6.7±4.6 %), *S. aureus* + *E. coli* + *Streptococcus* spp. + *Candida albicans* (6.7±4.6 %), *S. epidermidis* + *S. albicans* + *Streptococcus* spp. + *C. albicans* (6.7±4.6 %). Other mixed cultures were isolated in no more than 29.9±8.4 % of cases each (Table 2). Moreover, *C. albicans* strains were the most common mixed cultures in the group of patients with AO.

Control patients tooth sockets contained mixed cultures of *S. epidermidis*, *S. salivarius*, and *Veillonella* spp. (20.0±8.9 %), *Streptococcus* spp., *Clostridium* spp. and *Corinebacterium* spp. (20.0±8.9 %), *S. aureus*, *S. epidermidis*

**Table 1**  
**Species composition of microorganisms isolated from sockets of extracted teeth**

Microorganisms	Sighting frequency, %	
	Patients with alveolar osteitis n=30	Control group n=20
<i>Staphylococcus aureus</i>	90.0±5.5	45.0±11.1
<i>Staphylococcus epidermidis</i>	60.0±8.9	70.0±10.2
<i>Streptococcus</i> spp.	53.3±9.1	75.0±9.7
<i>Streptococcus intermedius</i>	36.7±8.8	0
<i>Streptococcus salivarius</i>	0	25.0±9.7
<i>Peptostreptococcus</i> spp.	36.7±8.8	0
<i>Enterococcus faecium</i>	30.0±8.4	10.0±6.7
<i>Escherichia coli</i>	46.7±9.1	15.0±8.0
<i>Corinebacterium</i> spp.	33.3±8.6	15.0±8.0
<i>Clostridium</i> spp.	56.7±9.0	75.0±9.7
<i>Bacteroides</i> spp.	60.0±8.9	0
<i>Veillonella</i> spp.	0	70.0±10.2
<i>Haemophilus</i> spp.	16.7±6.8	5.0±4.8
<i>Candida albicans</i>	53.3±9.1	10.0±6.7



**Fig. 1. Distribution of bacteria species associated with AO into physiological groups according to their relation to oxygen**

**Table 2**

**Microbial mixed cultures of microorganisms isolated from socket of extracted teeth of patients with alveolar osteitis**

No.	Mixed cultures	Frequency
1	<i>C. albicans</i> + <i>Streptococcus</i> spp. + <i>E. coli</i>	16.7±6.8
2	<i>S. aureus</i> + <i>Streptococcus</i> spp. + <i>C. albicans</i>	13.3±6.2
3	<i>S. aureus</i> + <i>Bacteroides</i> spp. + <i>C. albicans</i>	10±5.5
4	<i>C. albicans</i> + <i>Clostridium</i> spp. + <i>S. aureus</i>	10±5.5
5	<i>S. aureus</i> + <i>E. coli</i> + <i>Streptococcus</i> spp. + <i>C. albicans</i>	6.7±4.6
6	<i>E. coli</i> + <i>S. intermedius</i> + <i>E. faecium</i> + <i>Clostridium</i> spp. + <i>S. epidermidis</i>	6.7±4.6
7	<i>S. epidermidis</i> + <i>S. aureus</i> + <i>Streptococcus</i> spp. + <i>C. albicans</i>	6.7±4.6
8	Other	29.9±8.4

and *Clostridium* spp. (15.0±7.9 %). Only in a few cases different mixed cultures of 2 to 3 species of microorganisms with *C. albicans* or *Enterobacteriaceae* in their composition were isolated.

Since the dominant microorganisms that were isolated from the tooth socket of patients with AO were *Staphylococcus* spp., *E. coli* and *Streptococcus* spp., as well as unregulated anaerobic bacteria, we investigated their susceptibility to antibiotics that can be used in the treatment of AO.

As a result of these studies, amoxicillin, ceftriaxone and ciprofloxacin were the most active antibiotics that inhibited the growth of over 90 % of *S. aureus*, *S. epidermidis* and *E. coli* strains (Table 3).

Ampicillin inhibited the growth of only 11.1–16.7 % of the strains of these microorganisms. Tetracycline was most active against 74.1 % of *S. aureus* strains and only 57.1 % of *E. coli* strains.

As for *Streptococcus* bacteria, both *Streptococcus* spp., and *S. intermedius* were the most susceptible to amoxicillin, clindamycin, and

ciprofloxacin, which inhibited growth from 81.3±9.7 to 90.9±8.7 % of the tested strains of these microorganisms (Fig. 2). Ceftriaxone was active against 75.0±10.8 % of *Streptococcus* spp. strains and 81.8±11.6 % of *S. epidermidis* strains. Cefazolin and tetracycline were the most active among the studied antibiotics. Between 45.5±15.0 % and 63.6±12.0 % of *Streptococcus* strains isolated from tooth sockets of patients with AO were susceptible to these drugs.

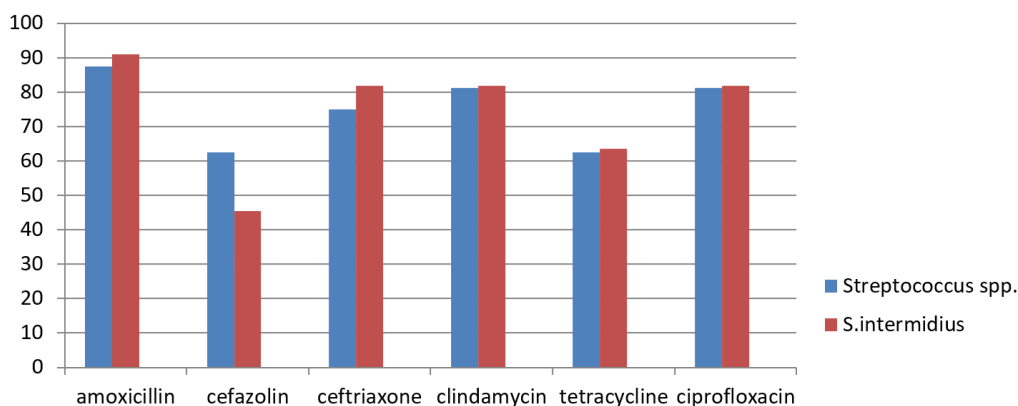
Bacterial isolates were the most susceptible to colistin and meropenem, which inhibited the growth of all the studied strains of *Clostridium* spp. and *Bacteroides* spp. (Table 4). Clindamycin was active against 88.2±7.8 % of the strains of *Clostridium* spp. and 94.4±5.4 % of the strains of *Bacteroides* spp. Other antibiotics inhibited the growth of 55.6–66.7 % of the strains studied.

It should be noted that we have not found any strains of microorganisms with multiple resistance to antibiotics, which confirms endogenous origin of AO pathogens in the examined patients.

**Table 3**

**Susceptibility to antimicrobial agents of *S. aureus*, *S. epidermidis*, and *E. coli* strains isolated from patients with alveolar osteitis**

Antibiotic	Number of susceptible strains (%)		
	<i>S. aureus</i> n=27	<i>S. epidermidis</i> n=18	<i>E. coli</i> n=14
Ampicillin	11.1±6.0	16.7±8.8	14.3±9.4
Amoxicillin	96.3±5.1	100	85.7±9.4
Cefazolin	74.1±8.4	66.7±11.1	71.4±12.1
Ceftriaxone	92.6±5.0	100	100
Gentamicin	81.5±7.4	83.3±8.8	85.7±9.4
Clindamycin	100	100	0
Tetracycline	74.1±8.4	66.7±11.1	57.1±13.2
Ciprofloxacin	96.3±5.1	88.9±7.4	92.9±6.9



**Fig. 2. Susceptibility to antibiotics of *Streptococcus* bacteria strains seen in patients with AO**

**Table 4**

**Susceptibility to antimicrobial agents of obligate anaerobes strains detected in patients with alveolar osteitis**

Antibiotic	Number of susceptible strains (%)	
	<i>Clostridium</i> spp. n=17	<i>Bacteroides</i> spp. n=18
Metronidazole	58.8±11.9	66.7±11.1
Ampicillin	58.8±11.9	55.6±11.7
Clindamycin	88.2±7.8	94.4±5.4
Imipenem	58.8±11.9	55.6±11.7
Colistin	100	100
Meropenem	100	100

**Discussion.** Today, despite the introduction of modern methods of prevention and treatment of inflammation which can occur after tooth extraction, serous and purulent AO remain a serious problem of oral and maxillofacial surgery. According to various authors, AO can comprise from 2.8 to 35 % of complications after extraction of teeth [10, 11]. The development of AO is influenced by a significant number of factors such as patient age, presence of somatic and infectious diseases, prolonged and complicated surgical treatment, patient's failure to follow the postoperative regimen, presence of prolonged post-extraction bleeding, presence of a clot in the socket due to the use of disinfectants or its elimination, disregard of a doctor's recommendations (smoking, mouthwash after the surgery, alcohol consumption), etc. [12–17]. However, a special place belongs to AO. Microorganisms penetrate into socket from odontogenic infectious sources, as well as from nasal and nasopharyngeal cavities. Infections in the socket when the tooth is extracted due to acute or chronic periodontitis or exacerbation of periodontitis can also be the cause [11].

There are different approaches to AO treatment. These include the use of antimicrobial drugs injected directly into the socket of the extracted tooth, the use of low-intensity laser impregnation, the use of osteoplastic preparations, drugs that stimulate immunological reactivity of the body, etc. [12].

However, if local treatment is ineffective, severe complications such as chronic osteomyelitis can develop. In these cases, there is a need for parenteral or intravenous administration of broad-spectrum antibiotics. In this case, it is necessary to take into account the data on the species composition of AO pathogens, as well as their susceptibility to antibiotics. This approach is based on the fact that through a wide and often irrational use of antibiotics, the species composition of the progenitors of infectious diseases tends to change and their level of antibiotic resistance tends to increase. This trend applies to oral cavity microbiota as well.

The data on the species composition of microorganisms that colonize tooth socket are contradictory. Thus, Borodulina I.I. and co-workers



showed that 2 to 5 species of microorganisms were found in socket of patients with AO [11]. In this case, mainly *C. albicans*, *S. saprophyticus*, *Enterococcus* were identified from biological material. Other investigators showed prevalence of *Streptococcus* spp. (over 70 %), *Actinomyces* spp. (24.3 %), *Peptostreptococcus* spp. (15.98 %), *Escherichia coli* (16.23 %), *Bacteroides* spp. (6.81 %), and *Candida* spp. (8.9 %) [12].

The results of our research showed that the tooth socket of patients with AO are colonized mainly by endogenous oral microflora, namely *S. aureus*, *S. epidermidis*, *Streptococcus* spp. and *E. coli*. However, unlike other authors, a significant increase in the number of isolated anaerobic bacteria, namely *Bacteroides* spp., *Clostridium* spp. as well as *C. aliphilus* was identified. These microorganisms were seen as mixed cultures of 3–5 species of microorganisms, which included *C. albicans* strains, while in patients without AO, mixed cultures of 2–3 species of normal oral microbiota were isolated from tooth socket. This indicates that AO develops amid disruption of the quantitative and qualitative composition of the oral cavity microbiota, which can realize its pathogenic potential and cause inflammation of the pit of the extracted tooth. Thus, the data obtained indicate a possible etiological role of endogenous microbiota in the development of AO, as well as confirm the presence of oral cavity dysbiosis in patients with this pathology.

The treatment strategy for AO includes the mandatory use of antimicrobial drugs. The choice of drugs is often based on the physician's subjective assessment of the clinical picture and depends on his qualification. There are a lot of recommendations for treatment of AO. Some authors recommend using a combined method of AO treatment using Gramicidin C, prednisolone, benzocaine and low-intensity laser imaging, which allows to relieve pain syndrome and achieve significant antibacterial effect [10]. Others describe the use of various antibacterial drugs in combination with osteoplastic materials [18–21]. Therefore, the data on the leading pathogens of AO as well as their susceptibility to antibiotics and antiseptics are important for the selection of drugs for their extensive use. To determine the list of antimicrobial drugs that can be used, first of all for the treatment of patients with complicated forms of AO, for which not only local application of antiseptics is indicated, as well as parenteral administration of antibiotics, we determined the

susceptibility of dominant pathogens of AO to antibiotics that are used in dentistry.

It was found that the microorganism strains studied did not have multiple resistance to antibiotics, which confirms their endogenous origin. Amoxicillin, gentamicin, ceftriaxone, and ciprofloxacin were the most active against *S. aureus*, *S. epidermidis*, and *E. coli*. In relation to strains of *Clostridium* spp. and *Bacteroides* spp. – colistin and meropenem, which inhibited the growth of all these microorganisms studied. Amoxicillin, clindamycin and ciprofloxacin were the most active against *Streptococcus* bacteria, inhibiting the growth of 81.3 to 90.9 % of the strains of these microorganisms studied.

### Conclusions

1. AO is associated with pathological colonization of extracted tooth's socket by representatives of endogenous microbiota, namely *S. aureus*, *S. epidermidis*, *Streptococcus* spp., *E. coli*, which were identified mainly in the mixed cultures with *C. albicans*.

2. For empirical antibiotic therapy of complicated forms of AO amoxicillin or ceftriaxone or ciprofloxacin in complex with colistin or meropenem can be used, since these drugs inhibit the growth of about 90 % of the isolated strains of aerobic, facultative and obligate anaerobic microorganisms, which are most often isolated from tooth sockets of patients with AO and are potential pathogens of its purulent forms.

## ВИДОВИЙ СКЛАД І ЧУТЛИВІСТЬ ДО АНТИМІКРОБНИХ ПРЕПАРАТІВ МІКРООРГАНІЗМІВ, ВИДІЛЕНИХ З АЛЬВЕОЛ ВИДАЛЕНИХ ЗУБІВ ПРИ АЛЬВЕОЛІТАХ

*В.О. Маланчук<sup>1</sup>, А. Джавадіасл<sup>1</sup>,  
А.В. Рибачук<sup>1</sup>, М.В. Облал<sup>1</sup>,  
В.В. Поточилова<sup>2</sup>*

<sup>1</sup> Національний медичний університет  
імені О.О. Богомольця, проспект Перемоги, 34,  
Київ, 01601, Україна

<sup>2</sup> Київська обласна клінічна лікарня,  
вул. Багговутівська, 1, Київ, 04106, Україна

### Резюме

Серед інфекційних ускладнень після видалення зубів одне з провідних місць посідають альвеоліти. Дані щодо видового складу збудників альвеолітів та їх чутливості до антимікробних препаратів

можуть стати основою для емпіричного використання останніх при загостренні запального процесу. **Мета.** Встановити видовий склад і чутливість до антимікробних препаратів мікроорганізмів, що виділялись у хворих альвеолітом, які звертались за медичною допомогою в хірургічне відділення Стоматологічного медичного центру НМУ імені О.О. Богомольця. **Методи.** Протягом 2018–2021 рр. мікробіологічно досліджено виділення з альвеол 30 пацієнтів із альвеолітом і 20 пацієнтів – без альвеоліту. Досліджуваний біологічний матеріал засівали на відповідні поживні середовища для виділення аеробних, факультативно- та облігатно-анаеробних мікроорганізмів. Умови анаеробіозу досягали в анаеростатах GENbox 7,0 L та GENbox 2,5 L за допомогою анаеропакетів GENbox anaer («Biomérieux», Франція). Родову і видову належність виділених бактерій визначали за Bergey. Чутливість виділених штамів до антибіотиків визначали диско-дифузійним методом. **Результати.** Встановлено, що при альвеолітах із альвеол зубів виділяються переважно *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Escherichia coli*, *Bacteroides* spp., *Clostridium* spp., дріжджоподібні гриби роду *Candida*, а також

їх мікст-культури з 3–5 видів мікроорганізмів. Ці аеробні та факультативно-анаеробні бактерії були чутливими до амоксицилін/клавуланової кислоти, цефтріаксону і ципрофлоксацину у 92,6–100 % випадків. Ріст облігатно-анаеробних бактерій у 100 % випадків пригнічувався колістином і меропенемом. **Висновки.** Альвеоліти розвиваються на тлі патологічної колонізації альвеол видаленого зуба представниками індигенної мікробіоти, а саме: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Escherichia coli*, що виділяються переважно у складі мікст-культур з *Candida albicans*. Для емпіричної антибіотикотерапії ускладнених форм альвеолітів слід використовувати амоксицилін/клавуланову кислоту або цефтріаксон, або ципрофлоксацин у комплексі з колістином або меропенемом, оскільки зазначені препарати пригнічують ріст 92,6–100 % штамів аеробних, факультативно- і облігатно-анаеробних мікроорганізмів, що є потенційними збудниками гнійних форм альвеолітів.

*Ключові слова:* альвеоліти, збудники альвеолітів, ускладнення після видалення зубів, чутливість до антимікробних препаратів.

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