

CLUSTER ANALYSIS REVEALED ANTIBIOTICS WITH THE HIGHEST EFFICACY AGAINST BACTERIA ISOLATED FROM PATIENTS WITH INFECTIOUS AND INFLAMMATORY DISEASES OF THE SOFT TISSUES OF THE FACIAL AREA

Klastrová analýza antibiotík s najvyššou účinnosťou proti baktériám izolovaným od pacientov s infekčnými a zápalovými ochoreniami mäkkých tkanív vo faciálnej oblasti

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Abstract

Background: Incorrect prescription of antibiotics in dental surgery leads to the acquisition of antibiotic resistance of microorganisms.

Aim: The aim of the given study was to conduct a retrospective cluster analysis of antibacterial drugs according to their efficacy against bacteria isolated from patients with infectious inflammatory diseases of the facial soft tissue.

Materials and methods: The cross-sectional study involved 351 patients who were treated for in the department of maxillofacial surgery during 2019 – 2022.

Results and conclusions: According to the results of cluster analysis, clinical isolates of *S. aureus* isolated from foci of infectious and inflammatory diseases of the facial soft tissue retain sensitivity to vancomycin, moxifloxacin, ciprofloxacin, levofloxacin, norfloxacin and ceftiofloxacin. Coagulase-negative *Staphylococcus spp.* are most sensitive to norfloxacin, erythromycin, azithromycin, benzylpenicillin, kanamycin, gentamicin and clindamycin and *Enterococcus ssp.* to norfloxacin and tigecycline. *Kocuria*, *Streptococcus*, drug *S. viridans* isolates retained a stable sensitivity only to vancomycin in a retrospective analysis (Tab. 1, Fig. 11, Ref. 39). Text in PDF www.lekarsky.herba.sk.

KEY WORDS: antibiotics, resistance to antimicrobial substances, clinical pathogens, dental surgery, facial area.
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Abstrakt

Úvod: Nesprávne predpisovanie antibiotík v stomatochirurgii vedie k získaniu antibiotickej rezistencie mikroorganizmov.

Cieľ: Vykonať retrospektívnu klastrovú analýzu antibakteriálnych liečiv podľa ich účinnosti proti baktériám izolovaným od pacientov s infekčnými zápalovými ochoreniami mäkkého tkaniva tváre.

Materiály a metódy: Na prierezovej štúdií sa zúčastnilo 351 pacientov, ktorí boli liečení na oddelení maxilofaciálnej chirurgie v rokoch 2019 – 2022.

Výsledky a záver: Podľa výsledkov analýzy izoláty *S. aureus* izolované z ložísk infekčných a zápalových ochorení mäkkých tkanív tváre zachovávajú citlivosť na vankomycín, moxifloxacín, ciprofloxacín, levofloxacín, norfloxacín a ceftiofloxacín. Koaguláza-negatívne *Staphylococcus spp.* sú najcitlivejšie na norfloxacín, erytromycín, azitromycín, benzylpenicilín, kanamycín, gentamicín, klindamycín a *Enterococcus ssp.* na norfloxacín a tige-cyklín. Rod *Kocuria*, Rod *Streptococcus*, drug *S. viridans* v retrospektívnej analýze zachovali stabilnú citlivosť iba na vankomycín (tab. 1, obr. 11, lit. 39). Text v PDF www.lekarsky.herba.sk.

KLÚČOVÉ SLOVÁ: antibiotiká, rezistencia na antimikrobiálne látky, klinické patogény, stomatochirurgia, faciálna oblasť.

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Introduction

About 6.5 million visits to a physician in the United States and around 700 K in Ukraine each year are associated with infectious inflammatory diseases of the skin and soft tissue, and the rate of hospital admissions for these infections has increased by almost 30.0% in recent years (4, 8, 17). Despite the rapid development of surgical dentistry, there is not only an increase in the number of patients with facial soft tissue infections but also a significant complication in their course and treatment (16). This may be related to the change in the qualitative composition of the microbiota of the foci of infections in recent years (1, 16, 34). Previously *Staphylococcus aureus* was considered as the dominant pathogen of facial soft tissue infections, but now no more than 15.0% of cases of its isolation have been established. In general, *Staphylococcus* spp. are isolated from patients with facial soft tissue infections with a frequency of about 30.0%. Moreover, coagulase-negative species, such as *Staphylococcus epidermidis*, are becoming more common (36).

Considering the fact that a destroyed tooth that serves as a source of infection is often revealed during facial soft tissue infections, representatives of the genus *Streptococcus* play an important role in these processes. Thus, in about a third of cases of facial soft tissue infections, alpha haemolytic viridans streptococci group are found (3, 29). However, with the development of microbiological diagnostic methods, the number of anaerobic pathogens is constantly increasing, which are usually representatives of such genera as *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Parvimonas* (5, 6).

Facial soft tissue infections usually result in purulent-inflammatory processes, such as abscesses and phlegmons of the facial area. They are characterized by a rapid aggressive course against the background of a sharp deterioration in the general condition of the patient with subsequent spread of inflammation from one anatomical part to another (35). It should be noted that almost 10% of these patients develop severe complications, leading to hospital admission to an intensive care unit, multiple operations, prolonged intubation and powerful complexes of systemic antibiotic administration (16, 35, 37). More than half of all antibiotic treatment courses worldwide are prescribed for patients with upper respiratory tract diseases, urogenital system diseases and soft tissue and skin diseases (8). It is known that most dentists prescribe antibiotics unnecessarily to prevent surgical complications (22). Undoubtedly, this leads to the development of dysbiosis, allergic reactions in patients and the acquisition of antibiotic resistance by microorganisms (2, 16, 33).

Penicillins, cephalosporins and macrolides, active against Gram-positive cocci and fluoroquinolones with a broad spectrum of action against Gram-positive and Gram-negative bacteria are used commonly. However, resistance to them among microorganisms isolated from foci of soft tissue infections of the head and neck has been found to be almost 10% higher than

that among general surgical pathogens. In addition, resistance to other groups of antibiotics has a disappointing prognosis for the near future (29).

Therefore, ongoing and timely monitoring of the sensitivity of the bacteria predominantly isolated from patients with infectious and inflammatory diseases of the facial soft tissue to antibiotics is very important today. This will make it possible to identify, based on retrospective data, a number of drugs to which the Gram-positive bacteria retain sensitivity and can therefore be effectively used for treatment.

Ukraine has been at the epicenter of military operations for a long time, which, accordingly, aggravates the situation with antimicrobial resistance in the country (20). However, the fragmentary nature of the data does not provide a clear summary of the development of antibiotic resistance among dominant microorganisms, which could contribute to the revision of infection treatment tactics (18). For this purpose, it is possible to apply the methods of multivariate statistical research with the subsequent ordering of the collected data according to the principle of homogeneity, based on the frequency of isolation of isolates sensitive to antibiotics. Thus, Ward's method will allow classifying antibiotics according to the frequency with which Gram-positive bacteria retain sensitivity to them, which, in turn, will outline the circle of drugs that continue to be effective in the fight against infections (31).

The aim of the given study was to conduct a retrospective cluster analysis of antibiotics according to their efficacy against the bacteria predominantly isolated from patients with infectious inflammatory diseases of the facial soft tissue.

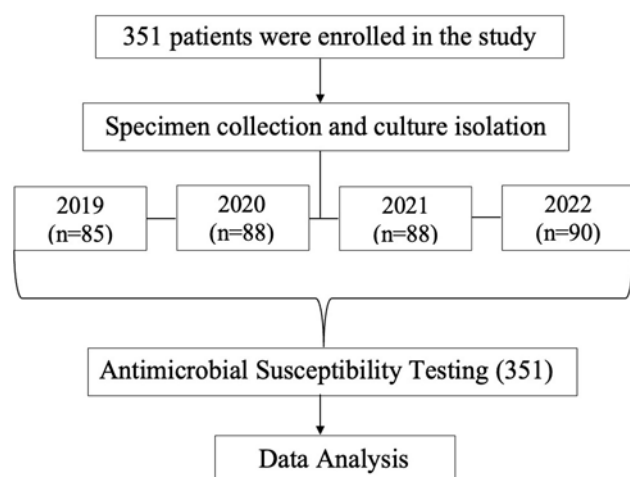
Materials and methods

Study population

The cross-sectional study involved 351 patients who were treated for infectious inflammatory diseases of the soft tissues of the facial area in the department of maxillofacial surgery of the "Poltava Regional Dental Centre - Dental Clinical Polyclinic" of the Poltava Regional Council (Ukraine) during 2019 – 2022 (Fig. 1). All patients were SARS-CoV-2 negative, anti-epidemic measures were followed. Patients with suspected infection until receiving the PCR test were managed according to the protocol of the management of the disease of COVID-19 (12, 13, 23). Pain management was also implemented due to possible post-covid complications (9 – 11).

Inclusion criteria for patients were diagnosis L00-L08 – Infectious skin and subcutaneous tissue diseases according to ICD-10 with consent to participate in the study. Exclusion criteria were non-compliance with L00-L08 diagnosis according to ICD-10, pregnancy, diabetes mellitus, presence of congenital or acquired immunodeficiencies, mental disorders, taking antibiotics before admission to a medical institution on their own and refusal to participate in the study.

Figure 1. A flow chart of the study design.



In addition, written informed consent was obtained from each subject after a detailed explanation of the study objectives and protocol, which was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. The study was approved by the Biomedical Ethics Committee of the Ukrainian Medical Dental Academy (protocol No. 165a of 17.05.2018) (38).

Specimen collection and culture isolation

Purulent exudate was sampled after dissection of the focus of infection using sterile tampons that were placed in tubes with AMIES transport medium. The samples were cultured on blood agar, saline agar, and thioglycol nutrient media at 37°C for 48 hours aerobically followed by culture isolation by standard culture method. Final identification of the isolates was performed using a Vitek 2 Compact automatic bacteriological analyzer (BioMerieux, France) according to the manufacturer's instructions.

Determining the sensitivity of micro-organisms to antibiotics

A standardised Kirby-Bauer disk diffusion method calibrated to EUCAST clinic MIC breakpoints was used to determine the antibiotic sensitivity of the microorganisms examined (15, 24, 28). Unsupplemented Mueller-Hinton agar was used to cultivate the microorganisms, with the addition of 5% mechanically defibrinated horse blood if necessary.

Inoculum suspension was prepared from an overnight microbial culture from a McFarland 0.5 standard followed by incorporation in Petri dishes with agar. For the next 15 min, standard antibiotic disks were placed on the surface of the agar with the microorganisms, followed by incubation at 35±1°C for 20 hours.

The result was assessed by the size of the zone diameter of inhibition of the microorganism around the antibiotic disk and expressed in mm. Zone diameters were interpreted according to the EUCAST clinical

threshold tables, according to which micro-organisms were classified as sensitive, susceptible with increased antibiotic exposure or resistant (15).

Statistical analysis

The sample size was based on available data. The normality of the data distribution was assessed using the Shapiro-Wilk test. Hypothesis testing was two-way. Data are expressed as mean (SD) and median (minimum-maximum), number and percentage (n, %). $p < 0.05$ was considered statistically significant. Categorical data were compared using chi-square test (χ^2), Yates' continuity correction and Fisher's exact test.

In order to determine the groups of antibiotics to which the studied microorganisms retain sensitivity, a hierarchical cluster analysis was performed using the Ward's method (31). The method aims at combining closely spaced clusters and produces clusters of small size. The distance between clusters is the increase in the sum of the squares of the distances of objects to the centers of clusters, resulting from combining them. Variance analysis techniques were used to estimate the distances between clusters. At each step of the algorithm, two clusters were combined that resulted in the minimum increase in the target function, that is, the within-group sum of squares.

Statistical analysis was performed using the standard software 2022 GraphPad Software and IBM SPSS Statistics version 22.0.

Results

A total of 351 clinical isolates were isolated from patients with facial soft tissue infectious inflammatory diseases during 2019 – 2022, including 126 *S. aureus*, 50 coagulase-negative *Staphylococcus* spp. (19 strains *Staphylococcus epidermidis*, 10 strains *Staphylococcus saprophyticus*, 10 strains *Staphylococcus haemolyticus*, 6 strains *Staphylococcus warneri*, 5 strains *Staphylococcus hominis*) and 82 *Enterococcus* spp. (64 strains *Enterococcus faecalis*, 18 strains *Enterococcus faecium*), 60 isolates of viridans streptococci group (10 strains *Streptococcus salivarius*, 17 strains *Streptococcus sanguis*, 26 strains *Streptococcus mitis*, 7 strains *Streptococcus anginosus*) and 33 isolates members of the genus *Kocuria* (20 strains *Kocuria kristinae*, 13 strains *Kocuria rosea*) (Tab. 1).

A retrospective study concerning the sensitivity of the bacteria predominantly isolated from patients with facial infectious and inflammatory diseases to antibiotics found that the sensitivity of *S. aureus* isolates to different antibiotics varied annually during 2019 – 2022 (Fig. 2). Thus, the sensitivity of representatives of this species remained at a stable high level only to vancomycin. It should be noted that in 2020 there was a slight increase in the sensitivity of *S. aureus* to benzylpenicillin, norfloxacin, gentamicin, erythromycin, clindamycin and tetracycline. Thus, the sensitivity of the studied microorganisms to erythromycin and tetracycline was 1.5 times higher in 2020 and 2.4 times higher to clindamycin compared to the corresponding figures in 2019 ($p < 0.05$).

Table 1. Frequency of Gram-positive bacteria isolation from the patients with facial infectious and inflammatory diseases of soft tissues.

Bacteria	Total		2019		2020		2021		2022	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
<i>S. aureus</i>	126	35.9	30	35.3	32	36.4	32	36.4	32	35.6
coagulase-negative <i>Staphylococcus</i> spp.	50	14.2	12	14.1	13	14.8	13	14.8	12	13.3
<i>Enterococcus</i> spp.	82	23.4	20	23.5	20	22.7	20	22.7	22	24.4
viridans streptococci group	60	17.1	15	17.6	15	17.0	15	17.0	15	16.7
<i>Kocuria</i> spp.	33	9.4	8	9.4	8	9.1	8	9.1	9	10.0

However, during 2021 – 2022, we recorded a downward trend in sensitivity among *S. aureus* to benzylpenicillin, amikacin, gentamicin, erythromycin and tetracycline. Moreover, the percentage of susceptible isolates to amikacin and gentamicin decreased to 46.9% and 34.4%, respectively, which were the lowest results recorded since 2019.

Based on the Ward’s cluster analysis results concerning the sensitivity of clinical isolates of *S. aureus* to various antibacterial drugs during 2019 – 2022, 5 clusters were identified in the first step (Fig. 3). Macrolides (erythromycin, azithromycin and clarithromycin), tetracycline, penicillins (benzylpenicillin, oxacillin), lincosamides (clindamycin) and aminoglycosides (amikacin) were combined into one cluster (Ia), the sensitivity of *S. aureus* isolates to which ranged from 52 to 62%; gentamicin was singled out as an antibiotic with the lowest activity against these microorganisms (cluster Ib); vancomycin and moxifloxacin were grouped into cluster Ic as antibiotics with the highest antimicrobial activity against *S. aureus*; the next cluster (Id) consisted of fluoroquinolones: norfloxacin, levofloxacin and ciprofloxacin, to which 70 – 80% of isolates were sensitive; cefoxitin was

also assigned to a separate cluster (Ie) with the share of *S. aureus* sensitive to it (69%).

However, as early as the second step, only two main clusters were identified of the studied antibiotics according to their efficacy against clinical *S. aureus* isolates. Thus, cluster IIa combined Ia and Ib, thereby separating out the antibiotics with the lowest activity against *S. aureus* clinical isolates during 2019 – 2022. In turn, cluster IIb included clusters Ic, Id and Ie (moxifloxacin, vancomycin, ciprofloxacin, levofloxacin, norfloxacin and cefoxitin), identifying the group of antibiotics to which *S. aureus* isolates retained the highest sensitivity during 4 years.

The results of an antibiotic sensitivity study of coagulase-negative *Staphylococcus* spp. indicated an annual decrease in benzylpenicillin efficacy from 58.3% to 46.2% during 2019 – 2022 (Fig. 4). Characterizing the sensitivity of these microorganisms to other penicillins, a slight increase in the percentage of susceptible isolates was found in 2020, but a downward trend appeared in the following years. Thus, the proportion of coagulase-negative staphylococci susceptible to ampicillin and oxacillin reached a critical low of 30.8% in 2022.

At the same time, sensitivity to cefoxitin has hardly changed over the past 4 years and has remained at a level not exceeding 50%. Overall, an analysis of the sensitivity of coagulase-negative *Staphylococcus* spp. to fluoroquinolones showed a worse result compared to the sensitivity of clinical *S. aureus* isolates to this group of antibiotics. While the sensitivity of coagulase-negative *Staphylococcus* spp. to norfloxacin tended to decrease during 2019 – 2022, but did not reach values below 53.8%, the sensitivity result of the studied microorganisms to ciprofloxacin indicated the development of their complete resistance. In addition, coagulase-negative *Staphylococcus* spp. showed variable sensitivity to aminoglycosides in recent years. Despite an increase in the proportion of sensitive isolates to kanamycin in 2021, the overall percentage of *Staphylococcus* spp. susceptible to this antibiotic decreased by 12.9% during

Figure 2. Sensitivity of *S. aureus* isolated from patients with infectious and inflammatory diseases of the facial soft tissues to antibiotics during 2019 (n=30), 2020 (n=32), 2021 (n=32), 2022 (n=32), % per year.

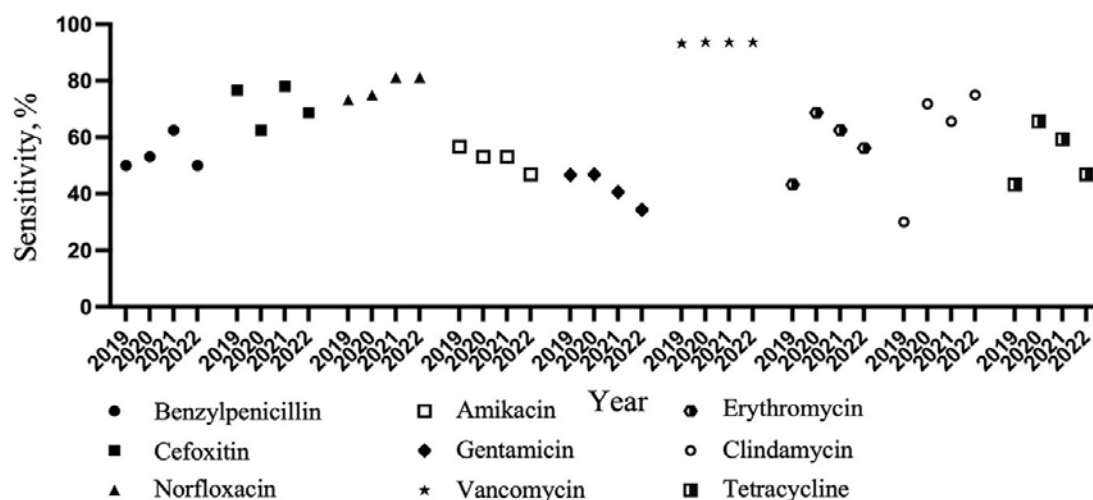


Figure 3. Ward's dendrogram of cluster analysis of antibacterial drugs according to their efficacy against clinical isolates of *S. aureus* (n=126) during 2019 - 2022.

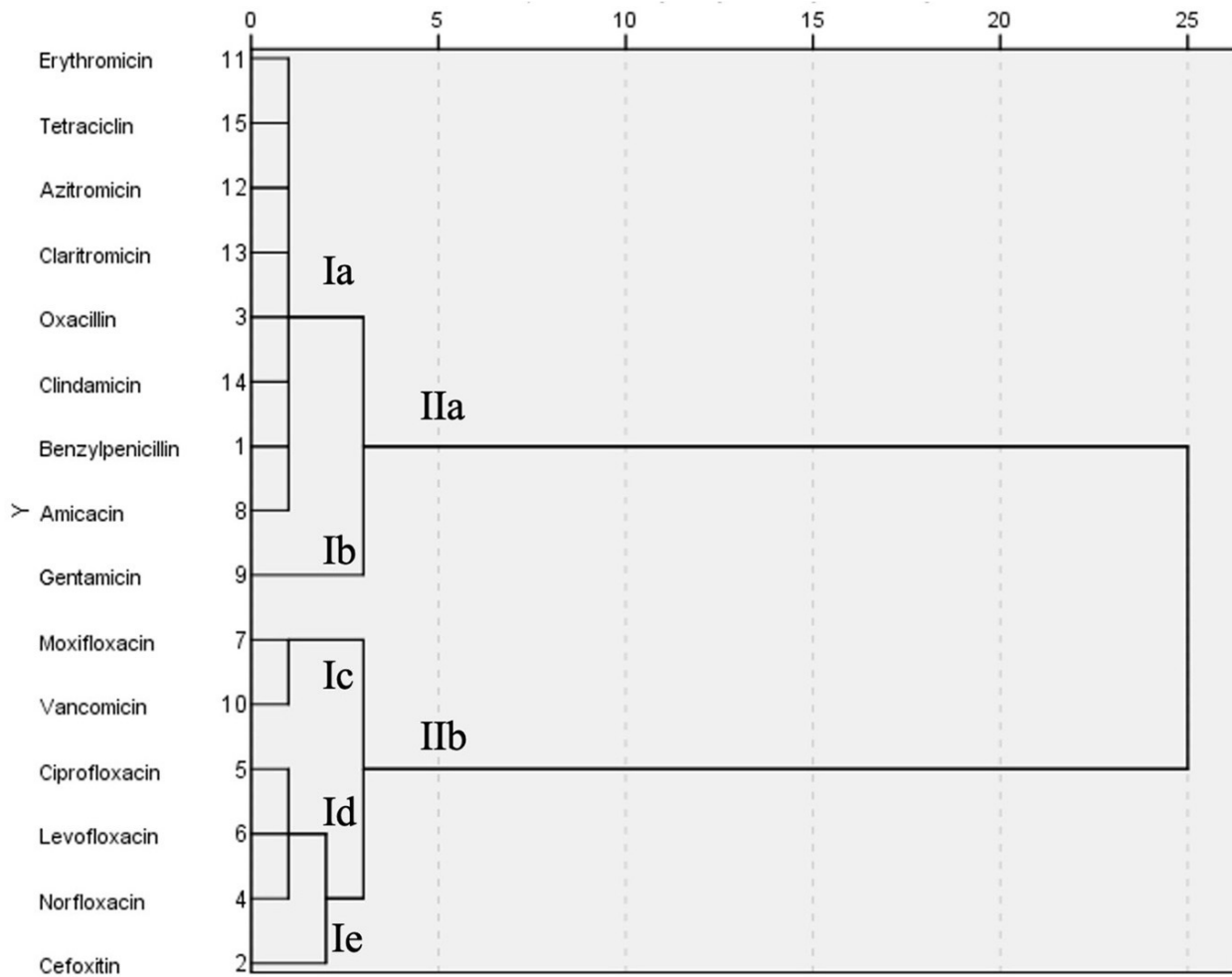
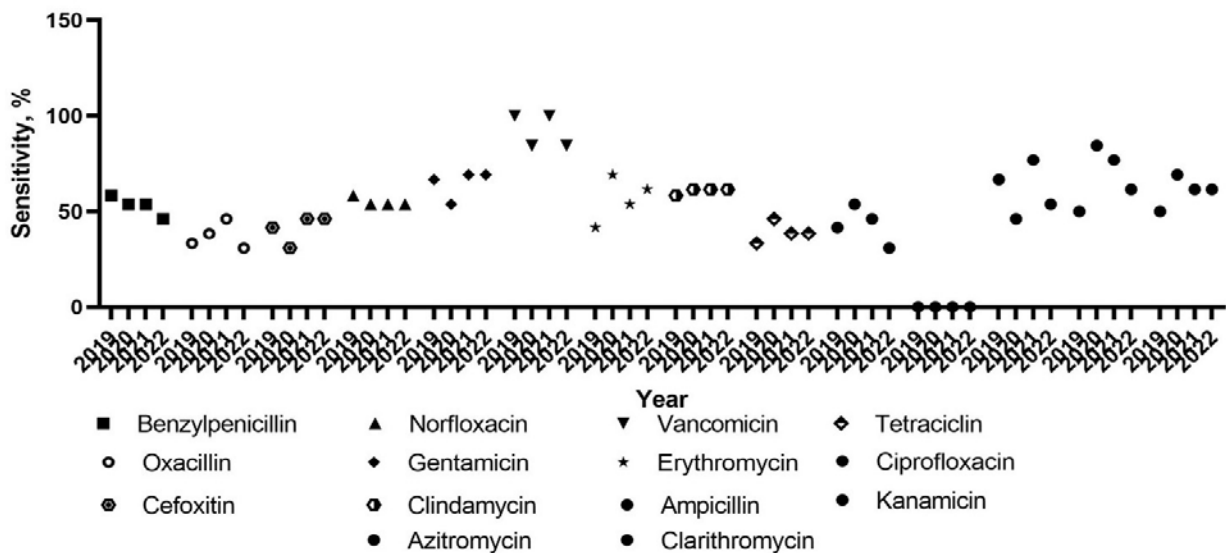


Figure 4. Sensitivity of clinical isolates of coagulase-negative *Staphylococcus* spp. isolated from patients with infectious and inflammatory diseases of the facial soft tissues to antibiotics during 2019 (n=12), 2020 (n=13), 2021 (n=13), 2022 (n=12), % per year.



2022. Whereas sensitivity to gentamicin remained almost at the same level, not exceeding 69.2% it should be noted that clinical isolates of coagulase-negative *Staphylococcus* spp. showed consistently high sensitivity to vancomycin, similar to *S. aureus* isolates. We recorded an increase in macrolide sensitivity among coagulase-negative *Staphylococcus* spp. During 2019 – 2022, the proportions of susceptible test microorganisms to erythromycin, azithromycin and clarithromycin were 20.0%, 11.5% and 11.5% higher, respectively, than their data. Isolates of coagulase-negative *Staphylococcus* spp. to clindamycin and tetracycline did not change significantly during the last 4 years and averaged 60.0% and 40.0%, respectively.

Following Ward's cluster analysis, the antibiotics for which sensitivity was determined among clinical isolates of coagulase-negative *Staphylococcus* spp. were combined into 5 clusters in the first step (Fig. 5). Cluster Ia included norfloxacin, erythromycin and benzylpenicillin, with the proportion of susceptible microorganisms being between 50.0 – 56.0%.

Cluster Ib included kanamycin, clindamycin, gentamicin and azithromycin, with the proportion of susceptible coagulase-negative *Staphylococcus* spp. not exceeding 68.0%. Vancomycin was highly effective against the isolates examined and therefore constituted a separate cluster Ic. Oxacillin, tetracycline, ampicillin and clarithromycin were included in cluster Id, with a sensitivity of 30.0 – 44.0% to the microorganisms. Only ciprofloxacin, as an antibiotic with no antimicrobial effect on coagulase-negative *Staphylococcus* spp.

Attention should be drawn to the result of the third clustering step, which resulted in the formation of cluster IIIa. Its peculiarity was the joining of clusters Ia and Ib to cluster I, thus defining the list of antibacterial drugs that retain activity against coagulase-negative *Staphylococcus* spp.: norfloxacin, erythromycin, benzylpenicillin, kanamycin, clindamycin, gentamicin. In the fourth step of the clustering of antibacterial drugs according to their sensitivity to coagulase-negative isolates of *Staphylococcus* spp. clusters Id and Ie were grouped into cluster IVa. This included oxacillin, tetracycline, ampicillin, clarithromycin and ciprofloxacin, forming the group of antibiotics with the lowest efficacy against coagulase-negative isolates of *Staphylococcus* spp.

The study revealed an increase in ampicillin sensitivity of clinical isolates of the genus *Enterococcus* from 55.0% to 70% during 2019-2021 (Fig. 6). However, in 2022, we observed a rapid decline in the proportion of the genus susceptible to ampicillin to 40.9%. Imipenem showed low efficacy against *Enterococcus* spp., but the percentage of sensitive isolates tended to increase in 2022 (54.5%). High sensitivity of these microorganisms to norfloxacin during all 4 years of the study indicated the effectiveness of fluoroquinolones against isolates of the genus *Enterococcus*.

The sensitivity of *Enterococcus* spp. to gentamicin remained almost unchanged during 2019 – 2022, remaining at 45.0 – 50.0%. It should be noted that the sen-

sitivity of *Enterococcus* spp. to vancomycin was lower than that of *Staphylococcus* spp. A high rate (68.2%) of sensitive isolates of this genus to vancomycin was recorded in 2022. Tigecycline, as a relatively new antibacterial agent, showed high antimicrobial activity against enterococci during the study. However, the percentage of susceptible isolates tended to decrease every year for the last 4 years. Yes, the percentage of *Enterococcus* spp. susceptible to tigecycline in 2022 was 26.8% lower compared to this figure for 2019. The efficacy of linezolid on *Enterococcus* spp. in 2021 and 2022 decreased compared to this figure during 2019 – 2020, but the percentage of susceptible isolates did not exceed 55.0%.

The first step of the Ward's cluster analysis of antibiotics according to their sensitivity to clinical isolates of *Enterococcus* spp. during 2019 – 2022 identified 4 clusters (Fig. 7). Cluster Ia combined imipenem, gentamicin and linezolid with susceptible micro-organism particles not exceeding 50.0%. Cluster Ib included ampicillin and vancomycin, whose percentages of susceptible *Enterococcus* were 58.5% and 64.6%, respectively. Cluster I included norfloxacin and cluster Id included tigecycline as antibiotics with high efficacy against clinical isolates of the examined genus, which were already combined in the next second clustering step into cluster IIa. Whereas the other antimicrobials studied (clusters Ia, Ib) were grouped in cluster IIIA in the third step of the cluster analysis, indicating their lower efficacy against clinical isolates of *Enterococcus* spp.

During 2019 – 2022, viridans streptococci group showed low sensitivity to all the antibiotics recommended for testing by the EUCAST committee, except vancomycin (Fig. 8). The sensitivity of these microorganisms to glycopeptide increased by 33.3% in 2022 compared to this figure in 2019 and was 100.0%. Clinical isolates of viridans streptococci group showed a similar pattern of changes in sensitivity to gentamicin and clindamycin during 2019 – 2022. Thus, in 2020, there was a slight increase in the proportion of the studied microorganisms sensitive to these antibiotics.

The antibiotics to which sensitivity was determined among viridans streptococci group isolates were divided into 3 clusters in the first step of the cluster analysis (Fig. 9). Cluster Ia consisted of benzylpenicillin, gentamicin and clindamycin, to which 35.0 – 43.4% of the isolates were sensitive. Moxifloxacin comprised cluster Ib as the antibiotic with the lowest proportion of susceptible streptococci. Cluster Ic included only vancomycin, which showed the highest efficacy (81.7%) against viridans streptococci group.

In the second step of the analysis, clusters Ia and Ib were combined into one cluster IIa, thus creating a group of antibiotics that had low efficacy against viridans streptococci group isolates. Alongside this, vancomycin was not joined to any of the antibiotics due to a lack of statistical similarity.

Clinical isolates of *Kocuria* spp. isolated from patients with facial soft tissue infectious inflammatory di-

Figure 5. Ward's cluster analysis dendrogram of antibacterial drugs according to their efficacy against clinical isolates of coagulase-negative *Staphylococcus* spp. (n=50) during 2019 – 2022.

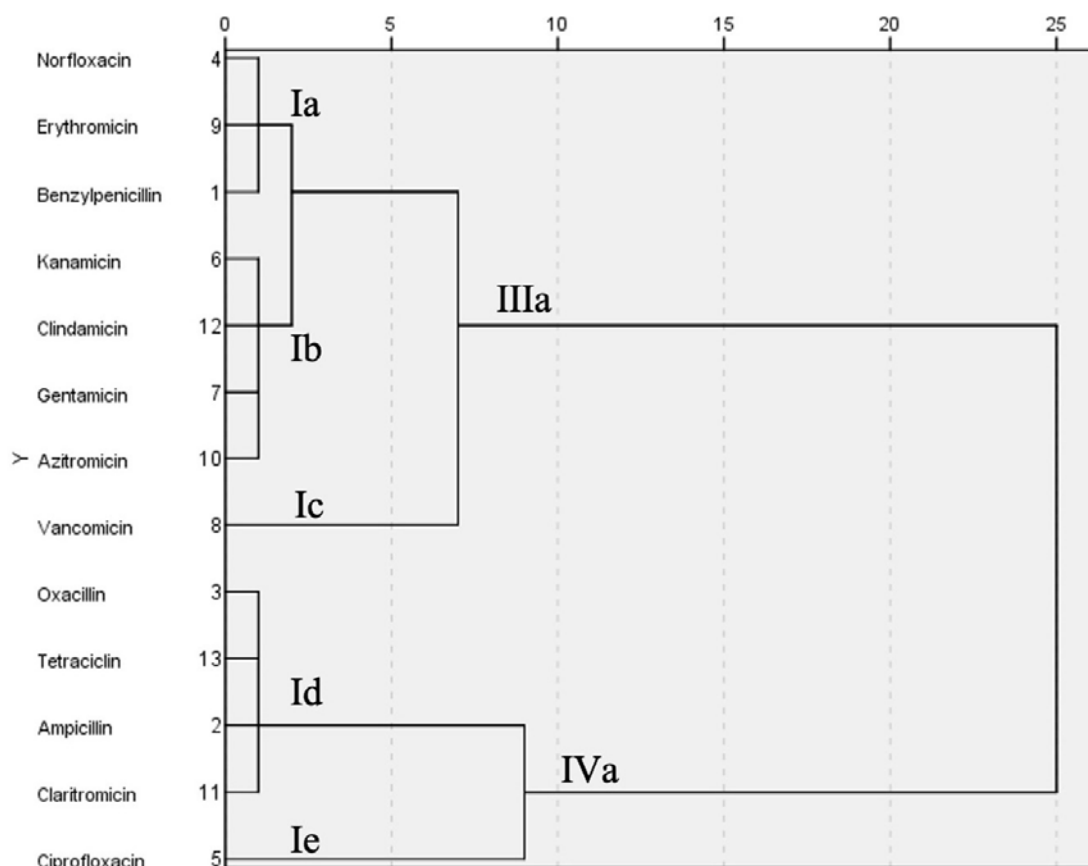
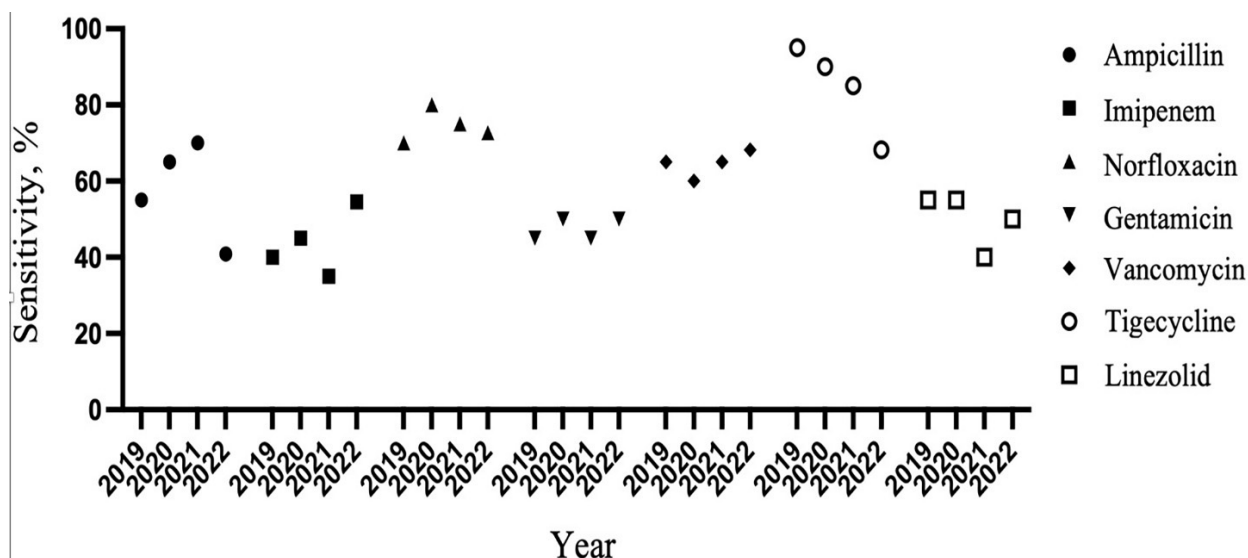


Figure 6. Sensitivity of clinical isolates of *Enterococcus* spp. isolated from patients with infectious and inflammatory diseases of the facial soft tissues to antibiotics during 2019 (n=20), 2020 (n=20), 2021 (n=20), 2022 (n=22), % per year.



seases showed variable sensitivity to all antibacterial drug groups during 2019 – 2022 (Fig. 10). However, their sensitivity to benzylpenicillin and fluoroquinolones (ciprofloxacin and moxifloxacin) showed a similar trend with a decrease in 2020 to a critically low level, followed by a rise in 2021.

A similar pattern in the sensitivity of isolates of the genus *Kocuria* to amoxicillin and cephalosporins (cefotaxime, ceftazidime) was established, the sensitivity to which remained at the same level in 2019 and 2020: 37.5%, 50.0%, 50.0, respectively. In 2021, an increase in the sensitivity of *Kocuria* spp. to the above-mentioned

Figure 7. Ward's cluster analysis dendrogram of antibacterial drugs according to their efficacy against clinical isolates of *Enterococcus* spp. (n=82) during 2019 – 2022.

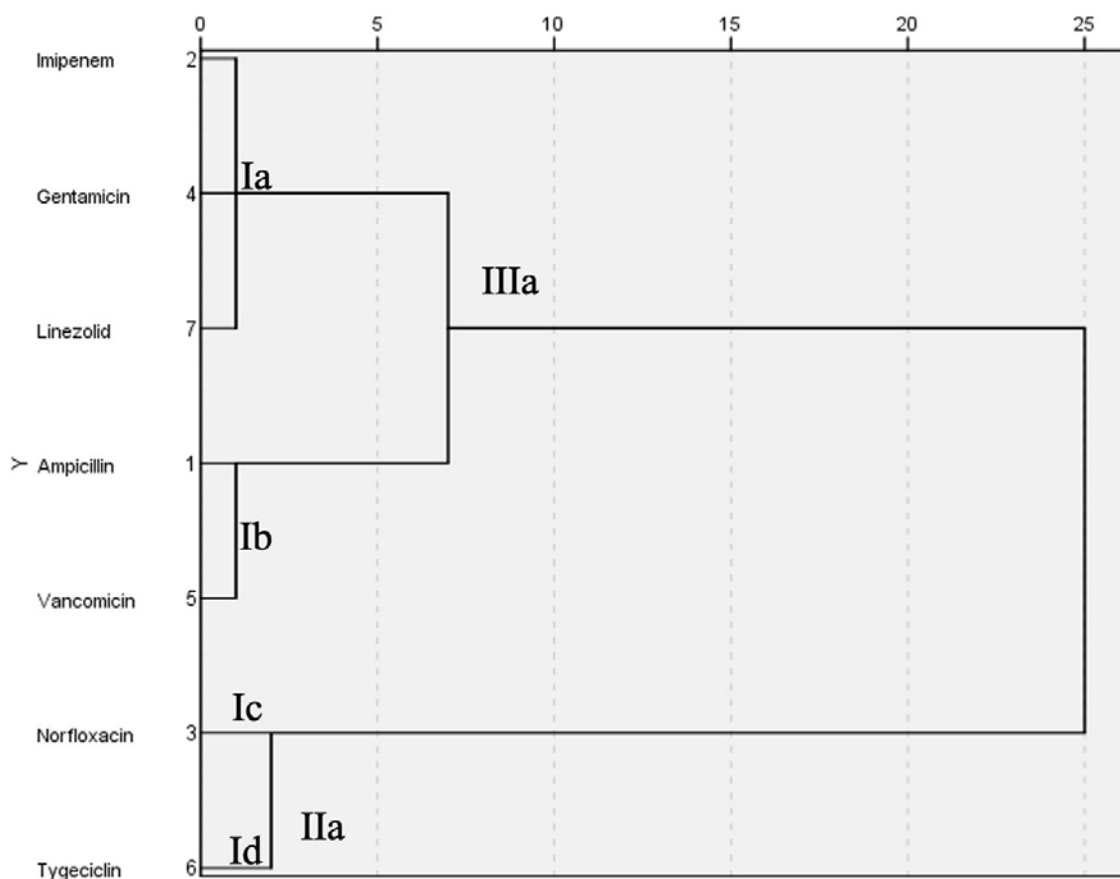
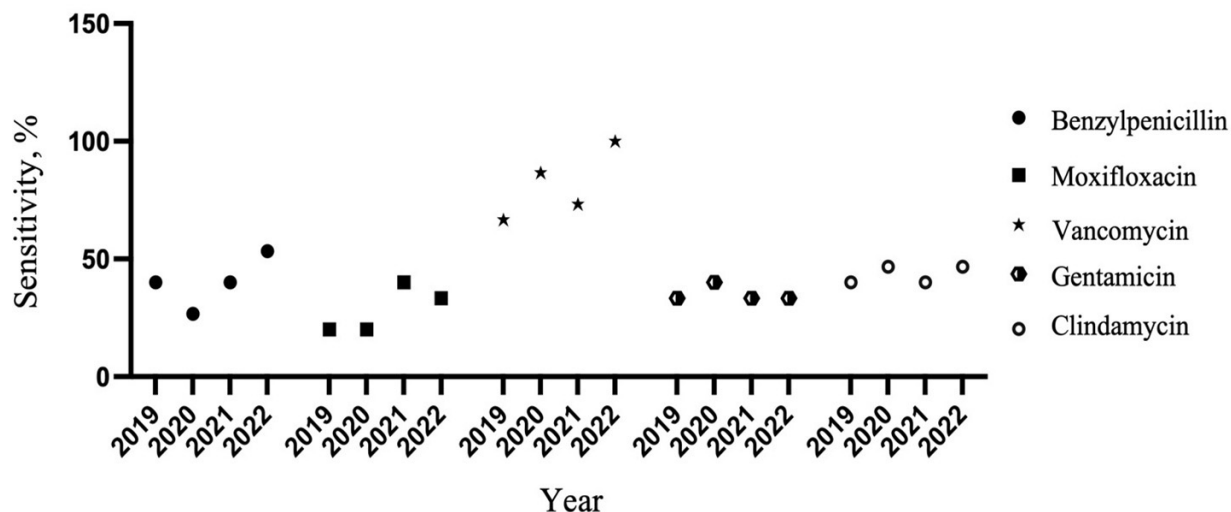


Figure 8. Sensitivity of clinical isolates of viridans streptococci group isolated from patients with infectious and inflammatory diseases of the facial soft tissues to antibiotics during 2019 (n=15), 2020 (n=15), 2021 (n=15), 2022 (n=15), % per year.



antibiotics was recorded to 62.5%, with a further decrease in 2022.

The efficacy of gentamicin against isolates of the genus *Kocuria* has declined annually since 2019, decreasing by 16.7% over 4 years in 2022. Undoubtedly, vancomycin demonstrated better antimicrobial activity against *Kocuria*. However, although the detection rate of susceptible isolates increased in 2020, it began to decline rapidly during 2021 – 2022. In turn, the sensi-

tivity of *Kocuria* spp. to meropenem remained constant (62.5%) during 2019-2021. In 2022, the sensitivity decreased by almost 7.0%.

The cluster analysis of antibacterial drugs, according to the sensitivity of *Kocuria* spp. isolates, resulted in 4 clusters in the first step (Fig. 11). Amoxicillin and gentamicin formed cluster Ia, to which *Kocuria* spp. had the lowest sensitivity. Cluster Ib included ciprofloxacin, moxifloxacin, cefotaxime, ceftazidime and benzylpenicillin,

Figure 9. Ward's cluster analysis dendrogram of antibacterial drugs according to their efficacy against clinical isolates of viridans streptococci group (n=60) during 2019 - 2022.

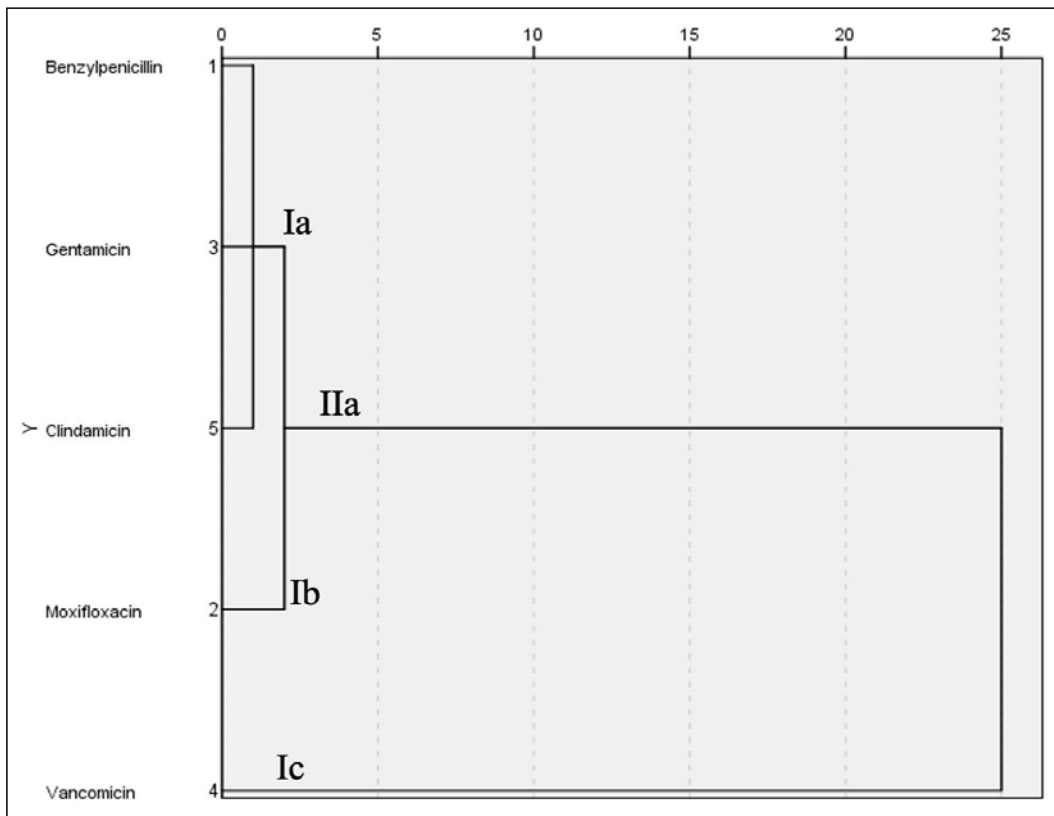
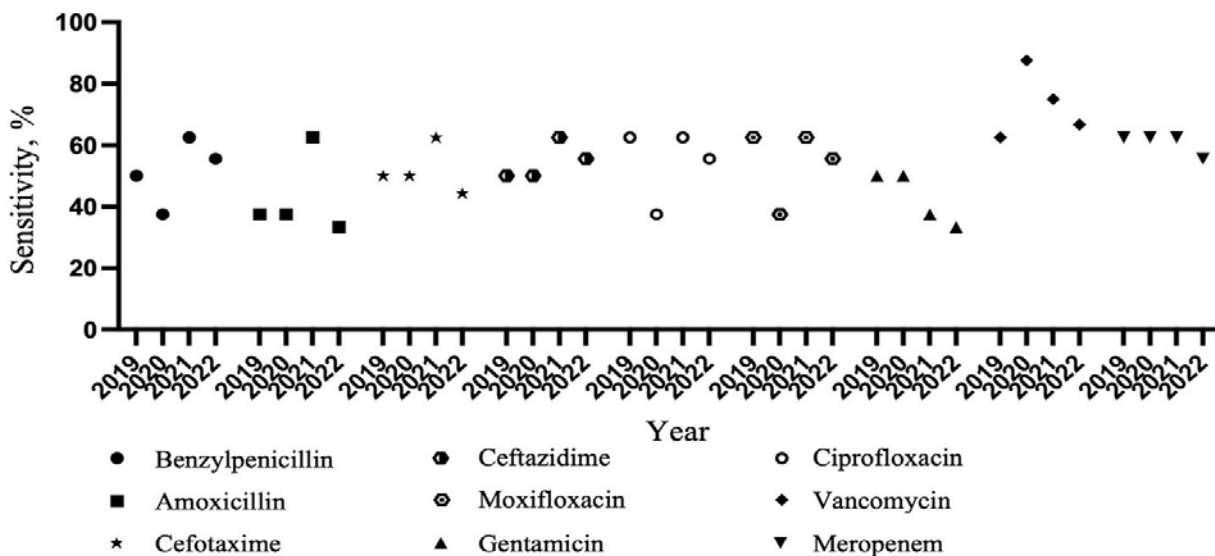


Figure 10. Sensitivity of clinical isolates of *Kocuria* spp. isolated from patients with infectious and inflammatory diseases of the facial soft tissues to antibiotics during 2019 (n=8), 2020 (n=8), 2021 (n=8), 2022 (n=9), % per year.



with the proportion of susceptible isolates of *Kocuria* spp. 50.0 - 55.0%. Meropenem constituted a separate cluster Ic, to which sensitivity among these microorganisms was 60.6%.

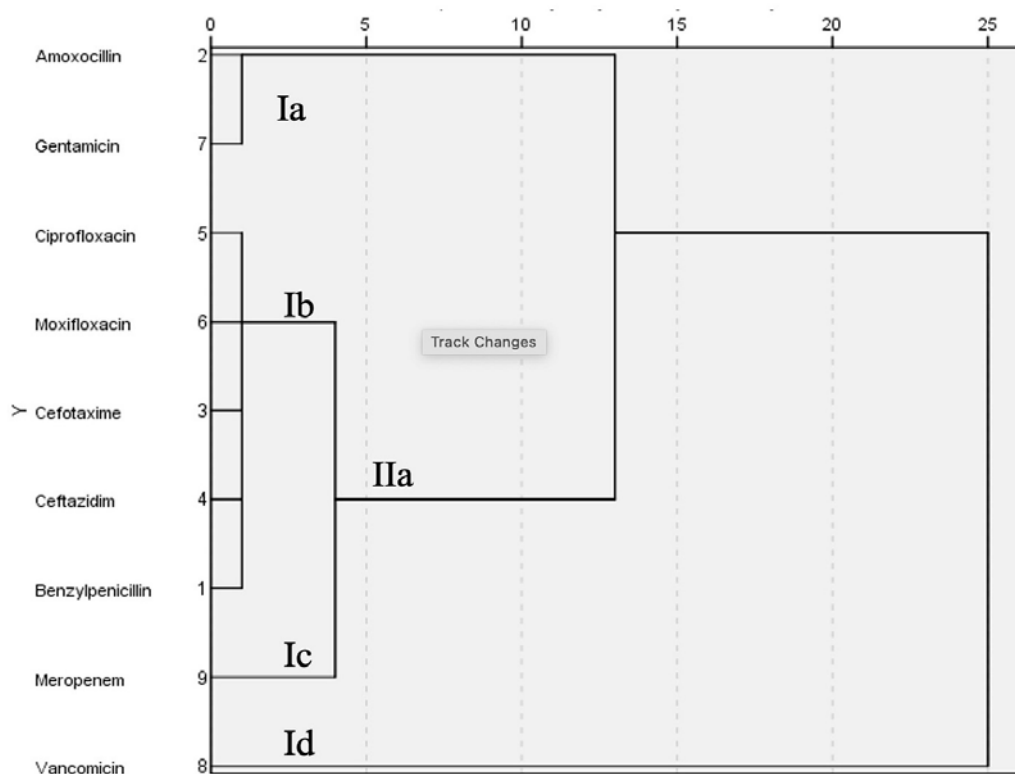
Cluster Id was represented by vancomycin, as an antibiotic of high efficacy against members of the genus *Kocuria*. In the second step of the analysis, clusters Ib and Ic were combined into cluster IIIa, which showed suscep-

tibility rates of *Kocuria* above 50.0%, but below that of vancomycin. Clusters with high and low rates of susceptible microorganisms were merged into clusters with others in later clustering steps with a large similarity distance.

Discussion

The results of susceptibility testing for pathogens of infectious and inflammatory diseases of the facial soft

Figure 11. Ward's cluster analysis dendrogram of antibacterial drugs according to their efficacy against clinical isolates of *Kocuria* spp. (n=33) during 2019 – 2022.



tissues over the past four years differed significantly depending on both the genus of the pathogen and the year of isolation of the microorganism. Undoubtedly, throughout the study, common patterns were observed regarding the variability in the sensitivity of Gram-positive microorganisms to betalactams, macrolides and aminoglycosides. Antibiotics of these groups are inactivated by bacterial cell enzymes, that is, they activate a common mechanism of resistance development (30).

As a result of the conducted research, an increase in the percentage of sensitive pathogens of infectious and inflammatory diseases of the soft tissues of the face to some antibiotics, mainly beta-lactams, was established during 2020 and 2021. Most likely, such changes were associated with an increase in the frequency of prescribing antibiotics in the treatment of co-infections during the COVID-19 pandemic in Ukraine, as in the whole world. During the outbreak, a large number of broad-spectrum antibacterial agents were used empirically (21, 25). An increase of antibiotics use and their active combination can lead to temporal increase of cases of sensitive to bacteria isolated at the start. However, at the same time, in the following year, we observed a rapid decrease in sensitivity to these antibiotics among the gram-positive microorganisms studied. A similar trend was detected by Chinese scientists, whose retrospective analysis indicated an increase in antibiotic resistance to carbapenems among enterococci by almost 40% after the peak of the pandemic in their country (26).

In general, the results of the annual sensitivity of pathogens of infectious and inflammatory diseases of the soft tissues of the face to antibiotics varied quite significantly during 2019 – 2022. This did not make it possible to draw unequivocal conclusions about the choice of antibacterial drugs appropriate for use in the treatment of these infections today. For this purpose, a cluster analysis of the data was carried out, which made it possible to combine antibiotics according to the sensitivity of microorganisms to them, identifying a number of drugs that still remain promising for therapy in surgical dentistry.

Thus, clinical isolates of *S. aureus* maintain sensitivity to the greatest extent to fluoroquinolones (moxifloxacin, ciprofloxacin, levofloxacin and norfloxacin) and, certainly, to vancomycin, as the drug of choice in the treatment of infections caused by resistant strains of *Staphylococcus aureus*. Such results confirm literature data on the low level of resistance of *S. aureus* to fluoroquinolones, the level of resistance to which does not exceed 12% (14, 27).

Coagulase-negative *Staphylococcus* spp. mainly demonstrated stable sensitivity to aminoglycosides and macrolides, which together with norfloxacin and benzylpenicillin form a cluster of antibiotics that are promising for the treatment of infections caused by them. It is worth noting that a similar trend of low sensitivity to fluoroquinolones and high sensitivity to aminoglycosides persists among coagulase-negative *Staphylococcus* spp. for several years (19). The prospects for the anti-

microbial activity of drugs against *Enterococcus* spp. are quite disappointing. A number of authors indicate the presence of genes that ensure resistance of enterococci to macrolides, penicillins, fluoroquinolones and even glycopeptides (7, 32, 39). That is why, as a result of our study, cluster analysis revealed only norfloxacin and tigecycline as antibiotics with the highest proportion of *Enterococcus* species sensitive to them. In turn, clinical isolates of viridans streptococci group and *Kocuria* spp. showed sensitivity only to vancomycin during 2019 – 2022, which did not allow any other antibiotic to be added to it.

Conclusions

According to the results of cluster analysis, clinical isolates of *S. aureus* isolated from foci of infectious and inflammatory diseases of the facial soft tissue retain sensitivity to vancomycin, moxifloxacin, ciprofloxacin, levofloxacin, norfloxacin and cefoxitin. Coagulase-negative *Staphylococcus* spp. are most sensitive to norfloxacin, erythromycin, azithromycin, benzylpenicillin, kanamycin, gentamicin and clindamycin and *Enterococcus* spp. to norfloxacin and tigecycline. Rod *Kocuria*, Rod *Streptococcus*, drug *S. viridans* isolates retained a stable sensitivity only to vancomycin in a retrospective analysis.*

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