Original article

Phenotypic virulence and antibiotic resistance features of microbial strains isolated from dental-plaque associated oral lesions

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Abstract

Background: Oral microbiota represents a highly complex microbial community living in a fine equilibrium with the host organism and any disturbance can lead to a series of oral pathologies. For this reason, investigating the resistance and virulence features of bacterial strains associated with oral pathology is essential for developing effective strategies with preventive or therapeutic value.

Objectives: This study focused on identifying a series of soluble virulence factors (hemolysins, lipase, gelatinase, caseinase, lecinthinase, esculinase, amylase, and DNase) as well as the antibiotic resistance profiles of bacterial and fungal strains isolated from the oral cavity of patients with dental plaque-associated pathologies.

Keywords virulence factors, antibiotic resistance, oral microbiota.

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The oral microbiota represents the second-largest bacterial community after the intestinal microbiota [1] and includes all resident microorganisms of the oral cavity [2-3]. In the oral cavity, microorganisms inhabit the supra- and subgingival tooth surface, tongue, oral mucosa, soft and hard palate, and saliva, with most of the bacteria in saliva being attached to human exfoliated epithelial cells[4]. The human oral microbiome comprises over 2000 taxa of bacteria and fungi co-existing in a complex delicate equilibrium[5-6]. However, under different conditions, such as poor oral hygiene, trauma, broad-spectrum antibiotics, immunosuppression, smoking, and denture wear, an imbalance can occur in the oral microbiota, exposing the organism to various oral and systemic diseases [7-10]. Dental plaque is a non-mineralized biofilm formed by aggregates of resident and/or pathogenic microorganisms and an extracellular matrix of a polymeric nature, structures attached to each other or attached to a solid surface [11-12]. Oral biofilms are the main etiological factor of various oral pathologies, such as dental caries, periodontal diseases, implant-related infections, and oropharyngeal candidiasis [13].

Infection occurs when the virulence, number, and exposure time supersede the local and general host’s defense, leading to a pathological reaction in the host’s tissues [14]. Virulence is defined as the ability of an organism to infect the host and cause disease. Virulence factors can be secreted extracellularly or associated with the cell envelope, the last category including molecules that allow bacteria to colonize the host at the cellular level [15]. The expression of these virulence factors, and therefore, the microbial pathogenicity level is dependent on the host condition [14, 16]. Hemolysins are virulence factors produced by various bacterial species. These compounds are responsible for cell membrane damage, cell lysis, and cell and tissue destruction to provide nutrients (and iron) to hemolysin-producing bacteria [17]. After spot cultivation on blood agar medium and incubation, hemolysis areas can be observed due to the lysis of red blood cells in the culture medium. Beta (complete) hemolysis is represented by the appearance of a clear, transparent halo around the bacterial colonies [18]. Alpha (partial) hemolysis is represented by the appearance of a pink or green halo around the bacterial colonies. Other extracellular enzymes are caseinase and gelatinase. They are proteases that hydrolyze proteins to peptides and amino acids, destroying the host’s tissues and the progression of the infection [18]. Gelatinase is useful in bacterial biofilm formation allowing bacterial cells to aggregate into microcolonies while also being able to destroy the host tissue [19]. Starch is a polysaccharide with a high molecular mass that cannot be transported through the cell membrane to the interior of the bacterial cell, the secretion of extracellular amylases being necessary to achieve its hydrolysis[20]. Esclulin (a glucoside) is hydrolyzed to glucose and esculentol. In the presence of iron citrate (FeC\textsubscript{6}H\textsubscript{5}O\textsubscript{7}) (Fe\textsuperscript{3+}) in the environment, esculentol released under the action of β-glucosidase (esculinate) leads to the formation of a black precipitate of ferric esculentol, a phenolic compound with Fe\textsuperscript{3+}, whose chemical structure is not fully known [18]. It has been shown that esculentol can fix iron chelators (such as those of the transferrin type), thus providing essential iron ions to bacterial cells to activate genes and express virulence factors. The role of the esculentol is particularly important for extracellular pathogenic bacteria because iron ions are present in small amounts in the extracellular environment, with most of the iron ions circulating in the bound form [18]. To test this virulence factor, the bacterial strains were seeded on esculin containing medium, the presence of a black precipitate around the microbial colonies indicating a positive result. Bacterial deoxyribonucleases (DNases) are enzymes that hydrolyze bacterial nucleic acids producing oligonucleotides used in their syntheses [20-21]. DNases can be involved in several important processes, such as bacterial growth and biofilm maturation, but are also involved in the ability of bacteria to escape the host immune system [20].

Antibiotic resistance is becoming increasingly problematic. It is necessary to know the antibiotic sensitivity or resistance profile of bacterial strains isolated from the oral cavity because bacterial resistance to certain antibiotics can affect the antibiotic treatments recommended in dental conditions. While facing changes in the oral microenvironment, microorganisms can express antibiotic resistance genes, ensuring their survival and genetic persistence, the oral cavity thus becoming a source of antibiotic resistance genes, causing an increase in the number of resistant bacterial infections [22]. Despite the clinical relevance and frequency of dental and oral-maxillofacial infections, there is a lack of recent data on the spectrum of clinical pathogens and associated antimicrobial resistance for those infections [23].

In this regard, this study provides the identification of soluble virulence factors (i.e., hemolysins, lipase, gelatinase, caseinase, lecithinase, esculinate, amyrase, and DNase) and the antibiotic resistance profiles of oral cavity-isolated microorganisms, aiming to offer a reliable framework for developing effective preventive and therapeutic strategies against dental pathological conditions.

Materials and Methods
The analyzed bacterial and fungal strains (125) were selected from previously isolates from various oral patholo-
cies, such as dental caries, periodontal diseases, implant-related infections, and oropharyngeal candidiasis, included in the Microbial Collection of the Research Institute of the University of Bucharest.

The identification of soluble virulence factors expressed by bacterial strains was analyzed by cultivation-dependent methods. A bacterial suspension with a density of 0.5 McFarland was obtained from the 24 hour bacterial strains previously cultivated on culture media, which was spotted in a volume of 10 μl on culture media supplemented with a specific substrate for the detection of virulence factors such as hemolysins, lipase, gelatinase, caseinase, lecithinase, esculinase, amylase, and DNase. The inoculated media was incubated for 24 h at 37°C to allow the production and detection of soluble virulence factors, with samples analyzed at 24, 48, and 72 h post-incubation. To evaluate the hemolysin production, the bacterial strains were seeded on blood agar. A positive reaction to the presence of hemolysins is indicated by the presence of a transparent halo around the bacterial colonies, indicating the hemolysis of erythrocytes in the composition of the culture medium. Tween 80 agar was used to evidence the presence of lipase with the formation of an opaque halo around the bacterial colonies, indicating a positive reaction and the absence of the halo a negative reaction [24-25]. Lecithinase was highlighted following cultivation on a culture medium supplemented with egg yolk substrate, the positive reaction being represented by an opaque zone (precipitation) and/or a clear zone around the culture spot. To show the production of proteases, the strains were spotted on solid media with the addition of casein or gelatin, and the presence of a precipitation/clarification zone around the growth area indicated the proteolysis of casein/gelatin (the presence of caseinase/gelatinase) [18, 25]. Esculinase was studied on agar culture medium with esculin by the appearance of a black compound around the bacterial colonies. Agar medium with starch was used to bring out the presence of amylase, and after incubation, Lugol solution was added over the plate with culture medium. The positive result consisted of the appearance of a yellow clarification zone around the bacterial colonies [25]. Bacterial strains were inoculated on agar culture medium with DNA to observe the presence of DNases. A positive response indicating the production of DNase is indicated by the appearance of a yellow halo around the colony on the blue agar.

To establish antibiotic resistance profiles, the inoculum used for seeding was represented by a bacterial suspension made from a pure bacterial culture developed on a solid culture medium (PCA medium), reported on a standard density scale of 0.5 MacFarland or 10⁶ CFU/ml. The bacterial inoculum was seeded with a sterile cotton pad after soaking it in bacterial suspension and removing the excess suspension on the tube’s inner walls [20]. Antibiotics were placed on the culture medium using a dispenser, after which the plates were incubated at 37°C for 24 hours. Reading the results was carried out by a graduated ruler used to measure the diameters of the inhibition zones around each disc with antibiotic [20]. The data obtained from the measurements was reported to standardized tables for the diffusimetric method, recommended by CLSI (Clinical and Laboratory Standards Institute). For anaerobic bacteria, the results obtained were reported by the diffusimetric method according to the EUCAST (The European Committee on Antimicrobial Susceptibility Testing) standard. Based on the results, it was established whether a bacterial strain is sensitive (S), resistant (R), or intermediately sensitive (I) [18].

Results and discussion

Virulence factors

The selected strains were divided into seven microbial groups: aerobic Gram-positive cocci, anaerobic Gram-positive cocci, Gram-positive bacilli, fermentative Gram-negative bacilli, non-fermentative Gram-negative bacilli, anaerobic bacteria, and yeasts. The virulence profiles of different groups are presented in Figure 1.

Regarding the virulence factors for strains belonging to the group of aerobic Gram-positive cocci, hemolysins predominated (Figure 1A). Lipases and lecithinases are included in the category of pore-forming enzymes at the level of the eukaryotic cell membrane leading to the destruction of the lipid content in the membrane structure [18].

Staphylococcus was the predominant genus identified in the gram-positive aerobic cocci-producing hemolysins group. In particular, S. aureus is known to exhibit numerous virulence factors such as capsule, coagulase, teichoic acid, polysaccharides, and adhesins; enzymes such as esterases, alpha, beta, gamma, and delta hemolysins, fatty acid modifying enzymes, various proteases, hydrolytic enzymes, catalase, β-lactamase, and various toxins such as leukocidin, enterotoxins, TSST-1 [26-27]. Staphylococcal hemolysins are predominant and the best characterized among the virulence factors expressed by S. aureus, being very important in the pathology of staphylococcal infections through their ability to destroy host cells, including cells of the immune system, allowing the spread of bacteria inside the host [27]. The most studied hemolysin from S. aureus is α-hemolysin, encoded by the hla gene, which causes lysis of host cells, such as epithelial cells, endothelial cells, erythrocytes, monocytes, and keratinocytes, causing cell membrane damage and their apoptosis [28]. Hemolysin β is a non-pore-forming hemo-
Figure 1. Virulence factors profiles in the analyzed strains. (A) Gram-positive aerobic cocci; (B) Gram positive anaerobic cocci; (C) Fermentative Gram-negative bacilli; (D) Gram-negative non-fermentative bacilli; (E) Actinomycoses spp; (F) Other Gram-positive bacilli; (G) Anaerobic bacteria; (H) Yeasts.
lysin, a neutral sphingomyelinase secreted by most strains of *S. aureus*, also called warm-cold hemolysin due to the enhanced hemolytic activity observed below 10°C after incubation at 37°C [28]. Gamma hemolysin increases the survival of the bacterial strain of *S. aureus* in human blood, with recent studies showing that strains that present gamma hemolysin are associated with bloodstream infections, including bacteremia and septic arthritis in mouse experiments and endophthalmitis in rabbits [28]. Also, in the study by Kim and Lee, 2015 [29] on *S. aureus* strains isolated from patients with periodontitis, virulence genes for hemolysins were detected, i.e.: hla, hlb, and hld.

Other frequent virulence factors are DNase, caseinase, lecithinase, and lipase, while amylase and esculine were less frequent in the analyzed strains. Lipase is an important enzyme that has implications for the pathogenesis of some microbial strains, which can form pores in the membranes of eukaryotic cells and alter the lipid content of these cells [18]. *S. aureus* secretes 2 lipases, lipase 1 (SAL1) and lipase 2 (SAL2), encoded by the gehA and gehB genes, respectively. The enzymatic activities of lipases are conferred by the catalytic triad, consisting of serine, aspartate, and histidine [30]. Although they share a similar catalytic mechanism, SAL1 and SAL2 are different, such that the SAL1 lipase functions optimally at pH 6 and is stable under acidic conditions but is inactivated when the pH is above 10. At the same time, the SAL2 lipase functions optimally around pH 8 and is inactive under acidic conditions [30].

In strains of Gram-positive anaerobic cocci, the predominant virulence factors were amylase and DNase (Figure 1B). Esculinase, caseinase, lecithinase, gelatinase, and lipase had medium frequency, while hemolysins were identified in fewer strains. The presence of a relatively high percentage of the proteases represented by caseinase and gelatinase could be explained by the presence of the *Streptococcus* genus within this group of bacteria, which is most often associated with the occurrence of dental caries, thus damaging the structure of the dental surface. Starch digestion involves enzymatic degradation, starting at the level of the oral cavity with the formation of maltose and maltodextrins, resulting in a high amount of carbohydrates for the nutrition of oral bacteria [31]. Oral streptococci are the commensal bacteria that colonize the oral cavity and dental plaque biofilm, with some strains exhibiting surface proteins that bind α-amylase, the predominant enzyme in the saliva of many mammalian species [32]. Amylase participates in the formation of the salivary film at the level of dental structures, the bacteria developing adhesion mechanisms to the film by binding to different amylase components, which leads to the initiation of bacterial dental plaque formation, which can also facilitate starch metabolism and bacterial development [32]. The amylase binding site is present in the enzyme’s glycosylated and non-glycosylated forms [31]. Salivary alpha-amylase exists as monomeric and dimeric forms with calcium and chloride ions, enhancing its enzymatic activity. The ability of α-amylase to bind to microorganisms is a calcium- and enzyme-activity-independent process [31]. Significant evidence supports salivary amylase’s role in the production of dental caries. Studies in animals infected with *S. mutans* revealed that a high-starch diet in the absence of sucrose resulted in a lower frequency of caries production; cultivation of the bacterial strain *S. mutans* on the starch substrate in the absence of sucrose produced small amounts of biofilms and glucans on saliva-coated hydroxyapatite discs [33]. *Enterococcus faecalis* can resist antimicrobial substances and survive in a hostile, oligotrophic environment with increased pH that can reach up to 11.5 [34]. The association of the bacterial strain *E. faecalis* with the failure of endodontic treatments is due to the ability of this bacteria to invade the dentinal tubules and adhere to the collagen fibers present in the dentin structure [35]. The increased virulence of the *E. faecalis* strain is due to enterococcal surface proteins, aggregating substances, serine proteases, hemolysins, gelatinases, and capsular polysaccharides [35]. Also, *Enterococcus faecalis* has been associated with endodontic infections, and studies by researchers on the virulence factors of this strain have shown that this species expresses factors such as gelatinase and hemolysins. Dahlén et al., 2012 [36], and Komiyama et al., 2016 [37] reported the presence of lipase, hemolysins, and gelatinase, while Khadijeha et al., 2019 [38] reported the presence of extracellular surface proteins and gelatinase.

Esculinase, gelatinase, and caseinase are the dominant virulence factors within the group of fermentative Gram-negative bacilli (Figure 1C). Hemolysins and DNase were present in a moderate percentage, and amylase, lipase, and lecithinase were in a lower number compared to the other virulence factors.

The results obtained for the group of non-fermentative Gram-negative bacilli (Figure 1D) indicated a high frequency for esculinase and gelatinase, moderate for lecithinase, lipase, caseinase, hemolysins, and DNase, and some reduced strains were positive for amylase.

The results obtained regarding the virulence factors for *Actinomyces* strains indicated the predominance of hemolysins (Figure 1E), followed by amylase, DNase, and esculin. Few details are known about the virulence factors produced by *Actinomyces* species but in general; these bacteria are present in polymicrobial communities where the factors produced by *Actinomyces* would contribute to the pathologi-
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In the dental process, these species being involved in the formation of dental plaque; *Actinomyces spp* also interact with other plaque bacteria such as *Fusobacterium, Prevotella* and *Vieillotella* maintaining the integrity of bacterial plaque [40].

Hemolysins, DNase, and esculinase were the virulence factors for strains of the predominant Gram-positive bacilli genera (Figure 1F). Factors with a moderate frequency are caseinase and lipase, while virulence factors with a low frequency are amylase, gelatinase, and lecithinase. Amylase, especially α-amylase, is an important biological product with wide applications in clinical practice and industry, and for this reason, microorganisms are considered cell suppliers to produce α-amylase, especially *Bacillus subtilis* [41].

The distribution of the virulence factors of the anaerobic bacteria in order of frequency was as follows: esculinase, DNase, amylase, lecithinase, lipase, hemolysins, and caseinase (Figure 1G).

Regarding the virulence factors of yeasts from the identified genera, respectively *Candida* and *Magnusiomyces*, in order of frequency, they are lipase, DNase, caseinase, esculin, hemolysins, amylase, and lecithinase (Figure 1H). The genus *Candida* expresses lipase as a major virulence factor, followed by gelatinase and DNase. The study by Neji et al., 2017 [39] on different strains of *Candida* revealed a high potential of yeast strains to produce caseinase, gelatinase, and hemolysins.

Antibiotics resistance profiles

The oral cavity is colonized by a characteristic and complex microbial community that develops as biofilms on all dental and oral mucosal surfaces [42]. The normal microbiota of the oral cavity is associated with various oral pathologies, one of which being periodontitis. Periodontal disease is caused by sessile and planktonic oral microbiota in saliva and dental plaque [43].

According to the protocol followed by clinicians, the treatment of periodontitis involves the mechanical removal of the microbial biofilm that causes inflammation and/or infection. However, in some cases, in addition to the mechanical removal of infected periodontal pockets, the clinical treatment plan for severe forms of periodontitis may involve the adjuvant use of antibiotics [44].

However, the inappropriate use of antibiotics can lead not only to an increase in the frequency of adverse reactions and healthcare costs but also to the risk of selecting antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and multidrug-resistant Gram-negative bacilli [45].

Figure 2. Antibiotic resistance profiles for (A) Staphylococcus spp; (B) Streptococcus spp; (C) Enterococcus spp.

Abbreviations: TE - Tetracycline; VA - Vancomycin; DA - Clindamycin; E - Erythromycin; P - Penicillin G; FOX - Cefoxitin; AK - Amikacin; AMC Anoxicillin/ clavulamic acid; CAZ - Ceftazidime; CTX - Cefotaxime; OX - Oxacillin; GM - Gentamicin; FEP - Cefepime; CRO - Ceftriaxone; LNZ - Linezolid; CN - Cefalexin.
Regarding the Gram-positive selected strains, *Staphylococcus* spp. (Figure 2A) were resistant to cefotaxime (n=7), penicillin (n=5) and erythromycin (n=5). Low resistance rates were recorded for antibiotics such as tetracycline, oxacillin, clindamycin, vancomycin, cefoxitin, amikacin, amoxicillin/clavulanic acid, ceftazidime, and gentamicin.

The study carried out by Garbacz et al., 2021 [46] on bacterial strains of staphylococci isolated from the oral cavity revealed that bacterial isolates were resistant to penicillin in a proportion of 62.5%, erythromycin (30.7%), followed by tetracycline (30.2%), cefoxitin/oxacillin (13.5%), clindamycin (15.1%), trimethoprim/sulfamethoxazole (10.4%), fusidic acid (7.8%) and chloramphenicol (4.7%), susceptibility of staphylococci was recorded in the case of vancomycin. Recent studies indicate increased rates of MRSA in the oral cavity [47]. The research carried out by Kim and Lee, 2015 [29] on bacterial strains of *Staphylococcus aureus* isolated from the oral cavity from patients with periodontitis highlighted the fact that most strains were susceptible to vancomycin, chloramphenicol, clindamycin, imipenem, and sulfamethoxazole. The resistance of the analyzed strains was observed in the highest proportion for penicillin and in a lower proportion for oxacillin, erythromycin, tetracycline, and gentamicin. Likewise, the research by Georgiev et al., 2009 [48] on the antibiotic resistance of staphylococci strains isolated from patients with generalized periodontitis revealed sensitivity to gentamicin, concluding that gentamicin is active on aerobic bacteria. In the study carried out by Malinda and Prisinda, 2022 [49] on the susceptibility to antibiotics on bacterial strains isolated from apical abscesses, the sensitivity of *Staphylococcus spp.* strains to penicillin and vancomycin and their resistance to clindamycin were revealed.

In this study, as in other studies, oral staphylococcal strains were resistant to erythromycin and penicillin, to which oxacillin and gentamicin are added in smaller proportions, were identified, with only one MRSA strain being identified.

In the case of the microbial strains belonging to the *Streptococcus* genus, a multi-drug resistance phenotype to antibiotics was observed, with sensitivity being observed for amoxicillin, ofloxacin, linezolid, and tetracycline (Figure 2B). The study carried out by Malinda and Prisinda, 2022 [49] on the susceptibility to antibiotics on bacterial strains isolated from apical abscesses, it revealed the sensitivity of...
strains belonging to the genus *Streptococcus spp.* to amoxicillin and resistance to clindamycin and vancomycin, similar results being obtained in this work.

*Enterococcus* strains were predominantly resistant to penicillin, vancomycin, cephalaxin, tetracycline, erythromycin, and linazolid and sensitive to ciprofloxacin and chloramphenicol (Figure 2C). Relatively similar results were observed in the study by Komiyama et al., 2016 [37] on *Enterococcus spp.* strains isolated from patients of different ages, which revealed that the strains showed high antibiotic resistance to tetracycline (53.8%), amoxicillin (12.3%), ampicillin (16.0%), and erythromycin (43.4%).

The study by Prado et al., 2017 [50] on antibiotic resistance of *E. faecium* and *E. faecalis* strains isolated from root canals revealed that *E. faecalis* was resistant to tetracycline, ciprofloxacin, and azithromycin, while *E. faecium* was sensitive to all antibiotics tested, suggesting that *E. faecium* showed higher susceptibility to antibiotics than *E. faecalis*.

Regarding the selected Gra.-negative isolates, the resistance profile for the strains of the *Enterobacter* genus was analyzed (Figure 3A), and it was observed that the strains of this genus were predominantly resistant to cefoxitin, ampicillin and cefazolin, ciprofloxacin, amoxicillin-clavulanic acid. The study by Jepsen et al., 2022 [51] on bacterial isolates from German periodontitis patients revealed increased resistance of *Enterobacter* spp. strains to ciprofloxacin, and amoxicillin-clavulanic acid, while bacterial isolates from patients with periodontitis from Rio de Janeiro were susceptible to ciprofloxacin. In the United States, *Enterobacter* is the second most common genus of carbapenem-resistant *Enterobacteriaceae*, contributing increasingly to the spread of infections with carbapenem-resistant bacteria [52]. Resistance to these antibiotics and the emergence of multidrug resistance have increased interest in these organisms because *Enterobacter cloacae* bacterial strains are nosocomial pathogens capable of producing various infections and septicemia [51].

After analyzing the antibiotic resistance profile of all microbial strains belonging to the *Enterobacteriaceae* family (Figure 3B), it was observed that these strains are resistant in the highest proportion to ampicillin, cephalaxin, piperacillin, cefoxitin, and tetracycline. Amoxicillin and moxifloxacin are antibiotics used in the prophylaxis of dental infections after tooth extraction. Diz Dios et al. 2006 [53] showed that the use of amoxicillin and moxifloxin reduced the prevalence and duration of post-extraction bacteremia, the study suggesting that moxifloxin is a promising alternative for the prevention of dental infections. A study carried out on bacterial strains that are part of the *Enterobacteriaceae* family, namely strains of the genera *Enterobacter*, *Klebsiella*, *Serratia*, *Escherichia*, and *Pantoea*, reported similar results regarding resistance to antibiotics such as ampicillin, amoxicillin, amoxicillin-clavulanic acid, cefotaxin, and in the case of imipenem and meropenem, sensitivity was preserved [54]. Also, in this study, it was observed that these strains are beta-lactamase producers, thus suggesting that in the oral cavity of people with endodontic problems could be reservoirs for these enzymatic resistance mechanisms.

Regarding the resistance profile of the microbial strains that are part of the group of non-enteric Gram-negative bacilli, high resistance to ceftazidime, cefotaxime, aztreonam, piperacillin, and imipenem was observed (Figure 3C). Among non-enteric bacilli, *Pseudomonas aeruginosa* strains gain access to the pharynx and oral cavities from external sources or transiently colonize the upper respiratory tract, but their presence in the oral cavity has not been investigated in detail [51]. The strains of the genus *Pseudomonas* isolated in this work were sensitive to almost all tested antibiotics except piperacillin and cephalexin (Figure 3D). In the case of anaerobic bacteria, according to EUCAST, two *Bacteroides urealyticus* bacterial strains were tested and proven susceptible to the two antibiotics (i.e., piperacillin-tazobactam and meropenem).

**Conclusions**

Regarding the distribution of soluble virulence factors, esculinase and proteases predominated both in the case of non-fermentative and fermentative Gram-negative bacilli, hemolysins were identified in the genus *Actinomycyes* and in the case of aerobic Gram-positive cocci, in anaerobic Gram-positive cocci amylase and DNase predominated while in the case of yeast strains, lipase and DNase.

*Enterobacteriaceae* strains showed high levels of resistance to penicillins (ampicillin) and first generation cephalosporins (cephalexin and cefazolin), non-enteric Gram-negative bacilli to piperacillin, penicillins with inhibitors, carbapenems, aminoglycosides, and quinolones, *Staphylococcus* sp. strains to penicillin and erythromycin, *Enterococcus* sp. strains to vancomycin and penicillin, and *Streptococcus* sp. strains to penicillin, clindamycin, erythromycin. Anaerobic bacterial strains revealed 100% sensitivity to most antibiotics tested.

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**References**


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27. Divyakolu S, Chikala R, Ratnakar KS, Sritharan V. Hemolysins of Staphylococcus aureus—an update on their biology, role in pathogenesis and as targets for anti-virulence therapy. Advances in Infectious Diseases, 2019, 9(2), 80-104.


