MIC-Directed Optimization of Antibiotic Therapeutics in Gram-Positive and Gram-Negative Bacterial Infections in the Oral Cavity

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Received: 02/04/2026
Accepted: 25/05/2026
Published: 30/06/2026
DOI: https://doi.org/10.65682/kjnhs.v2.i2.60-67

Abstract

Background: The presence of antimicrobial resistance in oral pathogens is a significant problem in dentistry, as antibiotics are commonly used to treat oral infections. The emergence of resistant bacteria related to dental caries, periodontal disease, and other oral conditions are related to inappropriate prescribing practices. A correct diagnosis and knowledge of antimicrobial susceptibility are crucial for proper treatment and stewardship.

Methods: Cross-sectional study of a laboratory sample of 112 oral specimens taken from subjects with plaque deposits and dental caries. Blood agar, MacConkey and chocolate agars were used for the culture of samples under suitable conditions. For preliminary classification Gram staining was done. The susceptibility of the antimicrobial agents was tested by the Kirby–Bauer disk diffusion method, and the identification of bacterial species and determination of the minimum inhibitory concentration (MIC) was done by VITEK 2 Compact automated system.

Results: The 112 specimens, 91 (81.3%) grew bacteria with 43 (47.3%) being Gram-positive and 48 (52.7%) Gram-negative bacteria. Resistance to a number of routinely used antibiotics was found to be high. The pathogens identified were Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella spp., Fusobacterium nucleatum, Streptococcus mutans, Staphylococcus aureus and Actinomyces spp. MIC analysis revealed that imipenem and meropenem had the highest antimicrobial activity. Clindamycin was found to be effective against multiple Gram-positive isolates, and ciprofloxacin was moderately active against Gram-negative bacteria.

Conclusion: There was high level of resistance of oral pathogens against the antibiotics which were commonly used. In the context of dental practice, the optimal treatment of the infection and to promote antimicrobial stewardship, routine identification of the microorganisms and appropriate antimicrobial treatment, based on their MIC, are recommended.

Keywords: Oral bacteria; Antibiotic susceptibility; Antimicrobial resistance.

1. Introduction

The advent of antibiotics transformed how bacterial diseases including those of an oral nature could be treated. Nevertheless, the empirical prescription and improper utilization of antimicrobial agents in the field of dentistry have largely been a contributory factor to the formation and spread of antimicrobial resistance (AMR) (Ramirez-Puebla et al., 2024). A significant percentage of outpatient antibiotic prescriptions is attributed to dentists, and research shows that a significant percentage of such prescriptions can be unnecessary or an inappropriate dosage and duration of treatment (Finn et al., 2021). This tendency of abuse has contributed to the emergence of resistant Gram-positive and Gram-negative pathogens in the oral cavity making the treatment effect more difficult and restricting the efficacy of first-line antibiotics (Anderson et al., 2023). To counter this increasing menace, it is now necessary to have rational antibiotic stewardship in dental practice. Prescribing evidence-based practice involves proper microbiological diagnosis which involves identification of bacteria and antimicrobial susceptibility testing (Bessa et al., 2022). Upon the integration of modern automated diagnostic systems with the establishment of minimum inhibitory concentration (MIC) values(Kaprou et al., 2021), specific characterization of pathogens is achieved and clinicians can individualize antibiotic treatment based on the profiles of resistance (Kadeřábková et al., 2024; Kiros et al., 2022). Laboratory-directed approaches are able to enhance clinical outcomes as well as maintain the efficacy of currently available antimicrobial agents (Tsavea et al., 2022). Thus, the current research will determine the most common types of bacteria related to oral infections and assess their antimicrobial susceptibility profiles including the distribution of the MIC(Farva et al., 2023; Kowalska-Krochmal & Dudek-Wicher, 2021). This study will help to add to the evidence-based framework of optimizing clinical decision-making and consolidating antimicrobial stewardship in modern dental care by producing local resistance data and evaluating the efficacy of antibiotics.

2. Materials and Methods

2.1. Sample Collection

In the present cross-sectional study, which is based on collaboration with Karbala University Clinics, oral samples of 112 patients (72 males and 40 females), were gathered based on their presentation with dental caries and plaque. These samples were collected with sterile swabs and placed in transport media that would not destroy the bacteria during transportation to the microbiology laboratory (Strathdee et al., 2023). The specimens were given codes and demographic and clinical data were annotated and further processed.

2.2. Bacterial Isolation

After receiving, the specimens were cultured on blood agar, MacConkey agar and chocolate agar to maximise the coverage of the various oral microbiota (Anderson et al., 2023). Enriched agar was selected as blood agar to provide the ability to grow fastidious microorganisms and also provide the opportunity to evaluate hemolytic activity (Bessa et al., 2022). MacConkey agar was used was because it is selective against Gram-negative bacteria and because it is also discriminatory to isolate lactose fermenters. Chocolate agar was used to cultivate specific anaerobic or microaerophilic bacteria in the condition of lowered-oxygen conditions (Casino et al., 2023). The plates inoculated were grown at 35-37 ºC over a period of 18 to 24 hours. Primary taxonomic placement was done through the systematic recording of morphology, pigmentation, and hemolytic features of the colonies (Khelaifia et al., 2023).

2.3. Gram Staining

Gram staining was used to isolate species of bacteria by single colony as a means of isolating them based on cell wall properties. To identify the Gram reaction and morphology of the cell, a microscopic analysis of the slide was done using the oil immersion technique (Khelaifia et al., 2023).

2.4. Antimicrobial activity

One hundred and twelve bacterial isolates were collected on patient specimens, and then the antimicrobial susceptibility was tested through the Kirby-Bauer disk diffusion on a Mueller-Hinton agar at 37ºC (Mishyna et al., 2018). The suspensions of bacteria were brought to 0.5 McFarland, placed on agar, and disks that were relevant to Gram-positive and Gram-negative bacteria were added. Measurement and interpretation of inhibition zones were done after 18-24 hours of incubation based on standard guidelines (Gabr et al., 2022).

2.5. Bacterial Identification and Minimum Inhibitory Concentrations (MIC)

The antimicrobial susceptibility testing and the definite bacterial identification was conducted through automated VITEK 2 Compact system (bioMerieux) (Djais et al., 2019). The bacterial suspensions were prepared in pure colonies that had been prepared in sterile saline and were standardized to the desired level of turbidity (Cieplik et al., 2019). These suspensions were added to identification (ID) and antimicrobial susceptibility testing (AST) cards with biochemical substrates/antibiotic dilutions (Mohammad et al., 2023). The cards were automatically integrated in the system that monitored the metabolism and developmental pattern of the organisms to identify the organisms and the minimum inhibitory concentrations (MICs) (Ghavami et al., 2025). The results were interpreted according to the set standards of clinical laboratory, therefore classifying the isolates as susceptible, intermediate and resistant.

2.6. Data Analysis

Findings of the microbial identifications and the profiles of antibiotic resistance were recorded and examined in a descriptive manner. A good antibiotic has been chosen according to MIC values, resistance patterns, potential adverse effects and cost-effectiveness and the object was to arrive at an appropriate therapeutic substitute to Gram-positive and Gram-negative isolates (Araújo et al., 2023).

3. Result

3.1. Culture Media

After culturing the specimens on the respective media, 36 of them posted growth on the MacConkey agar, 42 on the blood agar and 13 on chocolate agar. The cultivating conditions found no growth in 21 specimens (Figure 1). Regarding the demographic distribution of the samples, among females, 14 samples contained Gram-positive bacteria, 18 samples contained Gram-negative bacteria and 8 showed no growth. For males, 29 specimens gave Gram positive bacteria, 30 gave Gram negative bacteria and 13 showed no growth (Figure 2).

3.2. Bacterial Morphology

Gram staining was used on samples that showed growth on culture media. The analysis revealed that 43 isolates were Gram-positive and the remaining 48 isolates were Gram-negative. Out of the Gram-positive isolates, 22 were coccobacilli, and the rest were bacilli (rod-shaped). Conversely, in Gram-negative isolates, 19 of them exhibited bacillary morphology as compared to

the rest, which exhibited cocci morphology. Also, there are a few bacteria with unusual morphologies. Figure 2 represents these morphological features.

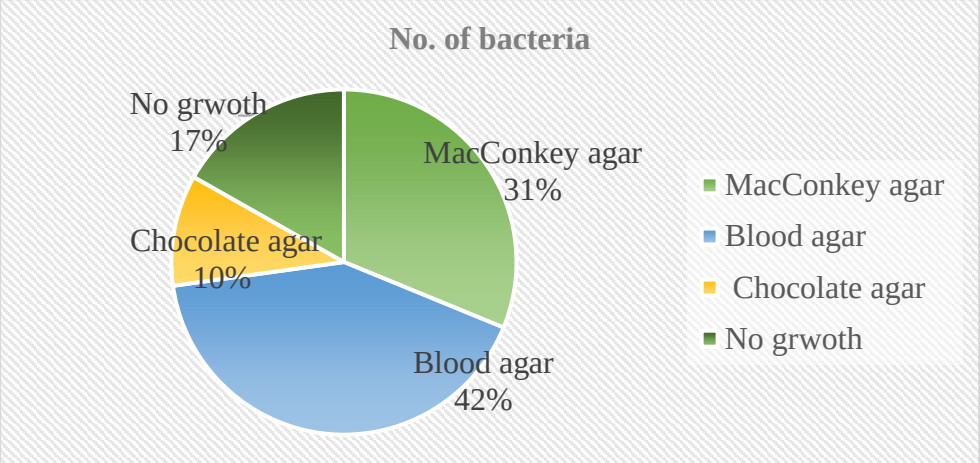

Figure 1. Number of bacterial in different media.

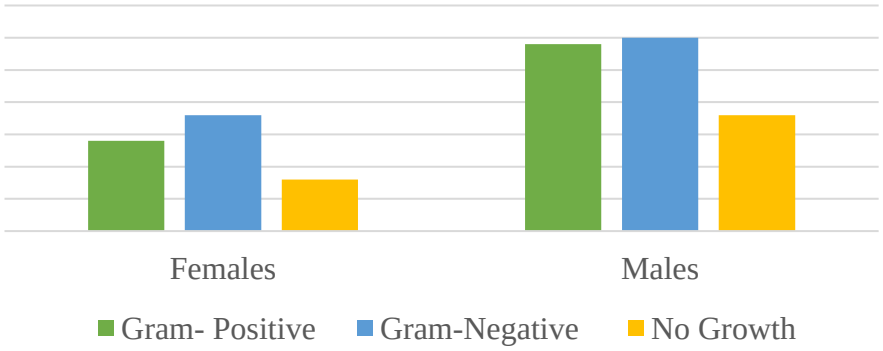

Figure 2. The demographic distribution of the samples, among females and males.

3.3. Antimicrobial activity

The antimicrobial susceptibility test showed the presence of significant patterns of resistance across the isolates. There were 23 Gram-positive isolates that were resistant to one of them, penicillin, vancomycin and ampicillin, and 19 Gram-negative isolates were resistant to one of them, ciprofloxacin and clindamycin (Figure 3). These results demonstrate that there is a variation in antimicrobial response and that there exist resistant bacterial populations in the recovered samples.

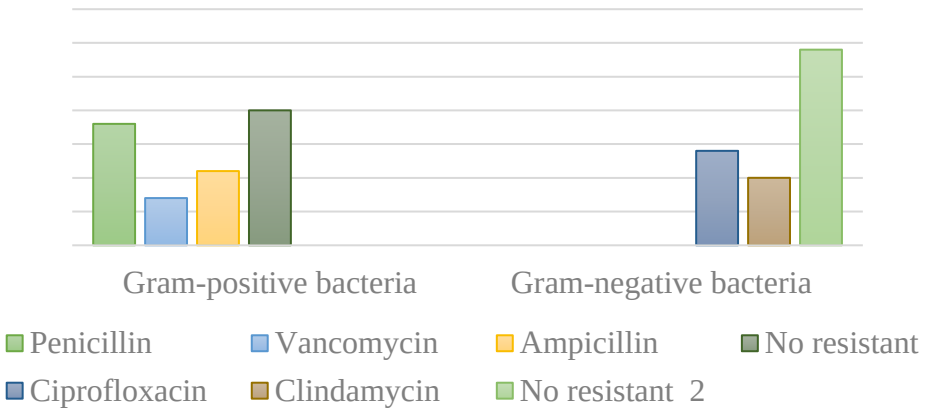

Figure 3. Patterns of antimicrobial resistance of Gram-positive and Gram-negative bacterial isolates.

3.4. Bacterial Identification and MIC

Twenty-one samples were also found to be normal oral flora constituent members, including *Capnocytophaga gingivalis, Streptococcus mitis, Streptococcus oralis,* and *Rothia dentocariosa.* Those with no resistance to antibiotics were 49 in number by way of antimicrobial susceptibility testing. Forty-two resistant isolates of bacteria were identified using the VITEK2 Compact system (BioMerieux). The pathogenic Gram-negative bacterial group comprised of twenty-two samples, *Porphyromonas gingivalis* (six samples), *Tannerella forsythia* (four samples), *Treponema denticola* (3 samples), *Serratia marcescens* (three samples), *Prevotella spp.* (two samples), *Citrobacter koseri* (two samples) and *Fusobacterium nucleatum* (two samples) as shown in Table 1. The Gram-positive pathogenic bacterial group consisted of twenty samples and among them there were: *Streptococcus mutans* (seven samples), *Staphylococcus aureus* (four samples), *Streptococcus anginosus* (four samples), *Lactobacillus salivarius* (three samples), and *Actinomyces* spp. (two samples) as shown in Table 2.

Table 1. Minimum Inhibitory Concentrations (MIC) for pathogenic Gram-negative bacterial group.

Antibiotic	**Minimum Inhibitory Concentrations (MIC) µg/mL**						
	P. gingivalis	*T. forsythia*	*T. denticola*	*S. marcescens*	*Prevotella spp.*	*C. koseri*	*F. nucleatum*
Amoxicillin	0.5	I	R	R	I	R	0.5
Penicillin	R	R	R	R	I	R	I
Erythromycin	R	R	R	R	R	R	R
Clindamycin	0.5	0.5	0.5	R	I	R	0.5
Imipenem	0.125	0.125	0.125	0.5	0.125	0.125	0.125
Azithromycin	1	1	1	R	2	R	1
Metronidazole	0.5	0.5	0.5	R	0.5	R	0.25
Doxycycline	I	I	I	1	R	1	I
Tetracycline	R	R	I	1	I	1	I
Ciprofloxacin	0.5	0.5	0.5	0.5	I	0.25	R
Gentamicin	R	R	R	I	R	1	R
TMP-SMX	R	R	R	0.5/9.5	R	0.5/9.5	R
Meropenem	0.125	0.125	0.125	0.25	0.125	0.25	0.125
Cefepime	R	R	R	1	R	0.5	R

Table 2. Minimum Inhibitory Concentrations (MIC) for pathogenic Gram-positive bacterial group.

Antibiotic	**Minimum Inhibitory Concentrations (MIC) µg/mL**				
	S. mutans	*S. aureus*	*S.anginosus*	*L. salivarius*	*Actinomyces* spp
Penicillin	0.12	R	I	0.25	I
Amoxicillin	0.25	R	0.25	I	0.25
Oxacillin	-	0.5	-	-	-
Cefoxitin	-	-	-	-	-
Erythromycin	R	R	R	1	I
Clindamycin	0.25	0.25	0.25	0.5	0.5
Azithromycin	I	R	I	1	I
Vancomycin	I	R	1	R	R
Linezolid	1	1	1	1	1

Antibiotic	Minimum Inhibitory Concentrations (MIC) µg/mL				
	S. mutans	*S. aureus*	*S.anginosus*	*L. salivarius*	*Actinomyces* spp
Doxycycline	I	0.5	I	I	I
Tetracycline	I	0.5	I	I	I
Gentamicin	R	1	R	R	R
Imipenem	0.06	0.5	0.06	0.125	0.06
Meropenem	0.06	0.25	0.06	0.125	0.06

4. Discussion

The current paper indicates the increasing difficulty of antimicrobial resistance of oral pathogenic bacteria and leads to the centrality of evidence-based antibiotic choice in dental infections. In this analysis, 112 patients (72 males and 40 females), were used and this study results yielded 43 Gram-positive and 48 Gram-negative, as well as no growth in 21 cases of caries. Result of the research was that there is a high level of antimicrobial resistance in the widely used antibiotics. The resistance to penicillin, ampicillin and gentamicin in Gram-negative isolates and Erythromycin and Gentamicin against Gram-positive isolates was developed, which shows the scary tendency of resistance to the first-line agents. This observation is in line with the reports in the world, which claim that the indiscriminate use of antibiotics in dentistry has increased the formation of resistance (Haque et al., 2019; Nizami et al., 2025). The MIC data also showed that carbapenems particularly imipenem and meropenem provided the best picture of antimicrobial activity since they act on both Gram-positive and Gram-negative pathogens. This is explicable by the fact that they are resistant to beta-lactamases, and have the ability of inhibiting cell wall synthesis in bacteria (Nizami et al., 2025). Ciprofloxacin proved to be average to perform particularly in certain Gram-negative isolates such as Serratia marcescens and Citrobacter koseri. This implies that it may be used as a cheap substitute in mild and moderate infections (Shariati et al., 2022). On the same note, Clindamycin was activity against Gram-positive pathogens. The paper advocates the transition of rational antibiotic stewardship in dental practice to prevent the further development of resistance without losing its therapeutic impact.

5. Conclusion

Clarified in this investigation, oral infections have a collection of gram-positive and gram-negative species of bacteria. The information has shown disastrous levels of resistance to the most commonly used antibiotics in clinical practice, especially penicillin, ampicillin. Carbapenems, particularly imipenem and meropenem, had better antibacterial efficacy against most isolates. Whilst these agents are seen to have a strong level of efficacy, they should solely be used in clinical practice on severe or recalcitrant infections to help curb the rapid development of antimicrobial resistance. Clindamycin and ciprofloxacin had moderate activity against some of the pathogens and can therefore be used as a cost-effective substitute for the milder infections, depending on the findings of susceptibility testing. Overall, the results emphasize a desperate need to use antibiotics with restraint in the field of dentistry. Systematic microbiological-profiling and antimicrobial susceptibility testing should guide the administration of antimicrobials to ensure the achievement of optimal clinical results, as well as to contain the development of resistant bacterial phenotypes. Evidence-based antibiotic stewardship use will be critical in improving the infection management and protection of the performance of remaining antimicrobial agents in oral dental practice.

6. References

Anderson, A. C., von Ohle, C., Frese, C., Boutin, S., Bridson, C., Schoilew, K., Peikert, S. A., Hellwig, E., Pelz, K., & Wittmer, A. (2023). The oral microbiota is a reservoir for antimicrobial resistance: resistome and phenotypic resistance characteristics of oral biofilm in health, caries, and periodontitis. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), 37.

Araújo, L. L., Lourenço, T. G. B., & Colombo, A. P. V. (2023). Periodontal disease severity is associated to pathogenic consortia comprising putative and candidate periodontal pathogens. *Journal of Applied Oral Science*, *31*, e20220359.

Bessa, L. J., Botelho, J., Machado, V., Alves, R., & Mendes, J. J. (2022). Managing oral health in the context of antimicrobial resistance. *International Journal of Environmental Research and Public Health*, *19*(24), 16448.

Casino, P., Lopez, A., Peiro, S., Terrones, I., Agusti, G., Terlevich, D., Asensio, D., Marqués, A. M., & Pique, N. (2023). Use of blood powder (ground and irradiated) for the manufacture of chocolate agar. *International Journal of Molecular Sciences*, *24*(9), 7965.

Cieplik, F., Kara, E., Muehler, D., Enax, J., Hiller, K., Maisch, T., & Buchalla, W. (2019). Antimicrobial efficacy of alternative compounds for use in oral care toward biofilms from caries-associated bacteria in vitro. *Microbiologyopen*, *8*(4), e00695.

Djais, A. I., Tahir, H., Hatta, M., Achmad, H., & Wahyuni, A. (2019). Differences of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Moringa Leaf Extract (Moringa Oliefera L.) on Bacteria Aggregatibacter Actinomycetemcomitans and Porphyromonas Gingivalis. *Indian Journal of Public Health Research & Development*, *10*(8).

Farva, K., Sattar, H., Ullah, H., Raziq, A., Mehmood, M. D., Tareen, A. K., Sultan, I. N., Zohra, Q., & Khan, M. W. (2023). Phenotypic analysis, molecular characterization, and antibiogram of caries-causing bacteria isolated from dental patients. *Microorganisms*, *11*(8), 1952.

Finn, E., Andersson, F. L., & Madin-Warburton, M. (2021). Burden of Clostridioides difficile infection (CDI)-a systematic review of the epidemiology of primary and recurrent CDI. *BMC Infectious Diseases*, *21*(1), 456.

Gabr, E. Z., Elgendy, M. M., & Halawa, S. M. (2022). Bacteriological characterization of pathogenic bacteria isolated from dental patients. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*, *14*(2), 41–55.

Ghavami, Q., Abd Ulhai Nasser, H., Vaziri, S. S., & Moghadam, M. T. (2025). A Focused Review on Commercially Available Automated Systems for Antibiotic Susceptibility Testing. *Journal of Tropical Medicine*, *2025*(1), 9694354.

Haque, M., Sartelli, M., & Haque, S. Z. (2019). Dental infection and resistance—global health consequences. *Dentistry Journal*, *7*(1), 22.

Kadeřábková, N., Mahmood, A. J. S., & Mavridou, D. A. I. (2024). Antibiotic susceptibility testing using minimum inhibitory concentration (MIC) assays. *Npj Antimicrobials and Resistance*, *2*(1), 37.

Kaprou, G. D., Bergšpica, I., Alexa, E. A., Alvarez-Ordonez, A., & Prieto, M. (2021). Rapid methods for antimicrobial resistance diagnostics. *Antibiotics*, *10*(2), 209.

Khelaifia, S., Virginie, P., Belkacemi, S., Tassery, H., Terrer, E., & Aboudharam, G. (2023). Culturing the human oral microbiota, updating methodologies and cultivation techniques. *Microorganisms*, *11*(4), 836.

Kiros, A., Saravanan, M., Niguse, S., Gebregziabher, D., Kahsay, G., Dhandapani, R., Paramasivam, R., Araya, T., & Asmelash, T. (2022). Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Factors among Dental Caries-Suspected Patients Attending the Ayder Comprehensive Specialized Hospital and Private Dental Clinic in Mekelle, Northern Ethiopia. *BioMed Research International*, *2022*(1), 3463472.

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens*, *10*(2), 165.

Mishyna, M., Mozgova, Y., Kovalenko, N., & Zamaziy, T. (2018). *Standard protocols to laboratory classes in special microbiology for the II and III year English media students of medical and dentistry faculties.(Part 3)*.

Mohammad, I., Kumar, A., Alam, P., & Khan, M. S. (2023). In vitro investigation of minimum inhibitory concentration and minimum bacterial concentration from combined extract of Salvadora persica L. and Zingiber officinale Rosc. against Streptococcus mutans. *Annals of Phytomedicine*, *12*(1), 776–782.

Nizami, M. Z. I., Yin, I. X., Niu, J. Y., Yu, O. Y., & Chu, C. H. (2025). Antibiotic Resistance in Dentistry: A Review. *Antibiotics*, *14*(12), 1259.

Ramirez-Puebla, S. T., Mark Welch, J. L., & Borisy, G. G. (2024). Improved visualization of oral microbial consortia. *Journal of Dental Research*, *103*(13), 1421–1427.

Shariati, A., Arshadi, M., Khosrojerdi, M. A., Abedinzadeh, M., Ganjalishahi, M., Maleki, A., Heidary, M., & Khoshnood, S. (2022). The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. *Frontiers in Public Health*, *10*, 1025633.

Strathdee, S. A., Hatfull, G. F., Mutalik, V. K., & Schooley, R. T. (2023). Phage therapy: From biological mechanisms to future directions. *Cell*, *186*(1), 17–31.

Tsavea, E., Vardaka, F.-P., Savvidaki, E., Kellil, A., Kanelis, D., Bucekova, M., Grigorakis, S., Godocikova, J., Gotsiou, P., & Dimou, M. (2022). Physicochemical characterization and biological properties of pine honey produced across Greece. *Foods*, *11*(7), 943.