ONLINE FIRST

ORIGINAL ARTICLE

Current Trends in the Antibiotic Sensitivity Profile and their Comparison with Chlorhexidine, Nisin, and their Combination on Oral Bacterial Isolates Using the Kirby-Bauer Method

Mehwash Kashif[1*], Mahmooda Kazmi[2], Farzeen Tanwir[3], Shazia Akbar[4], Somia Gul[5]

[1]*PhD Scholar, Jinnah University for Women & Professor and Chairperson, Department of Oral Pathology, Karachi Medical and Dental College, Karachi Metropolitan University, Karachi, Pakistan*
[2]*Chairperson, Dept. of Microbiology, Jinnah University for Women, Karachi, Pakistan*
[3]*Head of Department, Dept. of Periodontology, Bahria University of Health Sciences, Karachi, Pakistan*
[4]*Head of the Department, Dept. of Oral Pathology, Dow Dental College, Baba-e-Urdu Road, Karachi, Pakistan*
[5]*Professor, Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan*
Correspondence: *mehwashkashif@gmail.com*
doi: 10.22442/jlumhs.2026.01418

ABSTRACT

OBJECTIVE: To evaluate the sensitivity profile of antibiotics on different oral bacterial species using the Kirby-Bauer Method and to compare them with Nisin and Chlorhexidine and their combination.

METHODOLOGY: A prospective cross-sectional study, conducted at the Microbiology laboratory of Jinnah University for Women, from September 2023 to 2024. The supragingival plaque samples were collected from residents of Karachi, regardless of gender, who were at least eighteen years old and had a Loe and Silness plaque index of one to three. Individuals on antibiotic therapy within 12 weeks of sampling, with severe systemic illnesses, persistent systemic infections, or with known communicable diseases were excluded. Sensitivity profiling of five different antibiotic classes was assessed by using the Kirby-Bauer Method and compared with the zone of inhibition of a 10% solution of Nisin, 0.2% chlorhexidine, and their combination. SPSS version 25.0 was used for data analysis.

RESULTS: Clarithromycin showed the highest mean zone of inhibition, while Optochin showed no zone. Wilcoxon signed-rank tests revealed that Clarithromycin showed a statistically significant difference. Nisin and chlorhexidine showed statistically significant results of zone of inhibition with multiple antibiotics, while the combination showed substantial results only with Clarithromycin.

CONCLUSION: Clinically isolated oral bacterial species exhibited a wide range of antibiotic sensitivities, reflecting variations in microbial susceptibility and antimicrobial processes. Comparison of Nisin and chlorhexidine with commercially available antibiotics showed varying degrees of statistical significance. Nisin may be a helpful biocompatible alternative for the inhibition of bacterial growth in oral Supragingival plaque biofilms.

KEYWORDS: Antibiotics, Disk Diffusion, Antimicrobial Tests, Bacteria, Oral, Sensitivity

INTRODUCTION

Oral bacterial infections are among the most common medical conditions worldwide and often require antibiotic treatment as part of therapeutic management. Dental caries, gingivitis, and additional severe dental problems like periodontitis, endodontic infections, and odontogenic abscesses are examples of these infections[1]. Both commensal and pathogenic microorganisms, such as *Streptococcus* spp, like *Streptococcus sanguinis, S. mitis, S. gordonii, Actinomyces(A. naeslundii, A. viscosus)*, *Fusobacterium nucleatum, Porphyromonas gingivalis*, and *Staphylococcal spp.(aureus, epidermidis, hominis) etc.* are found in the oral cavity[2]. In ordinary dentistry treatment, where empirical usage of antibiotics is still frequent, the growing prevalence of antibiotic-resistant strains of these infections presents a serious concern[3,4].

Common dental and orofacial infections may become more challenging to treat due to antibiotic resistance against oral bacterial pathogens. Dental plaque and caries, periodontal disease, and endodontic infections are only a few of the disorders linked to the oral microbiota[5]. The development of multidrug-resistant strains is facilitated by the improper or empirical use of antibiotics in dental practice, which reduces therapeutic options and increases the risk of treatment failure[6].

A popular, easy, and affordable method for determining antimicrobial susceptibility is the Kirby-Bauer disk diffusion method, which is advised by the Clinical and Laboratory Standards Institute (CLSI)[7]. The Kirby-Bauer disk diffusion method enables a comparative analysis of antibiotic efficiency by determining the diameter of the inhibition zones encircling antimicrobial-impregnated discs applied to agar plates inoculated with bacterial isolates[8]. The agar well diffusion method involves the creation of wells on the MHA plate, followed by the addition of the test substance into the well, followed by observation of the zone of inhibition by the antimicrobial agent around the well. Agar-based methods for evaluating test compounds' in vitro antibacterial activity include Kirby-Bauer disk diffusion and agar well diffusion. Antimicrobial agents spread across the agar media in both techniques, preventing bacterial growth and creating quantifiable zones of inhibition. Both methods require consistent inoculum density and incubation conditions to guarantee reproducibility, and the diameters of these zones serve as comparative measures of antimicrobial potency[8].

By evaluating the zones of inhibition produced by antibiotics against oral bacterial isolates, this standardized technique helps guide evidence-based treatment regimens for the local population. In addition to selecting the best antibiotic, an accurate sensitivity profile is essential to prevent the misuse of broad-spectrum antibiotics, which hasten resistance.

The purpose of this study was to employ the Kirby-Bauer method for evaluating the antibiotic sensitivity profiles of lactams(Penicillin), Macrolides (Clarithromycin, Erythromycin), Glycopeptides(Vancomycin), Cephalosporin(Ceftazidime), and Quinine derivatives(Optochin) - against various oral bacterial species. It also compares the antibacterial activity of probiotic, Nisin, chlorhexidine, and their combination with the antibiotic sensitivity profile of the antibiotics used. The study aims to identify drugs with superior antibacterial activity, highlight potential resistance trends, and encourage sensible antibiotic prescribing in dental settings by analyzing the relative efficacy of different antibiotics. Improving patient outcomes, preventing the development of resistant strains, and guiding antimicrobial stewardship practices in clinical dentistry all depend on an understanding of the in vitro susceptibility patterns of oral bacteria to various antibiotics and biological compounds, such as Nisin. Therefore, the purpose of this study was to assess the antibiotic sensitivity profiles of different oral bacterial species using the Kirby-Bauer Method and to compare them with Nisin, Chlorhexidine, and their combination using the agar well diffusion method.

METHODOLOGY

In the Department of Microbiology at Jinnah University for Women, 101 bacterial isolates were recruited for this prospective, cross-sectional study between September 2023-24, using a non-probability, consecutive sampling technique. Jinnah University approved this study for Women with reference number DASR/92nd/March/2024. Participants were residents of Karachi, regardless of gender, at least 18 years old, and had a Loe and Silness plaque index of 1 to 3 [9]. Individuals who were on antibiotic therapy twelve weeks before sampling, had severe systemic illnesses, such as immunodeficiency disorders, were on chemotherapeutic medications, had persistent systemic infections, or had known communicable diseases were excluded. With a population size of 135, a margin of error of 5%, a population proportion of 50%, and a 95% confidence interval, the sample size was determined using an online sample size calculator. Supragingival plaque samples were collected from patients after taking informed verbal consent. After being moved to the laboratory, all samples were cultured in nutrient broth overnight at 37 °C. The standardized bacterial inoculum density was used (0.5 McFarland) to guarantee repeatability and reliable results comparability. Next, they were streaked over nutrient agar and incubated for a full day at 37ºC. Sensitivity profiling of six different antibiotic classes, which include β-lactams (Penicillin 10 µg), Macrolides (Clarithromycin 30 µg, Erythromycin 30 µg), Glycopeptides (Vancomycin 30 µg), Cephalosporin (Ceftazidime 30 µg), Quinine derivatives (Optochin 5 µg), by placing commercially available antibiotic discs (Thermo Scientific™ Oxoid™ Blank Antimicrobial Susceptibility discs) at equidistant intervals on a lawned plate of bacteria on Muller Hinton Agar.

The sensitivity profile of Nisin, chlorhexidine, and their 50% combination was assessed using the Kirby-Bauer method. Holes were created from a sterilized borer, followed by the pouring of 50 µg of 10% solution of Nisin in citric acid at pH 4, 0.2% solution of chlorhexidine in distilled water, and 50% solution of a combination of 10% solution of Nisin in citric acid at pH 4, 0.2% solution of chlorhexidine into each well. The plates were incubated at 37°C for the whole day. A customized proforma was used to record the highest zone of inhibition. The anonymity and confidentiality of the participants' data were maintained during the researcher's collection and analysis of results.

SPSS version 25.00 was used to analyze and enter the data. For the zone of inhibition (in millimeters) caused by six antibiotics, descriptive statistics were computed. The Friedman Test, which illustrates how effective certain antibiotics are. To assess the variations in antimicrobial efficacy among the drugs, the Wilcoxon Signed-Rank Test was used. A Bonferroni adjustment was used to reduce the risk of Type I error arising from multiple comparisons. The criterion for adjusted significance was established at $\alpha = 0.0033$ (0.05/15). The only p-values deemed statistically significant were those that fell below this modified alpha threshold.

RESULTS

Among 101 bacterial isolates, different species of Gram-positive and Gram-negative cocci and bacilli were found. (**Table I**)

Table I: Distribution and taxonomical classification of oral bacterial isolates

Phylum	Genus	Specie	Percentage	Cumulative %
Firmicutes	*Staphylococcus*	*aureus* *epidermidis* *caprae*	7.9 6.9 4.0	18.8
	Streptococcus	*mitis* *angiosus* *bovis* *canis* *intermedius*	2.0 1.0 1.0 1.0 3.0	8
	Alloicoccus	*otitis*	2.0	2
	Enterococcus	*feacalis* *feacium*	5.0 1.0	6
	Lactobacillus	*Salivarious* *casei*	6.9 11.9	18.8
	Bacillus	*coagulans* *megaterium* *licheniformis*	1.0 3.0 1.0	5
	Gamella	*haemolysans*	2.0	2
	Micrococcus	*luteus*	5.0	5
Actinobacteria	*Corynebacterium*	*matruchotii*	5.9	5.9
	Rothia	*mucilaginosa* *dentocariosa*	7.9 8.9	16.8
	Actinomyces	*odontolyticus* *viscosus*	4.0 1.0	5
Proteobacteria	*Acinetobacter*	*iwoffi* *calcoaceticus*	2.0 1.0	3
	Enterobacter	*gergovea*	1.0	3
	Citrobacter	*fruendii*	1.0	
	Neisseria	*mucosa*	1.0	

Means and standard deviations for each group are reported in **Table II**. These results indicate that not all antibiotics were equally effective.

Table II: Descriptive statistics of different variables

	Range	Mean Effectiveness	Clinical Insight	Mean ±SD	Std. Error	Mean Rank	Variance
Clarithromycin	43	High	Strong response	14.71±13.884	1.382	04.61	192.767
Erythromycin	40	High	Strong response	10.68±11.249	1.119	3.85	126.539
Penicillin	40	Moderate	Variable response	9.79±10.839	1.079	3.72	117.482
Vancomycin	39	Moderate-Low	Less effective response	8.74±9.762	.971	3.57	95.293
Ceftazidime	30	Low	Poor response	5.75±8.704	.866	3.02	75.768
Optochin	0	No	Clinically not useful	0	0	0	0

Table III shows the zone of inhibition analysis of all antibiotics tested and 15 pairwise comparisons based on the zones of inhibition. The Friedman test indicates that Clarithromycin is most effective, and Optochin is ineffective. Because the Friedman test showed a statistically significant result (p=0.00), the differences in antimicrobial effectiveness among the antibiotics were assessed using the Wilcoxon Signed-Rank Test.

Table III: Zone of inhibition analysis of 6 antibiotics (N = 101)

Compared To ↓ / Versus →	Penicillin	Erythromycin	Clarithromycin	Ceftazidime	Vancomycin
Penicillin	—	Z = -1.002 p = 0.316	Z = -4.034 p = 0.000*	Z = -2.775 p = 0.006	Z = -0.967 p = 0.334
Erythromycin	Z=-1.002 p=0.316	—	Z = -5.767 p = 0.000*	Z = -3.137 p = 0.002*	Z = -2.425 p = 0.015
Clarithromycin	Z=-4.634 p= 0.000*	Z=-5.767 p=0.000*	—	Z = -4.778 p =0 .000*	Z = -4.634 p = 0.000*
Ceftazidime	Z=-2.775 p=0.006	Z=-3.137 p=0.002*	Z=-4.778 p=0.000*	—	Z = -2.200 p=0.028
Vancomycin	Z= -.967 p= 0.334	Z= -2.425 p= 0.015	Z=-4.634 p=0.000*	Z=-2.200 p=0.028	—

Z = zone of inhibition
**Bonferroni adjustment = α = 0.0033*

The zones of inhibition of Nisin, Chlorhexidine, and their combination were cross-tabulated with the ZOI of various antibiotics. (**Table IV**).

Table IV: Cross-tabulation of probiotics and commercially available antibiotics

	Penicillin	**Erythromycin**	**Clarithromycin**	**Ceftazidime**	**Vancomycin**
Nisin 10 %	0.009	0.075	0.000*	0.978	1.000
Chlorhexidine 0.2%	0.000*	0.016	0.000*	0.827	0.056
Nisin+Chlorhexidine	0.463	0.145	0.009	0.274	0.998

**Bonferroni adjustment = α = 0.0033*

DISCUSSION

This cross-sectional study was conducted to evaluate the antibiotic sensitivity profiles of different oral bacterial species using the Kirby-Bauer Method and to compare them with Nisin, Chlorhexidine, and their combination. A variety of distinct Gram-positive and Gram-negative bacterial species have been identified in supragingival dental plaque from local isolates[2].

Descriptive statistics were applied for the zone of inhibition produced by different antibiotics. They showed strong, variable, moderate, and low responses, which are correlated with their mean effectiveness.

In this study, Clarithromycin exhibited the largest mean zone of inhibition (14.71±13.88 mm), indicating relatively higher antibacterial activity against the tested oral bacterial isolates. Clarithromycin's decisive action against Gram-positive organisms commonly encountered in oral infections may be explained by its ability to penetrate bacterial cells and inhibit protein synthesis[10]. Clarithromycin still frequently showed significant inhibitory effects in susceptibility tests compared with other antibiotics such as penicillin and Erythromycin, despite increasing resistance trends in the oral microbiota worldwide.

The study's small mean inhibition zones for penicillin (9.79 mm) and Erythromycin (10.68 mm) compared with Clarithromycin showed that these drugs have moderate but comparable antibacterial activity against the studied oral bacterial isolates. Penicillin, a β-lactam antibiotic that targets the formation of cell walls, also exhibits limited zones of inhibition against specific oral isolates, which is indicative of widespread resistance trends reported in recent studies on antimicrobial surveillance[11]. Similarly, oral bacterial isolates frequently exhibit varying sensitivity to Erythromycin, a macrolide that inhibits bacterial protein production, in part because of newly discovered resistance mechanisms reported in recent oral microbiome investigations[12]. To counteract growing antibiotic resistance, these findings encourage ongoing surveillance and cautious use of macrolides and β-lactams in the treatment of oral infections.

The mean inhibition zone of Vancomycin was 8.74 mm. Vancomycin is frequently used as a last-resort antibiotic. The smaller zone indicates potential intermediate resistance, which has been increasingly observed in oral isolates due of selective pressure and improper use[13], even though it remains only moderately effective. Considering that resistance may differ even within the same species, especially in complex biofilm settings, this underscores the importance of assessing strain-specific responses[14].

The mean inhibition zone of ceftazidime, a third-generation cephalosporin, was comparatively small at 5.75 mm. The growing resistance of mixed and Gram-negative oral flora, which renders many cephalosporins less effective, may be the cause of this low efficacy[15].

Notably, optochin (also known as ethylhydrocupreine hydrochloride) displayed no zone of inhibition. In dental plaque assessment, optochin testing is only considered as a confirmatory and differential method. Dental plaque contains a large number of alpha-hemolytic streptococci (viridans group), which can phenotypically mimic *S. pneumoniae* on blood agar[16]. By distinguishing optochin-sensitive *S. pneumoniae* from optochin-resistant viridans *Streptococci*, optochin sensitivity helps avoid misidentification.

Overall, Clarithromycin (4.61) was found to be the most effective antibiotic against the tested oral bacterial isolates based on the mean rank(MR) analysis. Its stability and improved intracellular penetration, which promote its therapeutic usage against Gram-positive pathogens, may be the cause of its increased activity when compared to other macrolides[17]. Erythromycin (MR=3.85) and penicillin (MR=3.72) demonstrated moderate sensitivity; nevertheless, changes in penicillin-binding proteins and β-lactam resistance mechanisms have

been linked to decreased penicillin susceptibility in oral streptococci[18]. According to its proven role against resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus*, Vancomycin (MR=3.57) showed moderate to low efficacy. Since third-generation cephalosporins are primarily active against Gram-negative bacteria, the reduced activity of ceftazidime (MR=3.02) is noteworthy. Since Gram-positive facultative *Streptococci* predominate in supragingival plaque and ceftazidime predominantly targets Gram-negative aerobic pathogens, it is not surprising that ceftazidime exhibited substantially reduced activity against supragingival dental plaque bacteria (MR = 3.02). Ceftazidime's efficacy in treating dental plaque-associated infections, which may lead to gingivitis and periodontitis, is further diminished by its restricted penetration into organized oral biofilms and the inherent resistance of oral *Streptococci*[19]. Recent research has revealed similar results, emphasizing the limited efficacy of third-generation cephalosporins in the management of oral biofilm-related infections and inflammations[20].

The results of the Wilcoxon Signed Ranks test were used to determine whether the antibiotics' efficacy against oral bacterial isolates was statistically significant or not (**Table III**). Pairwise comparisons showed that Penicillin and Vancomycin (p=0.000) were statistically significantly different from Clarithromycin. In comparative susceptibility analyses, penicillin and Vancomycin showed statistically significant associations with Clarithromycin, suggesting that these antibiotics differ considerably in their activity profiles against the tested bacterial isolates ($p = 0.000$), suggesting actual biological differences rather than chance variation because of their varied modes of action and ranges of activity, penicillin and Vancomycin may interact with Clarithromycin differently in terms of bacterial inhibition or resistance trends. When compared to β-lactams and glycopeptides, Clarithromycin, a macrolide that mainly targets Gram-positive cocci and atypical infections, frequently exhibits divergent efficacy, consistent with patterns of variable susceptibility observed in clinical isolates[21]. Because synergy or antagonism can vary greatly depending on the pathogen and resistance environment, recent data emphasize the importance of correctly interpreting combinatorial antibiotic outcomes. High rates of macrolide resistance among common Gram-positive pathogens are still being reported by current surveillance, which supports the clinical significance of statistically significant variations in antibiotic performance. This may reflect early signs of resistance development. These findings support recent reports of rising resistance to β-lactams and macrolides[22] and highlight the need for ongoing antimicrobial susceptibility testing to augment clinical decisions.

Erythromycin showed statistically significant results with all tested antibiotics, i.e., Clarithromycin, ceftazidime, and Vancomycin ($p = 0.000$, $p = 0.002$, $p = 0.015$, respectively), except penicillin ($p = 0.316$). Erythromycin frequently exhibits different efficacy profiles than Clarithromycin and glycopeptides like Vancomycin, consistent with recent data showing varying resistance patterns among macrolides and other antibiotic groups. The non-significant difference with penicillin, however, indicates comparable in vitro susceptibility among the tested oral isolates, which may reflect shared resistance mechanisms or levels to these antibiotics[23]. These results highlight the importance of local antibiotic susceptibility data for guiding empirical treatment in the face of evolving resistance patterns.

Clarithromycin showed statistically significant results with all antibiotics. These findings suggest a hierarchy of antibiotic effectiveness, with Clarithromycin as the most potent, followed by penicillin, Vancomycin, Erythromycin, and ceftazidime, which occupy an intermediate efficacy range. This information may guide clinical decision-making in selecting antibiotics for oral bacterial infections, emphasizing Clarithromycin's superior activity. However, factors such as resistance patterns, patient-specific considerations, and antibiotic stewardship principles should also inform treatment choices[24]. Clarithromycin exhibited vigorous activity, significantly outperforming all other antibiotics ($p < 0.001$). Ceftazidime

also showed strong inhibitory effects, substantially better than Erythromycin (p = 0.000) and Vancomycin (p = 0.000). Ceftazidime showed statistically significant results with Erythromycin (p= 0.002) and Clarithromycin (p=0.000). Given ceftazidime's primary activity against Gram-negative bacteria and macrolides' activity against Gram-positive and atypical organisms, the statistically significant associations between ceftazidime and macrolides such as Erythromycin (p = 0.002) and Clarithromycin (p = 0.000) likely reflect differences in susceptibility patterns and resistance mechanisms among bacterial isolates. When comparing susceptibility results across antibiotic classes in mixed-microbe investigations, differences in intrinsic spectra and modes of action can result in significant variation, highlighting that these correlations aren't caused by shared targets but rather by distinct microbial responses[25]. These statistically significant differences are crucial for directing antimicrobial stewardship and combinatorial therapy, particularly in areas where recent surveillance analyses have shown an increase in macrolide resistance.

Table IV showed a statistically significant comparative efficacy of Clarithromycin with Nisin, chlorhexidine and their combination. Chlorhexidine showed a statistically significant comparison with penicillin.

Nisin showed statistically significant values with Clarithromycin, while chlorhexidine individually exhibits statistically significant values with Clarithromycin and Penicillin, indicating vigorous comparative antibacterial activity with these antibiotics. Because of its membrane-active mechanism against Gram-positive bacteria, which can complement antibiotic action, Nisin's statistically significant association with Clarithromycin indicates that the bacteriocin can either enhance or reflect different antibacterial responses when compared with macrolide antibiotics[26]. Comparably, chlorhexidine's notable outcomes with penicillin and Clarithromycin highlight its potent, broad-spectrum antiseptic action, which can disrupt biofilms and enhance the effects of systemic antibiotics in vitro[27].

Compared with individual antibiotics, the combination of Nisin and chlorhexidine in this investigation did not show statistically significant differences in antibacterial activity, indicating a lack of synergistic effect in vitro compared with other antibiotic groups. This result is consistent with other studies that demonstrated that, when applied to multispecies biofilm models, Nisin did not enhance antibiofilm activity beyond that of chlorhexidine alone[28,29]. These findings emphasize the need for focused assessments of combination medicines and for additional in vitro studies, as not all antimicrobial combinations provide additive or synergistic benefits.

The clinical and research implications of the study revealed that certain antibiotics may be more successful than others; these findings can help direct empirical treatment for oral infections. It is helpful for empirical therapy selection in dental, ENT, or soft-tissue infections based on the organisms involved. From a scientific point of view, these results might call for further research into the mechanisms - such as resistance profiles, biofilm penetration, or the spectrum of action - that underlie the greater activity of some antibiotics.

The strengths of the study include comparisons of antibiotics and their effects on oral bacterial flora, statistical precision, paired evaluations, and determination of the impact of the most and least effective antibiotics on supragingival plaque bacteria. It also compares the effects of chemical-based compounds, i.e., antibiotics and chlorhexidine, with those of biologically derived compounds, such as the probiotic Nisin. This research serves as baseline data for upcoming research for the local population.

Limitations of the study included a cross-sectional design, a small sample size, a lack of uncultivable data, etc. For robust conclusions, in vivo results should also be considered.

It has been recommended that encouragement should be given for the use of evidence-based antibiotics related to specific bacterial species to improve dental care, create national guidelines for antibiotic sensitivity patterns of local bacterial isolates, boost services for

diagnostic microbiology associated with the oral cavity, train, and capacity building, and campaigns of public awareness regarding maintenance of oral hygiene.

Assessing antibiotic sensitivity and contrasting traditional antibiotics with substances like Nisin and chlorhexidine helps manage oral infections effectively, encourages sensible antimicrobial use, and helps combat antimicrobial resistance—all of which are essential goals under SDG 3, and it also indirectly supports SDG 12, responsible consumption and production by encouraging alternative and judicious use of antimicrobial agents. In addition, this study has achieved several socioeconomic benefits for Pakistan. It minimizes medical expenses by the effective use of antibiotics against oral bacterial isolates, reducing treatment failures, follow-up visits, and extended infections, which lowers costs for both patients and the healthcare system. It also enhances workforce efficiency, helps fight antibiotic resistance, and encourages pharmaceutical research and policy. The study's results can help policymakers and regional pharmaceutical businesses align production and regulation with real clinical needs. This study not only contributes meaningful insights into the selection of antibiotics for oral bacterial infections but also serves as a foundation for clinical guidelines, public health policies, and future research aimed at optimizing oral healthcare delivery in Pakistan and similar contexts.

CONCLUSION

Clinically isolated oral bacterial species exhibited a wide range of antibiotic sensitivities, reflecting variations in microbial susceptibility and antimicrobial processes. Compared with traditional antibiotics, Nisin and chlorhexidine showed varying degrees of statistical significance, suggesting that their antibacterial properties are organism-dependent and multifactorial. Nisin in comparison to chlorhexidine may be a helpful biocompatible alternative for the inhibition of bacterial growth in oral biofilms.

ACKNOWLEDGEMENTS

The authors greatly appreciate Dr Aman Ashar's assistance with data collection.

Ethical permission: Jinnah University for Women's ERC letter No. JUW/IERB/SCI-ARA-003/2025.
Conflict of interest: There is no conflict of interest between the authors.
Financial Disclosure / Grant Approval: It was a component of a doctoral project. Jinnah University for Women supplied all of the study's materials.
DISCLAIMER: The manuscript was part of a PhD thesis project in Microbiology
Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

All authors have contributed equally such as:
Substantial contributions to the concept or design of work, or the accusation, analysis, interpretation of data for the work, drafting the work or revising it critically for important intellectual content, final approval for publishing, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Neal TW, Schlieve T. Complications of severe odontogenic infections: a review. Biology. 2022 Dec 8;11(12):1784.
2. Kashif M, Kazmi M, Tanwir F, Akbar S, Mujtaba F, Gul S. Isolation, Identification and Phenotypic Analysis of the Oral Bacterial Species from Supragingival Dental Plaque and Their Association with Different Factors. Bangladesh J Med Sci. 2025; 24(4): 1153–1166. https://doi.org/10.3329/bjms.v24i4.84687.
3. Khan RT, Sharma V, Khan SS, Rasool S. Prevention and potential remedies for antibiotic resistance: current research and future prospects. Front Microbiol. 2024 Oct 3; 15: 1455759.
4. Abdullah FM, Hatim QY, Oraibi AI, Alsafar TH, Alsandook TA, Lutfi W et al. Antimicrobial management of dental infections: Updated review. Medicine. 2024 Jul 5; 103(27): e38630.
5. Kashif M, Ashar A. Important microbes in dentistry. 2024. Microbes Med Importance. IIP Series. 515-45.
6. Thompson W, Williams D, Pulcini C, Sanderson S, Calfon P, Verma M. Tackling antibiotic resistance: why dentistry matters. Int Dent J. 2021 Feb 10; 71(6): 450.
7. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the Clinical and Laboratory Standards Institute performance standards for antimicrobial susceptibility testing, M100. J Clin Microbiol. 2021 Nov 18; 59(12): 10-128.
8. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. European journal of microbiology & immunology. 2024; 14(2): 97–115. https://doi.org/10.1556/1886.2024.00035.
9. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontology. 1967 Nov; 38(6): 610-6.
10. Zaheer J, Khan MN, Rahman AU, Shahzad MA, Yaasir Z, Lateef M et al. Identification and Epidemiological Analysis of Antibiotic-Resistant Bacteria in the Oral Microbiome of the Population in Pakistan. Cureus. 2024 Oct 1; 16(10): e70666. doi: 10.7759/cureus.70666.
11. Zaheer J, Khan MN, Rahman AU, Ishfaq M, Shahzad MA, Lateef M et al. Characterization of Antibiotic Resistance Profiles in the Oral Microbiota of the Pakistani Population. Cureus. 2024; 16(10): e72617. https://doi.org/10.7759/cureus.72617.
12. Bayani M, Raisolvaezin K, Almasi-Hashiani A, Mirhoseini SH. Bacterial biofilm prevalence in dental unit waterlines: a systematic review and meta-analysis. BMC Oral Health. 2023 Mar 18; 23(1): 158.
13. Kashif M, Kazmi M. Probiotics, A New Frontier in Oral Health: Unraveling the Efficacy of Probiotic Metabolites vs. Chlorhexidine on Streptococcus mutans in Dental Plaque. J Pak Med Assoc. 2024 Mar 1; 74(3): 430-1.
14. Radaic A, de Jesus VC, Kapila YL. The oralome and its dysbiosis: New insights into oral microbiome-host interactions. Comput Struct Biotech J. 2022; 20: 141-152.
15. Chaiyasut C, Sivamaruthi BS, Sirilun S. Evaluation of Nisin-based products in dental applications: A review of in vitro and in vivo studies. J Oral Biosci. 2022; 64(1): 45-52.
16. Funk A, Jia Q, Janke L, Crawford A, Iverson A, Rosch J et al. Isolation and characterization of a novel alpha-hemolytic Streptococcus spp. from the oral cavity and blood of septicemic periparturient immunodeficient mice. Compar Med. 2023 Oct; 73(5): 346.
17. Alves DR. Antimicrobial activity of macrolides against oral pathogens: current perspectives. J Oral Microbiol. 2023; 15(1): 2184567.

18. Chen L. Penicillin resistance in oral streptococci: molecular mechanisms and clinical implications. Front Cell Infect Microbiol. 2022; 12: 949312.
19. Teymouri M, Mollazadeh S, Oroojalian F, Amani A, Arezumand R, Shakeri F. Liposomes used in the delivery of various antimicrobials to biofilm-producing infections: a systematic review. Nanomed J. 2025 Apr 1; 12(2).
20. Nayeem A, Suresh AS, Vellapandian C, Singh S, Elossaily GM, Prajapati BG. Comprehensive Insights into Cephalosporins: Spectrum, Generations, and Clinical Applications. Current Drug Therapy. 2024 Dec 17. https://doi.org/10.2174/0115748855339119241204071146.
21. Zhang X, Tan L, Ouyang P, Ma H, Peng J, Shi T et al. Analysis of distribution and antibiotic resistance of Gram-positive bacteria isolated from a tertiary-care hospital in southern China: an 8-year retrospective study. Front Microbiol. 2023 Sep 28; 14: 1220363.
22. Khan M, Samant PS, Chauhan R, Khan R, Siddiqui S, Sachan S. Comparative Evaluation of Effectiveness of Nisin, Amoxicillin/Clavulanic Acid (Augmentin) and Chlorhexidine on E. faecalis as an Intracanal Irrigant: An In-vitro Study. J Pharm Bioallied Sci. 2023 Jul; 15(Suppl 2): S1317–S1320.
23. Shivaee A, Kazemi F, Navidifar T. Global trends in macrolide and lincosamide resistance in Streptococcus species: a comprehensive systematic review and meta-analysis. BMC Infect Dis. 2025; 25: 1577. https://doi.org/10.1186/s12879-025-11884-5.
24. Amin AN, Dellinger EP, Harnett G, Kraft BD, LaPlante KL, LoVecchio F et al. It's about the patients: Practical antibiotic stewardship in outpatient settings in the United States. Front Med. 2022 Jul 27; 9: 901980. https://doi.org/10.3389/fmed.2022.901980.
25. Ahmad I, Kubaev A, Zwamel AH, Baldaniya L, Kaur J, Rani B et al. Insights into Haemophilus macrolide resistance: A comprehensive systematic review and meta-analysis. PLoS Negl Trop Dis. 2025 Mar 4; 19(3): e0012878. doi: 10.1371/journal.pntd.0012878.
26. Pinheiro ET, Karygianni L, Attin T, Thurnheer T. Antibacterial Effect of High-Purity Nisin Alone and in Combination with D-Amino Acids or Chlorhexidine in an Endodontic-Like Biofilm Model. Antibiotics (Basel). 2021 Feb 2; 10(2): 149. doi: 10.3390/antibiotics10020149.
27. Samgane G, Karaçam S, Tunçer Çağlayan S. Unveiling the synergistic potency of chlorhexidine and azithromycin in combined action. Naunyn-Schmiedeberg's Arch Pharmacol 2024; 397: 5975–987. https://doi.org/10.1007/s00210-024-03010-0.
28. Mitra D, Yadav A, Prithyani S, John LE, Rodrigues S, Shah R. The antiplaque efficacy of lantibiotic Nisin extract mouthrinse. J Indian Soc Periodontol. 2019 Jan-Feb; 23(1): 31-34. doi: 10.4103/jisp.jisp_326_18.
29. Abay Celkan İ, Özdemir FN, Bekhedda N, Mottaghizadeh F, Akçelik N. In Vitro Assessment of the Antimicrobial and Antibiofilm Efficacy of Chlorhexidine, EDTA, and NaOCl in Combination with Nisin Against Enterococcus faecalis Isolates. Mikrobiyol Bul. 2025 Oct; 59(4): 437-451. Turkish. doi: 10.5578/mb.20250419.