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IDENTIFICATION OF PATHOGENS IN ORAL INFECTIONS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS: A CROSS-SECTIONAL STUDY FROM SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

*Background: Oral infections are known to be highly polymicrobial, but growing rates of antimicrobial resistance (AMR) are leading to reduced efficacy of treatment, especially in South Asia, due to widespread avalability of non-prescription antibiotics. Objectives: To establish the pathogens involved in oral infections and their antibiotic resistance patterns to penicillin, linezolid, vancomycin, gentamycin, moxifloxacin, and TMP-SMX. Methods: In this experimental cross-sectional study carried out at the Laboratory of Khyber Medical University Institute of Health Sciences, Swabi from December 2025 to May 2026, oral swabs were collected from 153 patients having clinically diagnosed cases of oral infection. Bacterial cultures were grown on blood and MacConkey agars; their identification was done based on colony morphology and Gram stain reaction, while antibiotic resistance was determined using disc diffusion technique. Results: Total of ten bacteria were isolated, among which gram-negative bacteria were more commonly isolated than the gram-positive ones (greater than 57% prevalence rate). *Prevotella intermedia* and *Staphylococcus aureus* were the most common bacteria isolated (both at 14.4%), followed by *Streptococcus viridans* (13.1%) and *Tannerella forsythia* (11.1%). Antibiotic resistance rates were high; linezolid was the most sensitive antibiotic (30.7%) but vancomycin showed maximum resistance (27.5%). Only 22.2% isolates were sensitive to penicillin. Lowest resistance rates were observed in gentamycin (20.3%), but significant rates of intermediate susceptibility were observed in it too. Both moxifloxacin and TMP-SMX exhibited very poor antibiotic sensitivity rates (less than 25%). Most of the infections were present in middle-aged individuals; males were slightly more common. Conclusion: Pathogens of oral infections exhibit a high degree of AMR, which is compromising the antibiotic treatment efficacy. Penicillin cannot be reliably used any more for treating such infections. Culturing prior to starting empirical antibiotic treatment is a must.*

Introduction

Oral infection is among the most common infectious disease worldwide and remain a major public health concern. these infections affect the oral cavity ,teeth, gingiva ,periodontal tissues salivary glands, and surrounding maxillofacial structures. Factors such as poor oral hygiene, dental caries, periodontal diseases, malnutrition, tobacco use, trauma, systemic disorders, and immunocompromised conditions contribute to their development (1). If untreated, oral infections may progress to serious complications including cellulitis, osteomyelitis, septicemia, deep neck infections, and airway obstruction(2). The oral cavity a favorable environment for microbial growth due to its moisture, warmth, and continuous nutrient supply. More than 700 microbial species, including bacteria, fungi, viruses, and protozoa, inhabit the oral cavity (3). Under normal conditions, these microorganisms maintain a balanced ecosystem; however, disturbances in oral hygiene, diet, or immune status can promote the overgrowth of pathogenic organisms and lead to infection (4). Bacteria are the primary causative agents of oral infections, which are usually polymicrobial and involve both aerobic and anaerobic species. Common pathogens include *Streptococcus mutans, Streptococcus viridans, Staphylococcus aureus, Enterococcus faecalis, Porphyromonas gingivalis, Prevotella intermedia,* and *Fusobacterium nucleatum* (5). Anaerobic bacteria are particularly associated with periodontal diseases and odontogenic infections (6). Dental caries and periodontal diseases are the most prevalent oral infections worldwide. *Streptococcus mutans* plays a major role in dental caries because of its acidogenic properties (7) while pathogens such as *Porphyromonas gingivalis, Treponema denticola,* and *Tannerella forsythia* contribute significantly to periodontal disease progression (8). Odontogenic infections originate from teeth or supporting tissues and may spread to fascial spaces of the head and neck, becoming life-threatening if not treated promptly (9). Therefore, early diagnosis and identification of causative microorganisms are essential. Conventional microbiological methods such as Gram staining, culture techniques, colony morphology, and biochemical tests are commonly used for pathogen identification (10). Molecular techniques including polymerase chain reaction (PCR), DNA hybridization, and sequencing have further improved the rapid and accurate detection of oral pathogens (11). Antibiotics are widely prescribed in dentistry for the treatment and prevention of oral infections, especially in cases with systemic involvement such as fever, swelling, and diffuse infections (12). Commonly used antibiotics include penicillin, amoxicillin, metronidazole, clindamycin, erythromycin, tetracycline, and amoxicillin-clavulanic acid (13). However, the increasing emergence of antimicrobial resistance among oral pathogens due to inappropriate antibiotic use has become a major global health concern (14). Resistance in orally-associated microorganisms to commonly used drugs such as penicillin, erythromycin, and tetracycline has become one of the main problems, causing failure of the therapy and the occurrence of the same disease again. Factors responsible for this problem include irrational prescription, self-treatment, non-compliance with the full course of treatment, availability of over-the-counter drugs, and misuses of broad-spectrum antimicrobials (15). Repeated use of oral bacteria to antibiotics can alter the composition of the oral microflora and result in the emergence of new resistant strains. An important aspect of antimicrobial management is the determination of the sensitivity or resistance of microorganisms to the action of antimicrobials by means of various types of tests. They include the widely-used Kirby-Bauer disk diffusion test, the dilution broth method, and some other automated techniques (16). Differences in the susceptibility profiles of some oral microorganisms have been detected between various countries as a consequence of differences in the practice of antibiotic prescribing and administration (17). Thus, constant monitoring of the pathogenic microorganisms and their sensitivity is crucial. In addition to bacterial pathogens, fungal ones have also been recognized as responsible for oral infections. Among them, a major role belongs to Candida albicans; it causes the formation of lesions in immunocompromised persons, diabetics, denture users, and people taking long-term antibiotics (18). Growing resistance to antifungal medications, such as fluconazole, increases the significance of the examination of Candida strains' susceptibility to antifungals. The increasing number of cases of microbial resistance requires rational approaches to antibiotic prescribing. Therefore, the identification of pathogens in the mouth cavity and the estimation of their susceptibility to antibiotics are crucial for correct diagnosis, adequate treatment, and avoiding the development of resistance to antibiotics (19). The aim of the proposed study is to determine the most common pathogens responsible for oral infection and estimate the patterns of their sensitivity to antibiotics.

Literature Review

Oral infections are among the most common diseases worldwide and affect the teeth, gingiva, periodontal tissues, oral mucosa, salivary glands, and surrounding maxillofacial structures. Poor oral hygiene, smoking, malnutrition, diabetes mellitus, immunocompromised conditions, and prolonged antibiotic use are important predisposing factors (20). Untreated oral infections may lead to serious complications such as cellulitis, osteomyelitis, septicemia, and deep neck infections (21). The oral cavity contains a highly diverse microbial ecosystem composed of bacteria, fungi, viruses, and protozoa. More than 700 microbial species have been identified in the oral cavity (22). Under normal conditions, these microorganisms remain in balance with the host immune system; however, disturbances in oral environmental conditions may lead to pathogenic overgrowth and disease develpment (23). Dental plaque biofilm is considered a major etiological factor in oral infections. Dental caries is one of the most prevalent oral infectious diseases and is mainly associated with *Streptococcus mutans*, which produces acids that demineralize tooth enamel (24). *Lactobacillus* species also contribute to caries progression due to their aciduric properties (25). Periodontal diseases involve inflammation and destruction of tooth-supporting tissues and are strongly associated with anaerobic pathogens such as *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola* ((26)(27) These organisms produce virulence factors that contribute to tissue destruction and immune dysregulation. Odontogenic infections are polymicrobial infections originating from teeth or supporting structures. Commonly isolated pathogens include *Streptococcus, Prevotella, Fusobacterium,* and *Peptostreptococcus* species (28). If untreated, these infections may spread into facial spaces and become life-threatening. Conventional laboratory techniques such as Gram staining, culture methods, colony morphology, and biochemical tests are commonly used for pathogen identification (29). Molecular techniques including PCR and DNA sequencing have further improved the rapid and accurate detection of oral pathogens (30). Fungal infections, particularly oral candidiasis caused by *Candida albicans*, are common in immunocompromised individuals, diabetic patients, denture wearers, and patients receiving prolonged antibiotic therapy (31).Such findings emphasize the importance of local surveillance studies to support rational antibiotic prescribing. In summary, oral infections are polymicrobial diseases involving diverse bacterial and fungal pathogens. Identification of causative microorganisms and evaluation of their antibiotic susceptibility profiles are essential for effective treatment and prevention of complications. The increasing prevalence of antimicrobial resistance among oral pathogens highlights the need for continuous surveillance and rational antibiotic use in dental practice. Therefore, the present study aims to identify pathogens associated with oral infections and evaluate their antibiotic susceptibility patterns to support effective clinical management

Methods

An experimental-based cross-sectional study was conducted at the Laboratory of Khyber Medical University Institute of Health Sciences from December 2025 to May 2026 under standard laboratory conditions.A total of 153 participants with oral infections were included in the study. The sample size was selected based on a previously published study with similar objectives and methodology. Sterile oral swab samples were collected aseptically from infected oral sites, including gingival tissues, periodontal pockets, and oral lesions, from patients presenting with periodontal diseases and other oral infections.Samples were collected from patients attending the outpatient departments of District Headquarters Hospital Swabi, Tehsil Headquarters Hospital MTI Topi, and Bacha Khan Medical Complex MTI Shahmansoor. After obtaining informed consent, pus or exudate samples were collected aseptically using sterile swabs and transported to the microbiology laboratory within 1–2 hours in sterile transport media. Collected swabs were stored at 4°C until processing. Samples were cultured on Blood agar and MacConkey agar using standard microbiological techniques and incubated at 37°C for 24–48 hours. Culture plates were examined for colony morphology, pigmentation, and hemolytic characteristics. Distinct colonies were subcultured onto fresh media to obtain pure isolates for further identification and antibiotic susceptibility testing.

Results and Analysis

Sociodemographic Characteristics

Of the 153 participants enrolled, 85 (55.6%) were male and 68 (44.4%) were female, with no missing data for this variable. Across the five pre-defined age groups, the largest proportion of participants fell into age group 3 (28.8%), followed by age group 2 (23.5%), age group 1 (19.0%), age group 4 (18.3%), and age group 5 (10.5%) (Table 1).

Table 1. *Sociodemographic characteristics of study participants (N = 153)*

Variable	Category	n	%
Gender	Male	85	55.6
	Female	68	44.4
Age group[a]	Group 1	29	19.0
	Group 2	36	23.5
	Group 3	44	28.8
	Group 4	28	18.3
	Group 5	16	10.5
Total	–	**153**	**100.0**

Gram Staining Results of Isolated Organisms

Gram staining was performed on 153 specimens with no missing data. Gram-negative rods were the most common isolates, identified in 68 cases (44.4%), followed by gram-positive cocci in 65 cases (42.5%). Gram-negative coccobacilli and gram-negative spirochetes were detected in 11 (7.2%) and 9 (5.9%) cases, respectively. Overall, gram-negative organisms predominated, accounting for more than half of the isolates (Table 4.2).

Table 2. *Gram-stain morphology of isolated organisms (N = 153)*

Gram-Stain Category	N	%
Gram-negative rods	68	44.4
Gram-positive cocci	65	42.5
Gram-negative coccobacilli	11	7.2
Gram-negative spirochaetes	9	5.9
Total	153	100.0

Distribution of Bacterial Pathogens

Culture identified ten distinct bacterial species among the 153 isolates. *Prevotella intermedia* and *Staphylococcus aureus* were the most frequently isolated organisms, each accounting for 14.4% of isolates, followed by *Streptococcus viridans* (13.1%) and *Tannerella forsythia* (11.1%). *Fusobacterium nucleatum* (10.5%), *Streptococcus pneumoniae* (9.2%), *Porphyromonas gingivalis* (8.5%), *Aggregatibacter actinomycetemcomitans* (7.2%), and *Peptostreptococcus micros* and *Treponema denticola* (5.9% each) were identified less frequently (Table 3).

Table 3. *Distribution of bacterial pathogens isolated from oral infections (N = 153)*

Organism	n	%
Prevotella intermedia	22	14.4
Staphylococcus aureus	22	14.4
Streptococcus viridans	20	13.1
Tannerella forsythia	17	11.1
Fusobacterium nucleatum	16	10.5
Streptococcus pneumoniae	14	9.2
Porphyromonas gingivalis	13	8.5
Aggregatibacter actinomycetemcomitans	11	7.2
Peptostreptococcus micros	9	5.9
Treponema denticola	9	5.9
Total	153	100.0

Overall Antibiotic Susceptibility Pattern

Susceptibility testing demonstrated considerable resistance across all six antibiotics evaluated. Linezolid showed the highest sensitivity rate (30.7%), although resistance remained notable (26.1%). Vancomycin exhibited the highest resistance rate among the tested agents (27.5%). Sensitivity to penicillin was low (22.2%), with comparable proportions of resistant (22.2%) and intermediate (26.1%) isolates. Gentamicin showed the lowest resistance rate (20.3%) but predominantly intermediate susceptibility (29.4%). Moxifloxacin and trimethoprim-sulfamethoxazole both showed sensitivity rates below 25% (22.2% and 24.2%, respectively), with moxifloxacin showing the highest proportion of weak/variable responses among all antibiotics tested (34.0%) (Table 4).

Table 4. ***Overall in vitro antibiotic susceptibility pattern of isolates (N = 153)***

Antibiotic	IS n (%)	R n (%)	S n (%)	W n (%)
Penicillin	40 (26.1)	34 (22.2)	34 (22.2)	45 (29.4)
Linezolid	35 (22.9)	40 (26.1)	47 (30.7)	31 (20.3)
Vancomycin[b]	NR	42 (27.5)	NR	NR
Gentamicin	45 (29.4)	31 (20.3)	37 (24.2)	40 (26.1)
Moxifloxacin	36 (23.5)	31 (20.3)	34 (22.2)	52 (34.0)
Trimethoprim-sulfamethoxazole	44 (28.8)	30 (19.6)	37 (24.2)	42 (27.5)

IS, intermediate susceptibility; R, resistant; S, sensitive; W, weak/variable response; NR, not reported. [b] *A complete categorical breakdown (IS/S/W) for vancomycin was not available in the source dataset; only the resistant proportion could be verified.*

Organism-Specific Resistance Patterns

Organism-specific analysis of the four antibiotics with complete cross-tabulated data revealed marked variation in resistance by species. *Aggregatibacter actinomycetemcomitans* showed the highest resistance to gentamicin (45.5%) and penicillin (36.4%), while *Porphyromonas gingivalis* showed the highest resistance to moxifloxacin (38.5%). *Staphylococcus aureus* demonstrated comparatively high resistance to penicillin (31.8%) and trimethoprim-sulfamethoxazole (27.3%) but low resistance to gentamicin (4.5%). *Treponema denticola* and *Streptococcus pneumoniae* showed no resistance to gentamicin and trimethoprim-sulfamethoxazole, respectively, within this sample (Table 5).

Table 5. ***Organism-specific resistance rates (%R) to four antibiotics (N = 153)***

Organism	Penicillin %R	Gentamicin %R	Moxifloxacin %R	TMP-SMX %R
Aggregatibacter actinomycetemcomitans (n=11)	36.4	45.5	18.2	9.1
Fusobacterium nucleatum (n=16)	18.8	18.8	25.0	25.0
Peptostreptococcus micros (n=9)	11.1	22.2	11.1	11.1
Porphyromonas gingivalis (n=13)	23.1	30.8	38.5	23.1
Prevotella intermedia (n=22)	18.2	22.7	9.1	22.7
Staphylococcus aureus (n=22)	31.8	4.5	18.2	27.3
Streptococcus pneumoniae (n=14)	14.3	21.4	28.6	0.0
Streptococcus viridans (n=20)	15.0	20.0	30.0	25.0
Tannerella forsythia (n=17)	29.4	23.5	5.9	23.5
Treponema denticola (n=9)	22.2	0.0	22.2	11.1
Overall (N=153)	**22.2**	**20.3**	**20.3**	**19.6**

TMP-SMX, trimethoprim-sulfamethoxazole. Organism-level cross-tabulated data for linezolid and vancomycin were not available in the source dataset.

Moxifloxacin Susceptibility Pattern

Among the 153 isolates, weak/variable response was most common (34.0%), followed by intermediate susceptibility (23.5%) and sensitivity (22.2%), while resistance was least observed (20.3%)

Trimethoprim Sulfamethoxazole Susceptibility Pattern

A total of 153 isolates were tested for susceptibility to Trimethoprim Sulfamethoxazole.

Intermediate susceptibility (IS) was observed in 28.8% of isolates, while 24.2% were sensitive (S) to the antibiotic.

Resistance (R) was detected in 19.6% of the isolates

DISCUSSION

The present study included 153 patients with oral infections, with a slight male predominance (55.6%), which is consistent with previous reports from similar clinical settings where males often present more frequently with acute dental infections and associated risk factors such as tobacco use and poorer oral hygiene practices. Gram staining revealed a predominance of Gram-negative organisms (57.5%), particularly Gram-negative rods (44.4%), followed by Gram-positive cocci (42.5%). This pattern aligns with established oral microbiology, where anaerobic Gram-negative bacteria play a key role in periodontal and odontogenic infections. The presence of Gram-positive cocci also reflects the contribution of Streptococcus and Staphylococcus species in mixed infections. Culture analysis identified ten bacterial species, with Prevotella intermedia and Staphylococcus aureus being the most prevalent (14.4% each), followed by Streptococcus viridans (13.1%) and Tannerella forsythia (11.1%). The high prevalence of anaerobic periodontal pathogens such as Prevotella intermedia, Porphyromonas gingivalis, and Tannerella forsythia supports their established role in periodontal disease. The detection of Staphylococcus aureus in notable proportions suggests possible opportunistic colonization or infection, particularly in compromised oral environments. The overall findings confirm the polymicrobial nature of oral infections. Antibiotic susceptibility patterns demonstrated variable resistance across all tested antibiotics, with no single agent showing consistently high effectiveness. Penicillin showed low sensitivity (22.2%) with considerable resistance and intermediate responses, supporting reports of increasing β-lactam resistance among oral pathogens. Linezolid showed relatively higher sensitivity (30.7%), but resistance was still significant (26.1%), indicating emerging reduced susceptibility even to reserve antibiotics. Vancomycin showed the highest resistance rate (27.5%), raising concern regarding reduced efficacy against Gram-positive oral pathogens. Gentamycin showed the lowest resistance rate (20.3%) but mainly intermediate susceptibility, likely reflecting its limited activity against anaerobic oral flora. Moxifloxacin demonstrated predominantly weak or variable responses (34.0%), suggesting reduced reliability in routine use. TMP-SMX showed relatively low resistance (19.6%) but limited sensitivity, indicating only partial therapeutic usefulness. Overall, susceptibility patterns indicate widespread antimicrobial resistance among oral pathogens in this population, with all tested antibiotics showing limited high-level sensitivity. These findings reflect global concerns regarding irrational antibiotic use, self-medication, and over-the-counter antibiotic availability, particularly in developing regions. The detection of resistance even among reserve antibiotics such as vancomycin and linezolid is particularly concerning and highlights the urgent need for antimicrobial stewardship, culture-guided therapy, and continuous local surveillance to guide rational antibiotic selection in dental practice.

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study analyzed the microbiological profile and antibiotic susceptibility patterns of oral infection pathogens in 153 patients. A slight male predominance (55.6%) was observed. Gram-negative organisms were most common (57.5%), particularly Gram-negative rods, confirming the major role of anaerobic bacteria in oral infections. Ten bacterial species were identified, with Prevotella intermedia and Staphylococcus aureus being most frequent (14.4% each), followed by Streptococcus viridans, Tannerella forsythia, and Fusobacterium nucleatum. The presence of key periodontal pathogens, including red complex organisms, confirms their clinical importance, while the notable isolation of Staphylococcus aureus suggests emerging opportunistic colonization patterns. Antibiotic susceptibility testing showed reduced effectiveness across all antibiotics. Resistance ranged from 19.6% (TMP-SMX) to 27.5% (vancomycin). Linezolid showed the highest sensitivity (30.7%), while gentamycin showed the lowest resistance (20.3%). Overall, no antibiotic demonstrated consistently reliable efficacy, indicating significant antimicrobial resistance. These findings highlight the need for culture-guided therapy, rational antibiotic use, and strengthened antimicrobial stewardship. Limitations include single-center design and non-standard susceptibility categorization, limiting generalizability. In conclusion, oral infections in this population are polymicrobial with significant antimicrobial resistance, including reduced susceptibility to reserve antibiotics, emphasizing the need for continuous surveillance and evidence-based antibiotic selection.

5.2 Recommendations

1.Antibiotic therapy for oral infections should be guided by culture and susceptibility testing rather than empirical prescribing.

2.Antimicrobial stewardship programs should be implemented in dental and hospital settings to monitor and rationalize antibiotic use.

3.Strict regulation is needed to prevent over-the-counter availability and misuse of antibiotics.

4.Combination antibiotic therapy may be considered in severe polymicrobial infections.

5.Further multicenter and longitudinal studies are recommended to monitor resistance trends and investigate molecular mechanisms.

6.Clinicians should be vigilant regarding Staphylococcus aureus, including potential resistant strains, and manage cases using culture-guided therapy.

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