RESEARCH ARTICLE

Antimicrobial resistance patterns and bacterial profiling among dental caries patients

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Abstract

Objective: To examine the prevalence of dental caries and their antibiotic resistance patterns.

Method: The cohort study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan, from November 2022 to April 2023, and comprised bacterial dental caries samples that were processed for different biochemical parameters and antibiotic susceptibility. Data was analysed using SPSS version 2022.

Results: Out of the 1,000 subjects from whom the samples were collected, 676(67.6%) were males and 324(32.4%) were females. Overall, 426(42.6%) subjects were aged 5-20 years. Of the total samples, 540(54%) were positive for pathogenic bacteria, with streptococcus mutans 256(25.6%) being the most common bacteria, followed by streptococcus mitis 194(19.4%), escherichia coli 69(6.9%) and pseudomonas aeruginosa 21(2.1%). Dental caries was more common among male patients 396 (73.3%), and the most affected age group was 5-20 years 261(48.3%). Illiterate patients 302 (56%) had more dental caries compared to the literate 238 (44.07%) patients. Antibiotic sensitivity tests revealed that most bacterial isolates were highly resistant to ampicillin, ciprofloxacin, and vancomycin.

Conclusion: Dental caries mostly affected males and those aged 5-20 years. Most bacterial isolates were resistant to ampicilin, ciprofloxacin and vancomycin.

Key Words: Anti-Bacterial, Vancomycin, Streptococcus mutans, Ciprofloxacin, Pseudomonas aeruginosa, Streptococcus mitis, Vaccinology, Dental Caries, Microbial, Ampicillin. (JPMA 75: 41; 2025)

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Introduction

The oral cavity is an aetiological niche of microorganisms, including both normal flora and opportunistic pathogens that can pose a risk of cross contamination and systemic infection. Oral microbiota consists of viruses, protozoa, yeast and bacteria, forming a diverse ecological environment in the mouth. The oral microbiota plays a vital role in oral health and management, but in unfavourable conditions, commensals in oral cavity lead to dental infections.[1]

There are thousands of microbial species residing in the oral cavity that are responsible for dental caries and even oral cancer. Dental caries is an odontological biofilm sugar-driven, mediated, multifactorial microbial infection that results in localised destruction of calcified tissues. Caries can damage the crown and expose the surface of both primary and permanent dentition.[2] Acids can damage the enamel by detaching calcium and phosphate that causes dental caries.[3-4] Some acid-producing bacteria, such as veillonella[5], scardovia[6], lactobacillus[7] and Propionibacterium, have been associated with dental caries.[8] Streptococcus (S.) mutans and S. sorbrinus are also crucial in this regard, producing organic acids from dietary sugars and assisting bacterial colonisation. Low potential of hydrogen (pH) in plaque is ideal for aciduric bacteria, such as streptococci, lactobacilli and bibidobacteria, as they are more competitive at low pH.[9] Dental decay causes tooth-loss, limiting a varied diet and reducing the intake of fruits, vegetables and non-starch polysaccharides. Tooth-loss can hinder dietary goals, reduce food enjoyment, and impact socialisation. The development of caries requires sugars and bacteria, influenced by tooth susceptibility, bacterial profile, saliva quality, and available fermentable dietary carbohydrates.[10]

A number of microorganisms associated with dental and oral-maxillofacial infections are also linked with antibiotic resistance, according to the World Health Organisation (WHO).[11] There are some concerns that persistent use and misuse of antibiotics could lead to the emergence of resistant strains. Pathogenic antimicrobial resistance (AMR) has been a global issue, leading to financial loss and a catastrophe in global health.[12] Some antibiotics can make bacteria innately resistant to them, and long-term selective pressure can make bacteria develop acquired resistance.

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Bacterial resistance to an antibiotic that it encounters owing to its inherent structural or functional characteristics is known as intrinsic resistance.[13]

Despite the clinical significance and frequency of dental and oral-maxillofacial infections, contemporary data, especially from multi-centre research, is lacking about the variety of clinical pathogens and associated antibiotic resistance for these types of infections. Such details are necessary to develop therapeutic guidelines and suggestions for the treatment of dental and oral-maxillofacial infections.[14]

The prevalence of permanent dental caries ranks first among 328 diseases. There are 2.44 billion dental decay patients worldwide.[15] A recent survey in Pakistan showed that around 84% of overall population is suffering from dental caries.[16] In China, 71.9% children aged <5 years and 38.5% at the age of 12 years are suffering from dental caries.[17] Around 7.8% of the total world population had untreated deciduous dental caries, while 29.4% had untreated permanent caries.[18]

The current study was planned to examine the prevalence of dental caries and their antibiotic resistance patterns.

Materials and Methods

The cohort study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan, from November 2022 to April 2023. After approval from the institutional ethics review committee, the sample size was calculated using the Cochran formula n=z2p(1-p)/d2, where n was the required sample size, Z was level of confidence 95% which was 1.96, P was 50% proportion which was 0.5, and d was the margin of error which was 0.05.[19] The sample was raised using consecutive sampling technique.

Bacteria-infected dental caries samples were collected from patients at the Sandeman Provincial Hospital, Quetta, who had complaints of toothache, sensitivity, visible holes or pits, swelling, pus, and loose teeth. The samples were transported for microbiological analysis to the CASVAB laboratory in an icebox using transport media. Before sample collection, informed consent was obtained from all the participants or their parents.

Data regarding gender, age, education and socioeconomic status (SES) was gathered using a pre-designed checklist. Both male and female patients were included, while children aged <5 years and individuals reluctant to provide data and sample were excluded.

The samples were inoculated in Brain heart infusion (BHI) medium and incubated at 37°C for 24 hours. The overnight bacterial cultures were streaked on selective and differential media plates, including mitis salivarius bacitracin (MSB) agar, eosin-methylene blue (EMB) agar, and cetrimide agar, according to the requirement of bacterial growth conditions.

The pure colonies were used for additional organism confirmation. Gram-staining and various biochemical tests, including Indole, Simmon citrate, Methyl red, Voges-Proskauer, Oxidase, Motility and Catalase assays, all these media were manufactured by (HiMedia, India) company were performed.

Deoxyribonucleic acid (DNA) was extracted using a DNA purification kit (Hiper Bacterial Genomic DNA Extraction Teaching Kit, India). After extraction, DNA templates were preserved for further usage at -20°C. Specific primers were used for each strain to amplify their respective polymerase chain reaction (PCR) fragments (Table 1). PCR reaction mixture (2x AmpMaste Taq) was used for the 5 different isolates, and they were subjected to thermal cycler, including initial denaturation 94°C for 5min, final denaturation 94°C for 1min, annealing at specific temperature and time based on their primers, initial extension 72 °C for 1min, and final extension 72°C for 10 min, followed by 30 cycles. The final PCR products were observed through gel electrophoresis by using 1.5% agarose gel under ultraviolet (UV) light.

To determine antimicrobial susceptibility, disc diffusion method was used as recommended by the Clinical and Laboratory Standards Institute (CLSI).[20] A Mueller-Hinton agar media plate (Oxoid, United Kingdom) was prepared with a suspension of bacterial cells (0.5 McFarland), which was then spread out on the plate's surface. The plate was then incubated at 37°C for 24 hours. Antimicrobial susceptibility of the isolates was checked with 20 strains of each isolate. The suspension of bacterial cells was used to determine sensitivity or resistivity to particular antimicrobial agents on the basis of inhibitory zones.[21]

Data was analysed using SPSS version 22. Demographic parameters of the patients were analysed using Pearson's chi-square test. $p \leq 0.05$ was considered significant.

Results

Of the 1,000 subjects from whom the samples were collected, 676(67.6%) were males and 324(32.4%) were females. Overall, 426(42.6%) subjects were aged 5-20 years. Of the total samples, 540(54%) were positive for pathogenic bacteria, with S. mutans 256(25.6%) being the most common bacteria, followed by S. mitis 194(19.4%), escherichia (E.) coli 69(6.9%) and

Table-1: The list of primers used for isolating bacteria from dental caries

Organisms	Direction	Primer sequence	Bp size	Annealing temperature	Reference
E. coli	F	5′ CCAAAAGCCAGACAGAGT 3′	623	60 sec at 53°C	(Alsanjary et al., 2022)31
	R	5` GCACAGCACATCCCCAAAGAG 3`			
Strep. Mitis	F	5′ TGG CTT ATC CTT CCT AGA TGG 3′	557	30 sec at 58°C	(Park et al., 2012)32
	R	5′ GAT TGC GGT CGA CAA 3′			
Pseudomonas aeruginosa	F	5′ATGGAAATGCTGAAATTCGGC 3′	956	30 sec at 58°C	(Gawad, et al.2022)33
	R	5′ CTTCTTCAGCTCGACGCGACG 3′			
Strep.Mutans	F	5′ TCG CGA AAA AGA TAA ACA AAC A 3′	479	30 sec at 55°C	(Palmer et al., 2012)34
	R	5′ GCC CCT TCA CAG TTG GTT AG 3′			

E: Escherichia, Strep: Streptococcus.

Table-2: Socio-demographic and clinical characteristics of patients with dental caries

Dental Caries Patients in Balochistan, Quetta City Demographic parameters		Positive values	Negative values	P-value
Samples (%)	Total number of samples = 1000	n=540; 54%	n=460; 46%	0.030*
Sex %	Female	n=144; 14.4%	n=180; 18%	0.030*
	Male	n=396; 39.6%	n=280; 28%	
Race%	Baloch	n=198; 19.8%	n= 180;18%	0.0008*
	Hazara	n=89; 8.9%	n= 85; 8.5%	
	Panjabi	n=65; 6.5%	n= 35; 3.5%	
	Pushtoon	n=188; 18.8%	n=160; 16%	
Class %	Higher	n=104; 10.4%	n= 90; 9%	0.001*
	Middle	n=138; 13.8%	n= 175; 17.5%	
	Lower	n=298; 29.8%	n=195; 19.5%	
Literacy %	Literate	n=238; 44.07%	n= 110; 11%	
	Illiterate	n=302; 56%	n=350; 35%	0.84
	5-20-years-old patients	n¬=261; 26.1%	n=165; 16.5%	
	21–40-years-old patients	n=149; 14.9%	n=125; 12.5%	0.000*
Age %	41-60-years-old patients	n=77; 7.7%	n=95;9.5%	
	more than 60 years	n=53; 5.3%	n=75; 7.5%	

E: Escherichia, Strep: Streptococcus.

Table-3: Biochemical and sugar fermentation tests for isolating bacteria from dental caries samples.

Selective media used for isolated Bacteria	Streptococcus mutans	Streptococcus mitis	Escherichia coli	Pseudomonas aeruginosa
Growth on selective media	Small rough blue colonies, 0.5-1mm	Blue smooth colonies with elliptical shape, 0.6-0.8 mm	Metallic sheen forming colonies, 2-3mm	Green colonies 1.5-3mm
Gram staining results of isolated bacteria from dental caries				
Gram staining	Gram positive cocci in pairs or chain, 0.5-0.75	Gram positive cocci in pairs or chain, 0.6-0.8	Gram negative rod shape, 1.0-2.0 mic long and 0.5 width	Gram negative rod shape, 1-5µ Long and 0.5-1.0 width
Biochemical tests results of isolated bacteria from dental caries				
Indole	+	-	+	-
Citrate	-	-	-	+
ethyl red	-	+	+	-
Voges-Proskauer	+	-	-	-
Oxidase	-	+	-	+
Motility	-	-	+	+
Catalase	-	-	+	+
Urease	-	-	-	+
Sugar fermentation tests results of isolated bacteria from dental caries				
Maltose	+	+	-	-
Glucose	+	+	+	-
Sucrose	+	+	-	-
Fructose	+	+	-	-
Mannose	+	+	-	-
Lactose	+	+	+	-

E: Escherichia, Strep: Streptococcus.

Table-4: Antibiotic sensitivity against pathogens isolated for dental caries samples.

Antibiotics	Conc (µg)	S. mutans n=20		S. mitis n=20		E. coli n=20		P. aeruginosa n=20	
		R	S	R	S	R	S	R	S
Ampicillin	10 µg	17/20	3/20	16/20	4/20	18/20	2/20	19/20	1/20
		85%	15%	80%	20%	90%	10%	95%	5%
Azithromycin	30 µg	1/20	19/20	2/20	18/20	17/20	3/20	18/20	2/20
		5%	95%	10%	90%	85%	15%	90%	10%
Ceftriaxone	30 µg	2/20	18/20	1/20	19/20	3/20	17/20	4/20	16/20
		10%	90%	5%	95%	15%	85%	20%	80%
Ciprofloxacin	5 µg	18/20	2/20	19/20	1/20	16/20	4/20	17/20	3/20
		90%	10%	95%	5%	80%	20%	85%	15%
Clindamycin	10 µg	3/20	17/20	1/20	19/20	18/20	2/20	16/20	4/20
		15%	85%	5%	95%	90%	10%	80%	20%
Erythromycin	15 µg	2/20	18/20	3/20	17/20	16/20	4/20	19/20	1/20
		10%	90%	15%	85%	80%	20%	95%	5%
Gentamicin	10 µg	19/20	1/20	4/20	16/20	3/20	17/20	2/20	18/20
		95%	5%	20%	80%	15%	85%	10%	90%
Tetracycline	30 µg	17/20	3/20	5/20	15/20	2/20	18/20	19/20	1/20
		85%	15%	25%	75%	10%	90%	95%	5%
Tobramycin	10 µg	16/20	4/20	3/20	17/20	1/20	19/20	18/20	2/20
		80%	20%	15%	85%	5%	85%	90%	10%
Vancomycin	30 µg	18/20	2/20	15/20	5/20	17/20	3/20	19/20	1/20
		90%	10%	75%	25%	85%	15%	85%	5%

S: Streptococcus, E: Escherichia, P: Pseudomonas, R: Resistant, S: Sensitive.

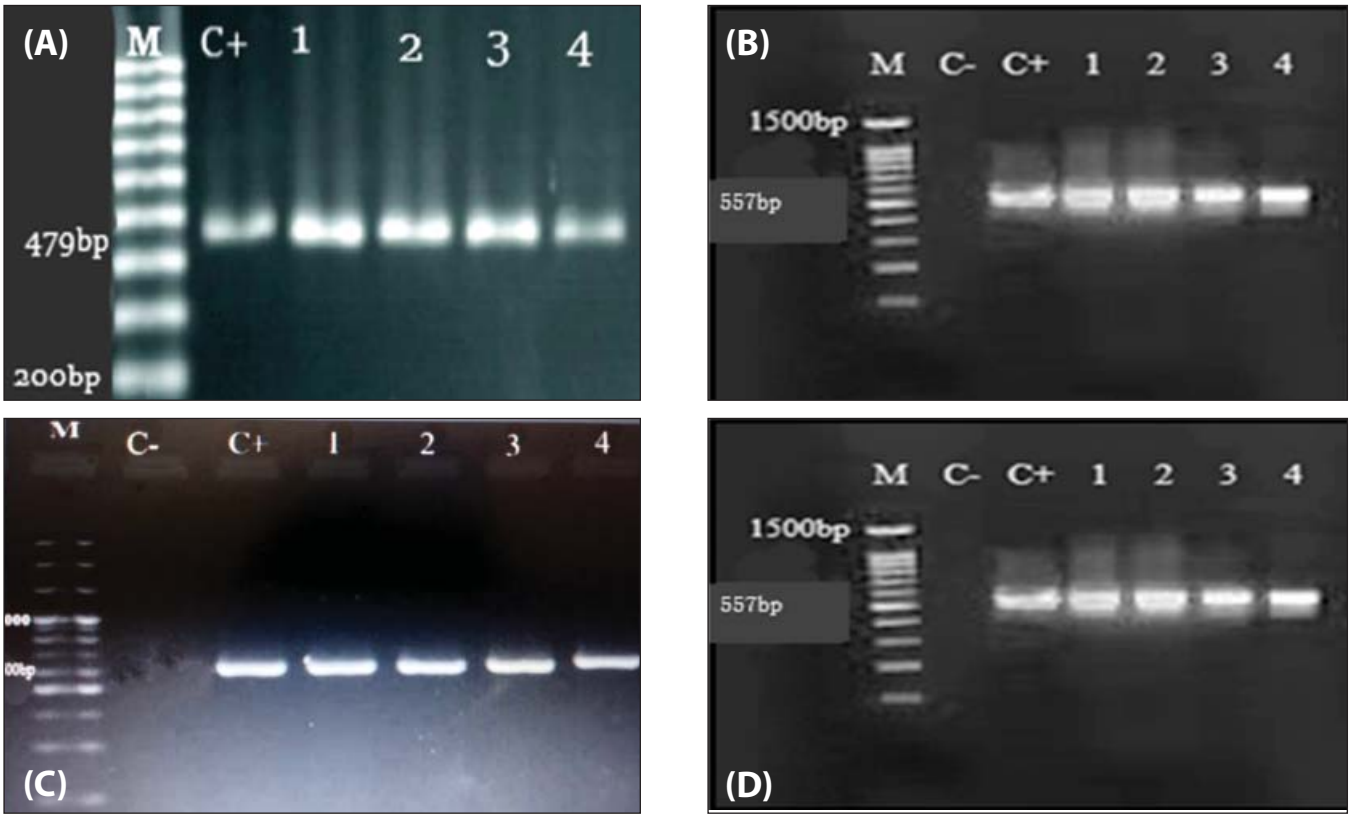

Figure: Polymerase chain reaction (PCR) base identification of bacterial isolates form dental caries. (A) Streptococcus (S.) mutans produced a specific size of 479bp fragment of the Sm479 gene. (B) S. mitis produced a specific size of 547bp fragment of the pheA gene. (C) Escherichia (E.) coli was identified with the uidA gene. (D) Pseudomonas (P.) aeruginosa produced a specific size of 956-bp fragment of the OprL gene

pseudomonas (P.) aeruginosa 21(2.1%). Dental caries was more common among male patients 396(73.3%), and the most affected age group was 5-20 years 261(48.3%). Illiterate patients 302 (56%) had more dental caries compared to the literate 238(44.07%) patients. Gender, SES, race and age were significant factors (Table 2). Data related to gram staining, biochemical tests and sugar fermentation tests was noted (Table 3).

E. coli and P. aeruginosa were highly resistant to ampiciilin, ciprofloxacin, clindamycin, erythromycin and vancomycin, while S. mutans and S. mitis showed resistance against ampicilin and vancomycin (Table 4).

PCR data for molecular base identification of the positive isolates was also noted (Figure).

Discussion

Dental caries, also referred to as tooth infection, is caused when bacteria and the tooth's surface interact and a biofilm form on the tooth's surface. During the dynamic process, the tooth's demineralisation and remineralisation phases change quickly. Particular caries lesions start to form at specific anatomical predilection places on the teeth if net demineralisation lasts for a long time. There must be a balance between the pathogenic and protective elements that influence dental caries' onset and progression. Protective variables promote remineralisation and lesion inhibition, while pathogenic factors tilt the scales in favour of dental caries and disease progression.[22]

Dental caries is one of the most common and chronic infections in the world. S. mutans was the most isolated bacteria from the dental samples in many studies,[21] while S. mitis, E. coli and P. aeruginosa were among the commonly isolated pathogens.[23] In the present study also, these organisms were isolated.

Previous studies revealed that males were infected more compared to females because of having more external environmental exposure and habits of unhealthy dietary lifestyle.[24] The current study also showed higher infection rate among male patients.

Dental caries infects various age groups, but it is most common in school-going children and teenagers as they have no proper hygiene maintenance and mostly eat junk food, including toffees and chocolates. The highest rate of infection was observed in the current study among those aged 5-20 years, followed by those aged 21-40 years, 41-60 years and those aged >60 years. The finding was similar to those reported earlier.[25,26] Hence, age is a significant determinant of dental caries risk. Children exhibit vulnerability in newly-erupted teeth due to immature enamel, whereas older adults are susceptible due to dietary modifications and decreased saliva production. Variations in oral hygiene practices across age groups also influence the prevention of decay. Customised interventions are essential, targetting lifelong habits and age-specific susceptibilities, to enhance oral health outcomes across diverse demographics.

Further, the importance of education in raising awareness about the importance of hygiene practices that can reduce the risk of infection among the population.

The current study also determined the relationship of SES with dental caries. This was comparable to findings reported in 2010.[27]

The current study identified variations among different ethnic groups, suggesting that the prevalence of dental caries is influenced by factors such as cultural practices, dietary habits and genetic makeup of individuals within specific ethnic communities.

A comprehensive range of biochemical tests were conducted to confirm the dental caries causing pathogens, which was consistent with earlier studies.[28-31]

In a recent study, molecular technique was employed to identify the pathogen-causing dental caries specifically for S. mutans, S. mitis, E. coil and P. aeruginosa by using specific primers for each pathogen related to the specific size of the gene.[31-32-33-34]

The antibiotic susceptibility test was conducted in the current study for 10 antibiotics. S. mutans and S. mitis were resistant to ampicillin, followed by ciprofloxacin and vancomycin, which was in line with previous studies.[35]

The current study has limitations. During dental caries sample collection, patient cooperation was a challenge due to anxiety or discomfort, hindering effective sample collection. Accessibility issues, especially in patients with limited jaw opening, may have restricted access to certain mouth areas. Diversity of the patient population was limited, affecting the generalisability of the findings. Sample collection methods, like drilling or biopsy, were considered invasive or unethical, and, as such, achieving a sufficient sample size for statistical significance was difficult. Time and resource constraints also limited the study's scope. Clinical setting variations may have introduced variability. Accurate diagnosis and identifying affected areas are crucial but challenging, especially in early cases, and prior dental treatments may have interfered with sample collection or could have affected microbial composition.

Conclusion

Dental caries mostly affected males and those aged 5-20 years. Most bacterial isolates were resistant to ampicilin, ciprofloxacin and vancomycin. In order to avoid dental infections and to enhance the quality of life, it is important for the people to be aware of personal hygiene, lifestyle choices, and timely reporting of dental caries.

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Author Contribution:
SJ: Literature search, writing and experiment.
MKT: Supervision and design.
MIK: Sample collection and approval.
MAK: Research and experiment.
AAS: Final draft.
SA: Data analysis.