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Integrative Morpho-Molecular Characterization and Antibiotic Resistance Profiling of Microbial Pathogens Associated with Dental Caries

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Abstract:

Background: Dental caries result from an imbalance in the oral environment, where bacteria produce acids that gradually dissolve tooth enamel over time. This study aims to isolate and characterize oral bacteria morphologically and genetically, and to determine the susceptibility of the bacteria to antibiotics.

Objective: the research is to identify the predominant bacterial isolates responsible for dental caries using the Polymerase Chain Reaction (PCR) technique.

Methods: A total of 150 samples were collected from tooth surfaces at the Specialized Dental Center in Babylon and cultured on suitable media under anaerobic conditions. A total of 87 bacterial isolates were obtained, showing the predominance of *Kocuria rosea* and *Streptococcus spp*., in addition to unidentified isolates identified using the Vitek 2 system. Antibiotic susceptibility testing was also performed using the same system, and *K.rosea* isolates showed resistance to several antibiotics, especially erythromycin, while remaining sensitive to

glycopeptides and tetracyclines. Polymerase Chain Reaction (PCR) was also conducted to detect the bacterial isolates.

Results: The isolates showed variable susceptibility to antimicrobial agents. A considerable proportion (41.7%) exhibited a multidrug-resistant (MDR) phenotype, with complete resistance to erythromycin and resistance to multiple antibiotic classes including macrolides, tetracyclines, aminoglycosides, fluoroquinolones, rifamycins, penicillins, and cephalosporins. However, all isolates remained susceptible to glycopeptides, oxazolidinones, and fosfomycin.

Conclusion: The study showed bacterial diversity in dental caries with the dominance of *K. rosea* and *Streptococcus spp*., along with the presence of multidrug-resistant isolates. PCR also confirmed the presence of virulence and resistance genes, highlighting their role in disease progression.

Keywords: Dental caries; Molecular detection; Antibiotic resistance; PCR.

Introduction: Dental caries is one of the most common bacterial diseases and is mainly caused by acid production, especially lactic acid, resulting from carbohydrate fermentation. This process leads to demineralization of tooth enamel and the formation of dental cavities[1]. The prevalence of dental caries increases with poor oral hygiene and high sugar intake due to an imbalance in the oral microbiota and the dominance of acidogenic bacteria. Among the bacteria associated with dental caries, *S.mutans, S. sobrinus*, and associated bacteria such as *K.rosea* play an important role in the development of dental lesions and oral infections [2,3].

K.rosea is considered part of the normal oral flora; however, it can become an opportunistic pathogen under immunocompromised conditions, causing oral infections that may progress to systemic complications such as septicemia and endocarditis [4]. Its virulence is associated with the production of extracellular enzymes and virulence genes, including urease-related genes (*UreA–UreG*), which enable its survival in acidic environments within the host [5]. In addition, the presence of resistance genes such as *ermA, ermB, and ermC* increases its ability to resist antibiotics and facilitates its spread [6].

The VITEK 2 system is one of the most widely used phenotypic diagnostic systems in clinical laboratories. It is based on rapid biochemical analysis for the identification of Gram-positive and Gram-negative bacteria with high accuracy. This system provides fast results within a short time, making it a valuable tool for routine laboratory diagnosis. However, it has some limitations in distinguishing closely related bacterial species with similar phenotypic characteristics, which may require additional confirmatory methods to ensure accurate identification [7,8].

In contrast, molecular diagnosis based on the *16S rRNA* gene is considered one of the most accurate and reliable methods for bacterial identification. It relies on sequence analysis of conserved and variable regions that allow precise differentiation between bacterial species. This technique has demonstrated high efficiency in confirming bacterial identity, reducing diagnostic errors associated with phenotypic methods, and is widely used in taxonomic studies and bacterial phylogenetic analysis [9]. The objective of this study is to perform a polyphasic identification of *Kocuria rosea* isolated from dental caries, integrating phenotypic screening via the VITEK 2 system with definitive molecular validation using 16S rRNA gene sequencing

Materials and methods:

Sample collection

A total of 150 oral samples were collected using sterile swabs from tooth surfaces of volunteers of different age groups and both sexes in several dental centers and clinics in Babylon province. Cotton swabs containing transport medium were used to ensure sample viability. The samples were placed in special transport tubes and transferred to the laboratory under appropriate cooling conditions to maintain integrity and ensure accurate results.

Identifying bacterial isolates: The samples were cultured on Mitis Salivarius Agar Base and Blood agar, then incubated anaerobically at 37°C for 48 hours using a candle jar. After incubation, preliminary tests and Gram staining were performed, followed by biochemical identification and final confirmation using the VITEK 2 automated system according to the manufacturer's instructions.

The study was designed to determine the antibiotic susceptibility patterns of *K.rosea* isolates (24 isolates) using the broth microdilution method in combination with the VITEK system. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, and isolates were classified as susceptible, intermediate, or resistant. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. Isolates showing higher resistance, particularly to erythromycin, were selected for molecular identification.

DNA Extraction and Molecular Detection of *K. rosea*

Genomic DNA was extracted from the bacterial isolates using the Geneaid DNA extraction kit (Taiwan) according to the manufacturer's instructions. The quality and integrity of the extracted DNA were assessed using electrophoresis on a 2% agarose gel.

Molecular detection was performed using polymerase chain reaction (PCR) for confirmation of bacterial identity. The *16S rRNA* gene was targeted for species-level identification of *K. rosea* using specific primers listed in Table 1. PCR primers were designed using NCBI and Primer3 software and synthesized by Scientific Research Co. Ltd., Iraq.The PCR reaction mixture contained Taq polymerase, dNTPs, $MgCl_2$, Tris-HCl, and KCl under optimized conditions. Amplification was carried out according to the thermocycling conditions presented in Table 2 to ensure accurate and specific detection of the target gene.

Table 1: PCR primers with nucleotide sequences and amplicon size.

Gene	Primer 5′–3′	Product Size	Gen bank
16S rRNA	F: GTACGGGCAGACTAGAGTGC	547 bp	CP127857.1
	R: CACCTTCCTCCGAGTTGACC		

Table 2: Thermocycling conditions for PCR.

No.	Gene name	PCR Amplicon	Initial denaturation Temp./time	Denaturation Temp./time	Annealing Temp./time	Extension Temp./time	Cycle	Final extension Temp./time	Hold Temp./time
1	16S rRNA	547 bp	95°C/ 5 min	95°C/ 30 sec	60°C/ 30 sec	72°C/ 55 sec	35	72°C/ 5 min	4°C/ forever

Results: The study demonstrated the isolation and purification of bacteria from 87 oral samples of patients with dental caries, where *K. rosea* was the most prevalent species (27.6%), followed by *Streptococcus spp*. (19.5%), with significant differences in distribution according to the Chi-square test, as shown in Table (3) and Figure (1).A total of 24 isolates were phenotypically identified and confirmed using the VITEK 2 Compact system, while molecular confirmation was performed for ten isolates using PCR targeting the *16S rRNA* gene with a 100% success rate. All isolates were found to carry virulence genes at a rate of 100%, and antimicrobial susceptibility testing according to CLSI guidelines revealed that 41.7% of the isolates were multidrug-resistant (MDR), with complete resistance to erythromycin, as shown in Table (4) and Figure (2).

Table (3): Frequency Distribution of Identified Bacterial Isolates

Bacterial isolate	**Frequency**	**Percentage (%)**
Kocuria rosea	**24**	**27.6%**
Streptococcus thoraltensis	7	8.0%
Streptococcus mitis	4	4.6%
Streptococcus sanguinis	3	3.4%
Streptococcus pseudoporcinus	3	3.4%
Streptococcus pluranimalium	3	3.4%
Kocuria kristinae	2	2.3%
Enterococcus raffinosus	1	1.1%
Granulicatella adiacens	1	1.1%

Dermacoccus nishinomiyaensis/Kytococcus sedentarius	1	1.1%
Granulicatella elegans	1	1.1%
Lactococcus lactis ssp cremoris	1	1.1%
Leuconostoc mesenteroides ssp cremoris	1	1.1%
Total isolates	**87**	**100%**

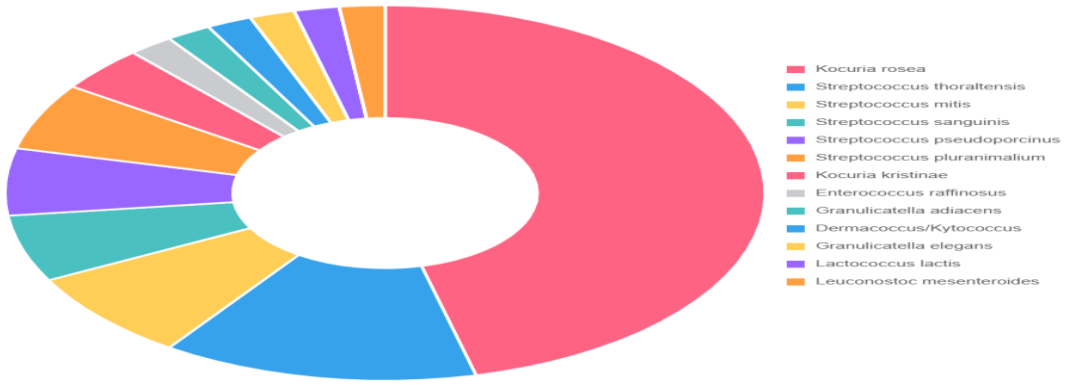

Figure (1): The Prevalence Distribution of bacterial isolates from mouth samples.

Table 4: Antibiotic Susceptibility of *Kocuria rosea* Isolates

Antibiotic Class	Antibiotic	Susceptible (S)	Intermediate (I)	Resistant (R)	% Susceptible
Glycopeptides	Vancomycin	**24**	0	0	**100%**
	Teicoplanin	**24**	0	0	**100%**
Oxazolidinones	Linezolid	**24**	0	0	**100%**
Tetracyclines	Tetracycline	**24**	0	0	**100%**
	Doxycycline	**24**	0	0	**100%**
Aminoglycosides	Gentamicin	**23**	**1**	0	**95.8%**
	Amikacin	**23**	**1**	0	**95.8%**
Fluoroquinolones	Levofloxacin	**24**	0	0	**100%**
	Ciprofloxacin	**23**	**1**	0	**95.8%**

Rifamycins	Rifampin	**24**	0	0	**100%**
Macrolides	Erythromycin	**14**	**4**	**6**	**58.3%**
Penicillins	Amoxicillin-Clavulanate	**21**	**3**	0	**87.5%**
Cephalosporins	Cefoxitin	**20**	**4**	0	**83.3%**
Folate Pathway Inhibitors	Trimethoprim-Sulfa	**18**	**4**	**2**	**75.0%**
Fosfomycin	Fosfomycin	**2**	**3**	**19**	**8.3%**

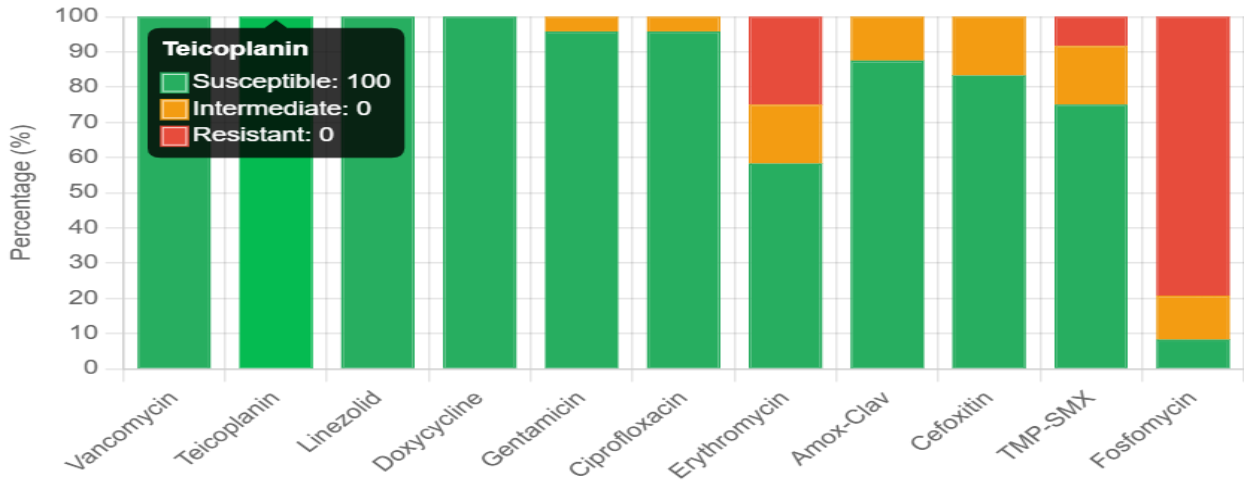

Figure (2): Overall Susceptibility Rates by Antibiotic

Molecular detection of the *16S rRNA* gene: molecular detection results for the 10 clinical multidrug-resistant (MDR) *K. rosea* isolates are presented in Figure (3) This figure shows the gel electrophoresis pattern of the PCR amplification targeting the *16S rRNA* gene. The PCR samples in agarose gel electrophoresis image was show a single, specific amplicon of approximately 547 bp was amplified in lanes S1 through S10, corresponding to each of the ten isolates. The bands were sharp, intense, and aligned precisely with the expected size, indicating a highly efficient and specific PCR reaction with no evidence of non-specific amplification or primer-dimer formation.

The positive result in every sample lane confirmed the identity of all isolates as *K. rosea* at the molecular level, achieving a 100% detection rate.

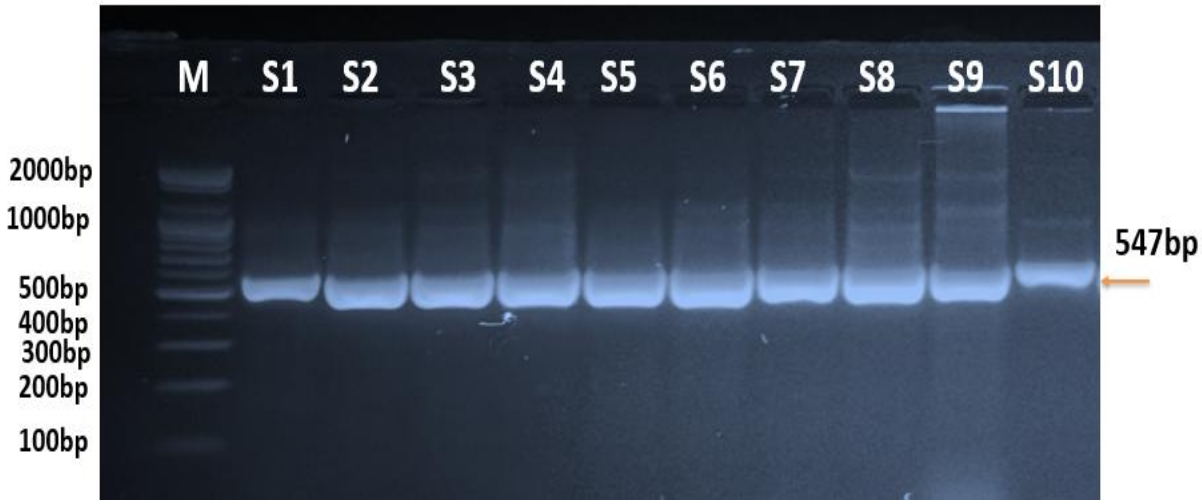

Figure 3: Agarose gel electrophoresis (2%) of *16S rRNA* gene PCR products from clinical MDR *K. rosea* isolates. Lane M: 100 bp DNA ladder. Lanes S1-S10: PCR products from ten clinical MDR *K. rosea* isolates. A specific amplicon of approximately 547 bp (indicated by the arrow) was successfully amplified in all isolates.

Discussion

The results of the present study indicate that the predominant bacterium among the clinical isolates associated with dental caries is *K.rosea*, suggesting a potential role for this genus as part of the normal oral microbiota with the ability to act as an opportunistic pathogen under certain conditions. The distribution of this organism is consistent with the findings of [10,11], who reported that members of the genus *K. rosea* may exist as commensals in the oral cavity and skin, with the potential to cause opportunistic infections when microbial balance is disturbed or immunity is compromised. This is further supported by [12], who emphasized its possible transition into a clinically relevant pathogen under specific conditions. The higher prevalence observed in this study compared to others may be attributed to differences in geographic location, dietary habits, oral hygiene status, and variations in isolation and identification techniques [13], which explains the variability among reported isolation rates.

In contrast, the results also demonstrated a clear presence of *Streptococcus spp*., which represents one of the core components of the normal oral flora in both healthy and diseased individuals, particularly species involved in dental plaque formation and acid production leading to dental caries. This finding is consistent with regional and local studies reporting the predominance of *streptococci* in oral samples, reflecting similarities in oral microbial composition across different populations [14,15,16]. Furthermore, a study conducted in Qatar reported that species such as *S. mutans* and *S. salivarius* constitute the highest proportion of oral isolates due to their strong ability to adhere to tooth surfaces and form biofilms [17], which explains their key role in the development of dental caries. At the phenotypic level, the VITEK 2 system demonstrated high efficiency in identifying Gram-positive bacterial isolates, with an overall accuracy of approximately 94.5% at the species level. This aligns with the findings of [18] regarding the system's reliability in routine diagnostic applications, as well as with [7], who reported its high accuracy in identifying Gram-positive cocci. This performance is attributed to its reliance on a wide range of biochemical tests, including enzymatic activities and carbohydrate fermentation profiles, generating a characteristic metabolic pattern compared against an extensive database. However, occasional misidentifications highlight limitations in distinguishing closely related species, as noted by [8].

Regarding antimicrobial susceptibility, the results revealed a considerable proportion of multidrug-resistant (MDR) isolates (41.7%), indicating an alarming increase in resistance patterns within the oral clinical environment. A marked resistance to erythromycin, a macrolide antibiotic, was observed, consistent with the findings of [19], who reported widespread macrolide resistance among *Kocuria spp*. This resistance is commonly associated with the presence of *erm* and *mef* genes, which modify the antibiotic binding site or mediate efflux mechanisms, thereby reducing drug efficacy. In contrast, the isolates showed high susceptibility to last-line antibiotics such as vancomycin, linezolid, and teicoplanin, in agreement with [20,21], who confirmed the continued effectiveness of these agents against multidrug-resistant Gram-positive cocci.

At the molecular level, PCR targeting the *16S rRNA* gene demonstrated excellent diagnostic accuracy, reaching 100% detection, with a single amplification band at the expected size (547 bp). This reflects the high specificity of the primers used, the efficiency of the protocol, and the absence of contamination or non-specific amplification. These findings are consistent with [22,23], who reported the superiority of molecular methods over conventional phenotypic identification.

Similarly, [24] confirmed the reliability of *16S rRNA* gene sequencing in identifying *K. rosea* from clinical samples. In contrast, [25] reported reduced sensitivity when using alternative genetic targets such as *rpoB*, highlighting the importance of selecting appropriate molecular markers for accurate bacterial identification. Overall, the study confirms that dental caries is associated with a diverse microbial community dominated by *streptococci*, with a notable emergence of *K.rosea* as a potential opportunistic organism. The integration of phenotypic identification using VITEK 2 and molecular detection using PCR significantly enhances diagnostic accuracy. However, the increasing prevalence of antimicrobial resistance underscores the need for rational antibiotic use and precise diagnostic approaches prior to treatment.

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