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Isolation and identification of pathogenic bacterial species from dental clinics in Iraq-Najaf city

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Abstract
Dental practices are potential breeding grounds for pathogenic microorganisms, due to the nature of dental procedures which often involve contact with body fluids including blood and saliva. Statistical analysis of the prevalence of isolated and identified pathogenic bacterial species from dental clinics in the Iraqi city of Al-Najaf. In total 75 samples were taken from dental chairs, instruments, and water supplies from various dental clinics, and the isolated bacteria were isolated as *Streptococcus mutans* 16 isolates (21.3%), *Staphylococcus aureus* (14 isolates) (18.7%), *Klebsiella pneumoniae* (15 isolates) (20%), *Escherichia coli* (18 isolates) (24%), and *Pseudomonas* spp. (12 isolates) (11%). To separate the isolated bacteria, the samples were grown in a range of the media, such manitol salte agar, nutritional agar, blood agar and MacConkey agar. Species identification was performed with automated identification tools (VITEK-2) and biochemical methods. The dental practice, continuously exposing to the oral cavity, is prone to the existence of various microorganisms, which urges dental clinicians to use advances sterilizing procedures and infection control system to avoid infection and cross-infection.

Keywords: Dental practices, pathogenic microorganisms, dental procedures, body fluids, blood and saliva

1. Introduction
Microbial Infection Education in Children: Patients and dental personnel are at risk of exposure to pathogenic microorganisms at dental clinics. Given that such close proximity with blood, oral secretions and contaminated tools within dental clinics leads to a persistent risk of nosocomial infections (Forbes *et al*., 2007) [1]. Pathogens such *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* have been commonly isolated from dental clinics (Jain & Varshney, 2011) [8]. Immunocompromised individuals and patients who receive invasive dental procedures are at significant risk from these microorganisms.
The increasing presence of antibiotic-resistant bacteria (Igbinosa & Okoh, 2010) [9] complicates the management of infections arising from dental clinics. Consequently, knowledge of the microbial flora in these circumstances facilitates the development of effective infection control measures.
Dental clinics are healthcare environments that have a relatively high risk of germ exposure for patients due to the nature of dental treatments and the close contact between patients' oral cavities and dental equipment and surfaces (Al-Taee, R., *et al*. 2021) [19]. When pathogenic bacteria exist in dental clinics, there is great potential for cross-infection, which can lead to serious health problems for both patients and dental health care workers (Smith *et al*., 2019) [2].
The human oral cavity is a complex ecosystem that contains a diverse community of microorganisms, including both beneficial and pathogenic bacteria. *Staphylococcus aureus* is a golden cluster which is associated with several cases includes soft tissue infection, while *Streptococcus mutans* is the most chronic dental caries (Al-Hashimi *et al*., 2020) [3]. Such gram-negative bacteria include *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas* spp. Because they lead to opportunistic infections, and therefore substantial morbidity and mortality, particularly in the immunocompromised. Which are also a cause for concern (Al-Moussawi *et al*., 2021) [4].
Antibiotic resistant bacteria can also spread in health care systems, including dentistry units, therefore there have been concerns regarding the prevalence of antibiotic-resistant bacteria

in healthcare systems in recent years. Infections caused by these pathogens are becoming increasingly difficult to treat due to the emergence of multidrug resistant strains caused by drug abuse and poor infection control practices (Hussein *et al*., 2022) [5]. Microbiological monitoring of dental clinics and proper application of the infection control measures are needed to prevent spread of pathogenic bacteria.

The objective of this study was to conduct statistical analysis to examine the relative public health significance of all isolated pathogenic bacterial species from the dental clinics in the Iraqi province of Al-Najaf.

2. Materials and Methods

2.1. Sample Collection

Seventy-five dental samples were collected from various surfaces, including water supply, dental chairs, as well as dental equipment. Samples of water were collected in sterile vials, and surface samples were taken using sterile swabs. The samples were taken to the lab for processing within two hours of collection.

2.2. Culture Media

The media were autoclaved for 15 min at 121 ˚C to sterilize. Urea agar base was supplemented with 20% sterile urea solution; blood agar base was supplemented with 5% human blood after cooling the medium to 45 ˚C and transferring it to sterile Petri dishes. (MacFaddin, 2000) [6].

2.3. Isolation and identification of the microbes

The samples were then inoculated into suitable media and incubated for 24 to 48 hours at 37 °C, after which colonies were examined for morphological characteristics and bacterial isolates were Gram stained. Biochemical tests (urease, oxidase, coagulase, and catalase) were used to identify the bacterial species.

2.4. Vitek - 2 for Identefication

GP, GN were used to identify Gram positive and Gram negative bacteria. Bacterial suspension of 0.5 McFarland standard was prepared in 2.5 mL of 0.45% sodium chloride solution and adjusted with a Vitek-2 device (bioMérieux, France). The inoculum preparation was never more than 30 min apart (And usually much less) from the card filling. It is a fully closed system so no need of adding reagents in the GP, GN identity card. The card was positioned onto the cassette designed for the Vitek-2 system, inserted in the device and then automatically filled into a vacuum chamber, barcoded, and subsequently incubated at 35.5˚C. The card underwent an automatic colorimetric assay every 15 minutes over a maximum period of 8 hours, executing a new readinghead assembly. The data were examined via Vitek-2, a database that allows for kinetic organism identification beginning at 180 min after the incubation period begins (Guido and Pascale, 2005) [7].

2.5. Statistical Analysis

The prevalence of bacterial species was expressed as a proportion of the total number of isolates. Chi-square tests were used to find significant differences in the incidence of bacteria at the various sampling locations. A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

Findings of the study revealed the presence of many pathogenic species of bacteria at dental clinics. *Escherichia coli* was the most frequently isolated bacterium (24%) followed by *Streptococcus mutans* (21.3%), Klebsiella pneumoniae (20%), *Staphylococcus aureus* (18.7%) and *Pseudomonas* spp. (11%). Other species including Proteus mirabilis (2%), and Enterococcus faecalis (3%), were also shown to be prevalent.

Statistical analysis using chi-square tests ($p < 0.05$) showed a significant difference in the proportion of bacteria between the various sampling sites. For example, 40.0% of dental chairs and 25.0% of equipment and 20.0% of water supplies were *Escherichia coli* positive. According to Al-Hilfi *et al*. [15] The authors of [29] similarly found that *Klebsiella pneumoniae* was isolated more frequently (30.0%) from water supplies than from dental chairs (15.0%) and implements (10.0%).

Conclusions this study proved that several damaging germs exist in dentistry facilities in Najaf Governorate In addition, high E. coli prevalent (24%) indicates that fecal matter could contaminate the sample resulting from poor hygiene practices or contaminated water supply (Al-Zubaidy, A.H., Al-Saadi, R.K. 2022). S. mutans, a known pathogen in dental caries, was frequently isolated (21.3%), suggesting intra-oral cross-infection between patients.

Their frequency is disturbing because *Staphylococcus aureus*, especially methicillin-resistant strains (MRSA) can cause serious infections (Tiwari *et al*., 2008; AAY Al-Akwa., *et al*., 2020) [13, 8]. Isolated from water sources, *Pseudomonas aeruginosa*, which is multidrug resistant, also indicates that dental unit waterlines are easily contaminated (Munro *et al*., 1994) [12]. The isolation of *Escherichia coli* and Klebsiella pneumonia, both associated with respiratory tract and urinary tract infections highlights the risk of cross-contamination in dental offices (Grossnickle, Roger Earl 1995) [20].

The results of this study were similar to previous studies conducted in Iraq, which also showed a high prevalence of pathogenic bacteria in medical settings (Al-Moussawi *et al*., 2021) [4]. However, the percentage distribution of the isolated bacteria in this study was slightly different from findings in previous studies, perhaps due to differences in sampling methods, geographic area and infection control policies.

Klebsiella spp. and Streptococcus spp. were recognised as the predominant bacterial isolates in this research. Replaced by E. Coli, Proteus species, *Pseudomonas* aureginosa, *Staphylococcus aureus* and Enterobacter species. These findings are in line with earlier studies. (Agrawal A, *et al*. 2014) [14].

The Centers for Disease Control and Prevention (CDC) estimates that antibiotic resistance is associated with 2 million infections and more than 20,000 deaths and 55 billion dollars in medical costs in the U.S. every year.

4. Conclusion

This study reveals that different pathogenic bacterial species exist in dental clinics in the province of Al-Najaf. The results emphasize the importance of effective sterilization processes and monitoring of water quality, as well as adherence to infection prevention guidelines, in order to minimize the transmission of disease in dental settings (Ramamurthy *et al*., 2014) [10]. Further research is required to address detachment of antibiotic resistance in these isolates that will help in formulating approaches to decrease nosocomial infections in the dentistry clinics.

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