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Original article

A Cross-Sectional Assessment of Bacterial Contamination in Fixed Prosthodontic Impressions Transferred from Clinics to Dental Laboratories: A Study in Tripoli, Libya

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Abstract
*Fixed prosthodontic impressions are essential in restorative dentistry but pose significant cross-contamination risks if inadequately disinfected, with adherence to protocols being particularly low in developing regions like Libya. This cross-sectional study evaluated bacterial contamination of 100 fixed prosthodontic impressions arriving at a central dental laboratory in Tripoli, collected from 23 private clinics, aiming to determine the prevalence and pathogenicity of implicated bacterial species. Microbiological analysis utilised selective and differential culture media under aerobic and anaerobic conditions, with isolates identified through colony morphology, Gram staining, and microscopic examination. Identified strains were classified as pathogenic, potentially pathogenic, or non-pathogenic, and the antibiotic susceptibility of pathogenic isolates was tested via Kirby-Bauer disk diffusion. Results revealed a high contamination rate, with 90% of impressions harbouring bacteria. Twenty-one bacterial species were identified, with pathogenic bacteria being predominant; Escherichia coli (13%), Streptococcus pyogenes (11%), and Staphylococcus aureus (9%) were the most common. Statistical analysis (Kruskal-Wallis test and Dunn-Bonferroni post-hoc tests) indicated significant differences in contamination rates between bacterial groups (*p* = 0.009), confirming pathogenic bacteria were significantly more prevalent than potentially pathogenic strains. The extensive prevalence of pathogenic microbes indicates a critical failure in current infection control practices within the sampled clinics. The urgent implementation of standardized disinfection protocols is essential to protect both laboratory personnel and patients' health. Future studies must examine the efficacy of diverse disinfection procedures and elucidate the molecular survival mechanisms of microbes on impression materials.*

Keywords. *Fixed Prosthodontic Impressions, Bacterial Contamination, Infection Control, Pathogenic Bacteria, Disinfection Protocols, Tripoli, Libya.*

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Introduction

Fixed prosthodontic impressions are fundamental to restorative dentistry, serving as precise negative replicas of the oral cavity used in fabricating crowns, bridges, and other fixed prostheses. Due to direct contact with saliva, blood, and oral microbiota, these impressions often carry significant microbial loads. Without proper disinfection, they can become vectors for cross-contamination between dental clinics and laboratories, posing risks of nosocomial transmission [1]. Previous research has consistently identified a range of pathogenic and opportunistic microorganisms on dental impressions, including *Escherichia coli*, *Staphylococcus aureus*, *Porphyromonas gingivalis*, and *Streptococcus pyogenes*. These organisms can persist on impression surfaces for extended periods, posing serious infection risks to laboratory personnel and patients alike [2,3].

To address these risks, organisations such as the Centres for Disease Control and Prevention (CDC) and the American Dental Association (ADA) have issued clear guidelines recommending that all dental impressions be disinfected before being transferred to laboratories. However, adherence to these protocols remains inconsistent, particularly in developing countries where infection control practices are often poorly enforced [4,5]. In Libya, there is a notable lack of published data and standardised disinfection protocols regarding infection control practices related to the transportation of these impressions from clinics to dental laboratories. Informal reports indicate that many private dental clinics may not consistently disinfect impressions prior to sending them to laboratories, raising concerns about occupational safety for laboratory personnel and the potential compromise of patient care quality.

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This cross-sectional study aims to evaluate bacterial contamination levels in fixed prosthodontic impressions received from private dental clinics, as these impressions represent potential vehicles for microbial transmission and cross-infection risk within laboratory environments

Methods

Study Design and Population

A cross-sectional study was conducted over a two-month period (April to May 2025) at a central laboratory in Tripoli. As the analysis involved only existing anonymized materials, with no collection or disclosure of personal or identifiable patient data and no anticipated risks.

Sample Collection

A total of 100 impressions were collected upon arrival at the laboratory. These samples were sourced from 23 private clinics, each contributing approximately 4 to 5 randomly selected impressions. All samples were related to fixed prosthodontic treatments (e.g., crowns and bridges) and were fabricated using commonly used materials like condensation silicone, valued for its affordability and precision [7]. Immediately upon receipt, each impression was labeled and swabbed using sterile cotton swabs, which were gently rubbed across multiple surface areas of the impression. Swabs were then placed in Amies transport medium and transferred to the microbiology lab within one hour to preserve viable microbial content [8].

Microbiological Processing

Each swab sample was inoculated onto four culture media: Blood agar for general bacterial growth and hemolysis assessment; MacConkey agar for selective isolation of Gram-negative enteric bacteria; Chocolate agar for fastidious organisms; and Mitis Salivarius agar for selection of oral streptococci [9]. All plates were incubated at 37°C for 24–48 hours under both aerobic and anaerobic conditions. Following incubation, colonies were evaluated based on morphology, pigmentation, and hemolytic patterns.

Antibiotic Susceptibility Testing

All identified pathogenic bacterial strains underwent antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. The antibiotics tested included amoxicillin-clavulanic acid, ciprofloxacin, erythromycin, clindamycin, and metronidazole (specifically for anaerobic strains). Zones of inhibition were measured after 24 hours of incubation and categorized as sensitive, intermediate, or resistant based on CLSI interpretive standards.

Bacterial Identification and Quantification

Cultured microorganisms were categorized as pathogenic bacteria (e.g., *E. coli*, *S. aureus*, *S. pyogenes*, *P. gingivalis*), commensal/oral flora (e.g., *Streptococcus salivarius*, *Gemella sanguinis*), or recorded as no growth. Quantitative analysis involved calculating the number and percentage of culture-positive versus culture-negative impressions and identifying the dominant bacterial species in each positive sample. Microscopic examination using Gram staining techniques was employed to differentiate Gram-positive and Gram-negative organisms based on cell wall characteristics; morphological features including cellular shape (*cocci*, *bacilli*), arrangement (chains, clusters), and staining reactions were documented under light microscopy to support colony identification. To ensure clarity in data interpretation, only the most dominant isolate per sample was considered in frequency calculations. The relative frequency for each dominant isolate was calculated as follows: Percentage (%) = (Number of samples with the dominant isolate / Total culture-positive samples) × 100. This approach facilitated a structured assessment of contamination patterns and associated risks.

Data Analysis

All analyses were performed using IBM SPSS Statistics (Version 28.0). Due to violation of normality assumptions in contamination-level data impressions, nonparametric Kruskal-Wallis H test was conducted to compare bacterial contamination across pathogenicity categories (Pathogenic, Non-Pathogenic, Potentially Pathogenic) value less than 0.05 were significant statistically.

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Results

Out of a total of 100 dental impression samples analyzed, 90% (n = 90) were found to be contaminated with one or more bacterial species. Microbiological analysis of the 90 positive samples revealed the presence of 21 distinct bacterial species, reflecting a broad spectrum of both gram-positive and gram-negative organisms.

Table 1: Prevalence and Pathogenicity Distribution of Bacterial Species Isolated from Fixed Prosthodontic Impressions in Tripoli, Libya (n=100 Samples).

Type of Bacteria	Pathogenicity	Percentage of total samples (out of 100 samples)	Percentage of infected samples (out of positive impressions)
Streptococcus mutans	Pathogenic	8%	9%
Porphyromonas gingivalis	Pathogenic	8%	9%
Staphylococcus aureus	Pathogenic	9%	10%
Escherichia coli	Pathogenic	13%	14%
Klebsiella oxytoca	Pathogenic	3%	3%
Streptococcus mitis	Non-Pathogenic	2%	2%
Citrobacter koseri	Pathogenic	4%	4%
Streptococcus gordonii	Non-Pathogenic	2%	2%
Streptococcus pyogenes	Pathogenic	11%	12%
Streptococcus infantis	Non-Pathogenic	4%	4%
Gemella sanguinis	Potentially Pathogenic	1%	1%
Hafnia alvei	Potentially Pathogenic	1%	1%
Aeromonas hydrophila	Pathogenic	3%	3%
Enterobacter cloacae	Pathogenic	5%	6%
Citrobacter freundii	Pathogenic	2%	2%
Serratia marcescens	Pathogenic	3%	3%
Campylobacter concisus	Pathogenic	2%	2%
Streptococcus salivarius	Non-Pathogenic	2%	3%
Klebsiella pneumonia	Pathogenic	3%	3%
Streptococcus clone	Potentially Pathogenic	2%	2%
Prevotella clone	Potentially Pathogenic	2%	2%

This study on the bacterial profiles of dental impressions taken for crown or bridge fabrication revealed a wide variety of bacteria with varying levels of infectious risk, as detailed in Table 1. Out of a hundred samples analysed, 90% showed signs of contamination, while only 10% were free of any detectable microbial growth. Among the bacteria identified, certain pathogens were particularly notable due to their higher prevalence. Escherichia coli was detected in 13% of the total samples and 14% of the infected samples (Figure 1). This indicates a significant presence of *E. coli*, highlighting its potential importance for patients undergoing dental procedures. Similarly, *Streptococcus pyogenes* was present in 11% of the total samples and 12% of the infected impressions cases (Figure 2), underscoring its commonality in the population.

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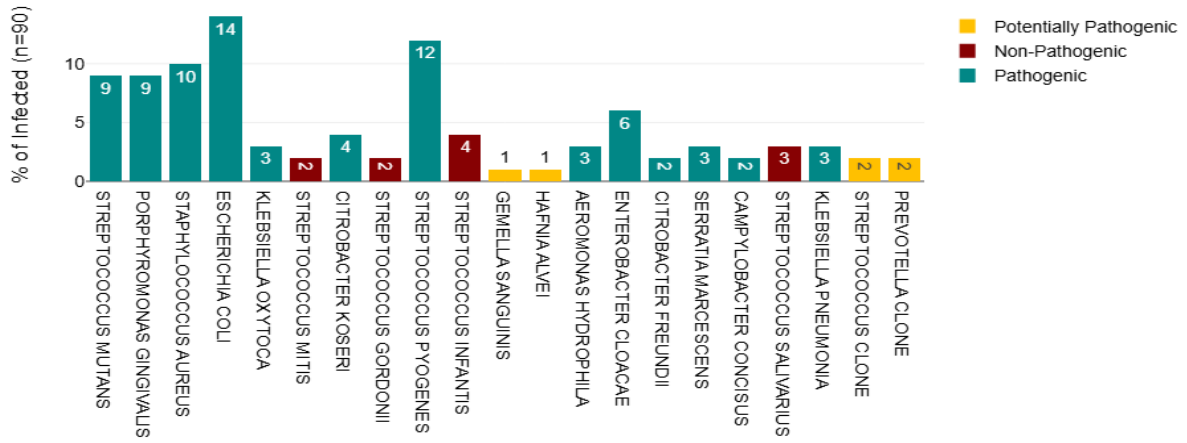

Figure 1: Pathogenicity distribution contaminated (total sample N=90)

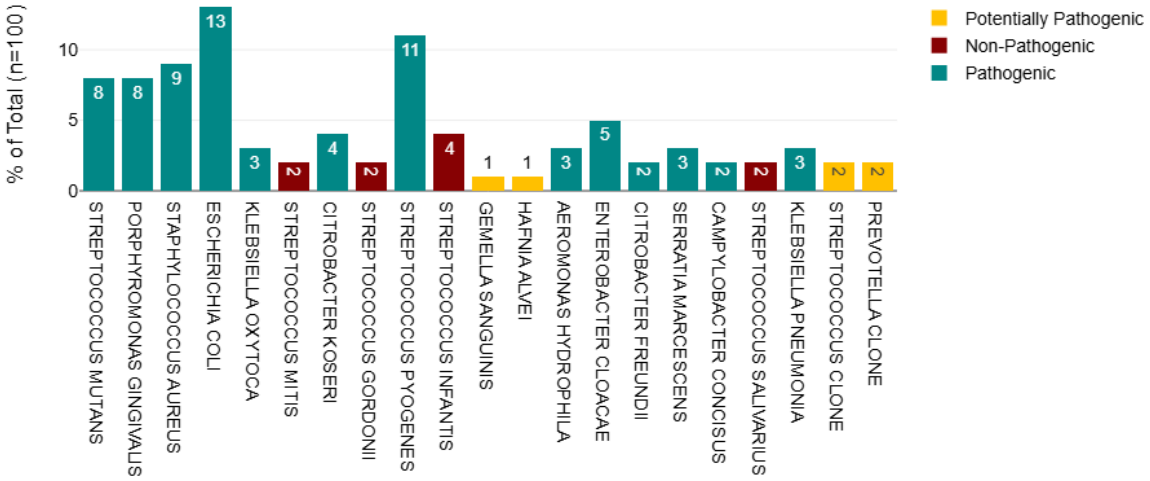

Figure 2: Prevalence infectious pathogens (total 100 sample).

Staphylococcus aureus was detected in 9% of total samples and 10% of infected impressions. Similarly, both *Streptococcus mutans* and *Porphyromonas gingivalis* were identified in 8% of total samples and 9% of infected impression. These findings highlight the persistent nature of these well-known pathogens, many of which are associated with oral or systemic infections. Several additional pathogens were identified at lower frequencies. *Enterobacter cloacae* was found in 5% of total samples and 6% of infected impressions. *Citrobacter koseri* was detected in 4% of both total and infected samples. Additionally, several species, including *Aeromonas hydrophila*, *Klebsiella oxytoca*, Serratia *marcescens*, *Klebsiella pneumoniae*, and *Campylobacter concisus*, were identified at a rate of 3%, with their proportions remaining unchanged in the infected group. *Citrobacter freundii* was the least prevalent, foundin 2% of both total samples and in the infected impressions.

Non-pathogenic bacteria were also identified, although they existed at a lower frequency among infected samples. *Streptococcus mitis and Streptococcus gordonii* were each found in 2% of total and infected samples. *Streptococcu* Infantus was found in 4% of both groups while *Streptococcu* Salivarius was detected in 2% of total and 3 % of infected samples.

Some species were categorized as "potentially pathogenic." These included *Gemella sanguinis*, *Hafnia alvei*, *Streptococcus Clone* and *Prevotella Clone*, all of which comprised 1-2% of total and infected samples. These findings suggest some limited but possible role in contamination transmission.

As shown in Figure 3, the analysis of the total impression sample(N=100), indicated that 5.69% were classified as pathogenic, 2.5% as non-pathogenic, and 1.5% as potentially pathogenic. This indicates that pathogenic samples represented the largest proportion within this group. For the subset of infected impression samples (N=90), the

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distribution was as follows: 6.15% classified as pathogenic, 2.75% as nonpathogenic and 1.5% as potentially pathogenic. Pathogenic bacteria continued to be predominant within this infected subgroup.

These finding highlights the significant presence of pathogenic microorganisms in dental impressions, which is critical for understanding infection control in dental practices. The predominance of pathogenic samples suggests a need for stringent disinfection protocols to mitigate the risk of cross-contamination and ensure patient safety.

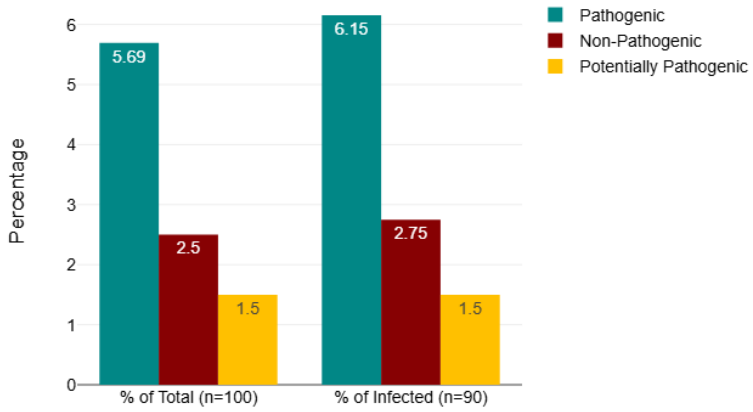

Figure 3: Distribution of bacterial isolates by pathogenicity category in total impressions (N=100) vs. contaminated impressions (N=90).

A Kruskal-Wallis test was performed on 90 infected dental impression samples to evaluate differences in bacterial contamination rates among three groups: pathogenic, non-pathogenic, and potentially pathogenic. Given that the data were nonparametric and did not meet the assumptions of normality required for parametric tests, the use of the Kruskal-Wallis test was appropriate. The analysis revealed significant differences between the groups, with χ^2 (2) = 9.37 and =0 .009, indicating variations in contamination levels associated with different types of bacteria.

Subsequent post-hoc Dunn-Bonferroni analysis identified a significant difference only between the pathogenic and potentially pathogenic groups (*adjusted p* = 0.009). In contrast, no significant differences were observed between the pathogenic and non-pathogenic groups (*adjusted p* = .514) or between the non-pathogenic and potentially pathogenic groups (*adjusted p* = .574). Therefore, it can be concluded that pathogenic bacteria were more frequently associated with contamination compared to potentially pathogenic bacteria (Figure 4).

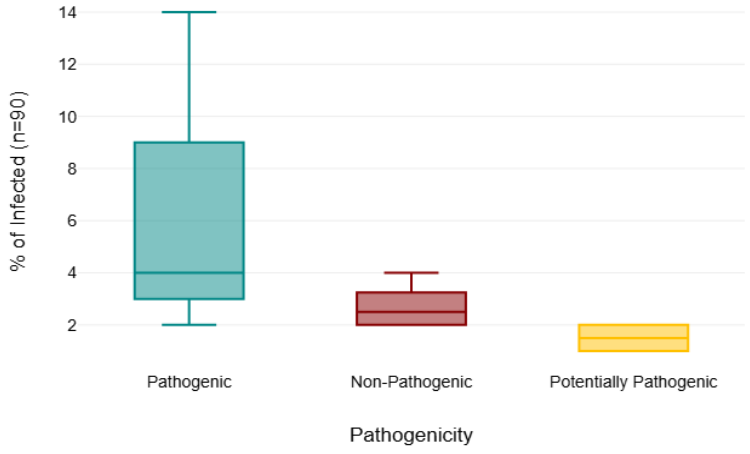

Figure 4: Kruskal-Wallis analysis of bacterial contamination frequency by pathogenicity category.

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Discussion

The present study reveals critical insights into the microbial contamination of dental impressions, with profound implications for infection control in dental practice. Analysis of 100 samples transferred from private clinics to a central dental laboratory in Tripoli, Libya, demonstrated an alarming 90% contamination rate, indicating near-ubiquitous bacterial presence. The diversity of isolate spanning 21 bacterial species including both oral and environmental pathogens highlight the complex bioburden and cross-contamination risk, aligning with known contaminants like *Staphylococci*, *Streptococci*, *Pseudomonas*, *Escherichia coli*, and *Candida spp.* [11]. Pathogenic species constituted the most significant proportion of contaminants (5.69% of total samples; 6.15% of infected samples), with high-prevalence pathogens including *Escherichia coli* (13% of total samples*), Streptococcus pyogenes (11%), and Staphylococcus aureus* (9%) posing serious clinical concerns. *E. coli* suggests possible hygiene lapses, while all three microorganisms are recognized for causing systemic infections in immunocompromised individuals [12]. Similarly, *S. pyogenes* and *S. aureus* are associated with pharyngitis, skin infections, and antibiotic-resistant complications. The presence of *Porphyromonas gingivalis* and *Streptococcus mutans* (8% each), associated with periodontal disease and dental caries respectively, is notable given research linking these organisms to infective endocarditis, cerebral hemorrhage, tumors, and inflammatory bowel disease [13]. These pathogens may transmit to dental staff via occupational exposure [14], and their prevalence aligns with previous studies [15, 16, 17, 18]. The Kruskal-Wallis test confirmed significant differences in contamination rates among pathogenicity groups ($\chi^2(2) = 9.37$, $p = .009$), with pathogenic bacteria significantly more prevalent than potentially pathogenic strains (adjusted $p = .009$). The lack of difference between pathogenic and non-pathogenic groups ($p = .514$) suggests non-pathogens may coexist without altering contamination severity. Detection of enteric pathogens (*Klebsiella spp*., *Citrobacter spp*.) and environmental species (*Aeromonas hydrophila*) indicates possible water/aerosol contamination or inadequate disinfection, while oral pathogens reflect direct transfer from patients' microbiota. Evidence links *P. gingivalis* to cardiovascular disorders, suggesting probiotic interventions might mitigate bacteremia risks [19]. Contributing factors to high contamination prevalence include: decreased disinfectant efficacy from improper dilution [20,21], inadequate water rinsing without chemical disinfection [22], limited microbial kill rates from insufficient contact time or subpar methods [23], lack of infection control training in private clinics [24], and undetected errors due to absent monitoring [25].

This study has several limitations. First, its restriction to impressions received at a single central laboratory in Tripoli may limit generalizability to other regions. Second, we did not assess the disinfection status of impressions upon arrival, precluding direct correlation between clinical practices and contamination levels. Third, the low prevalence of 'potentially pathogenic' species (e.g., *Gemella sanguinis*, *Prevotella Clone*) warrants further investigation to clarify their clinical significance, and the classification itself requires refinement through genomic virulence analysis. Finally, the 10% uncontaminated samples suggest variability in disinfection efficacy or sampling methods that merits targeted investigation.

Based on the 90% contamination rate identified in this study, we recommend implementing enhanced disinfection protocols using EPA-registered hospital-grade disinfectants such as glutaraldehyde or hydrogen peroxide solutions. Additionally, strict source control measures should be enforced, including the use of sterile trays, distilled water for rinsing, and rigorous hand hygiene during impression handling. Comprehensive staff training on pathogen transmission risks must be established alongside regular compliance audits. Finally, routine microbial monitoring of impressions is essential to evaluate disinfection efficacy.

Conclusion

This study unequivocally demonstrates that dental impressions serve as reservoirs for pathogenic bacteria, with contamination driven primarily by species linked to systemic infections. The predominance of high-risk pathogens necessitates immediate revision of infection control guidelines in dentistry. Future research should explore molecular mechanisms of biofilm formation on impression materials and evaluate novel disinfection technologies to mitigate this under recognized threat to patient safety.

Conflicts of interest

We declared no conflict of interest.

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