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Change in Oral Microbiota in Maxilla Facial Trauma Patients after Insertion of an Upper/Lower Arch Bar with Intermaxillary Fixation (IMF): A Prospective Randomized Study

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ABSTRACT

Background

Intermaxillary fixation (IMF) with upper and lower arch bars is commonly used for the management of maxillofacial fractures. However, prolonged fixation may alter the oral microbial environment, resulting in dysbiosis and increased antimicrobial resistance.

Aim

To evaluate changes in oral microbiota and antimicrobial susceptibility patterns in maxillofacial trauma patients following IMF with arch bars.

Methods

A prospective randomized observational study was conducted in the Department of Oral and Maxillofacial Surgery, H.P. Government Dental College and Hospital, Shimla, Himachal Pradesh, from November 2023 to October 2025. A total of 102 patients undergoing IMF were included. Oral samples were collected before IMF placement and after 4–6 weeks from the tongue, palate, gingival margins, dental plaque, and throat. Bacterial and fungal cultures were performed using blood and MacConkey agar, followed by Gram staining, biochemical identification, and antimicrobial susceptibility testing.

Results

The majority of participants were male (90.2%), with falls being the most common cause of trauma (56.9%). Significant weight loss was observed after IMF ($p < 0.001$), and poor oral hygiene was significantly associated with wound infection ($\chi^2 = 8.92$, $p = 0.0028$). Pre-IMF samples were predominantly characterized by Gram-positive commensals, particularly *Streptococcus*, whereas post-IMF samples showed a significant increase in Gram-negative and opportunistic organisms. Gram-negative isolates increased from 27.5% to 60.8% ($\chi^2 = 36.5$, $p < 0.001$). Although several organisms retained good antimicrobial susceptibility, increased resistance was observed in *Acinetobacter spp.*, *Klebsiella pneumoniae*, *Streptococcus mitis*, and *Lactobacillus* spp. Post-IMF isolates demonstrated a greater overall resistance burden.

Conclusion

Prolonged IMF is associated with significant alterations in oral microbiota and increased antimicrobial resistance. Regular oral hygiene, microbiological surveillance, and appropriate antimicrobial selection are therefore important during IMF treatment.

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KEYWORDS: *Intermaxillary fixation; Oral microbiota; Maxillofacial trauma; Antimicrobial resistance; Oral dysbiosis.*

1. INTRODUCTION

1.1. Background of the Study

Maxillofacial trauma is a major global health concern involving injuries to the facial bones, dentoalveolar structures, and surrounding soft tissues. Common causes include road traffic accidents, falls, interpersonal violence, sports injuries, and occupational accidents, with road traffic accidents being among the leading cause worldwide (Maniaci et al., 2025). Such injuries may impair essential functions such as mastication, speech, respiration, and facial aesthetics, substantially reducing quality of life and increasing the socioeconomic burden on patients and healthcare systems. Treatment aims to restore anatomical form, functional occlusion, fracture stability, and prevent infection and other complications. Depending on the type, severity, and displacement of the fracture, management may involve conservative treatment or surgical intervention, frequently requiring immobilisation of the jaws (Maniaci et al., 2025).

Intermaxillary fixation (IMF), also known as maxillomandibular fixation, is an established technique in the management of maxillofacial fractures. It may be used as a definitive treatment in selected cases or combined with open reduction and internal fixation to maintain occlusion and facilitate fracture healing (Papócs et al., 2026). Despite developments in rigid fixation methods, IMF remains widely used because it is relatively simple, economical, and suitable for resource-limited settings. Nevertheless, prolonged jaw immobilisation can restrict mandibular movement, interfere with nutrition, cause discomfort, and make oral hygiene difficult, thereby increasing plaque accumulation and the risk of oral infection (Pellegrini et al., 2023).

Among the available IMF techniques, the conventional Erich arch bar is frequently used. These stainless-steel bars are adapted to the dental arches and secured with circumdental wires, allowing fixation with elastics or ligatures (Fernandes et al., 2020). Although arch bars provide effective fracture stabilisation and occlusal control, they can make oral hygiene difficult and contribute to plaque accumulation, gingival trauma, periodontal injury, and occupational needle-stick injuries among clinicians (Kalluri et al., 2024). These problems are particularly important in trauma patients who may already have difficulty maintaining adequate oral hygiene.

The oral cavity is a complex and dynamic ecosystem influenced by salivary flow, microbial interactions, host immunity, diet, and mechanical forces. IMF with arch bars changes this environment by limiting mouth opening and reducing the effectiveness of mechanical plaque removal. The metallic, non-shedding surfaces of arch bars also provide suitable sites for microbial attachment and biofilm development. Reduced mastication may decrease salivary stimulation, while liquid or semi-solid diets, particularly those containing high levels of carbohydrates, can further support microbial growth. Together, these factors may promote microbial imbalance and overgrowth (Marino et al., 2021).

The oral microbiota consists of diverse bacteria, fungi, viruses, and archaea that normally exist in a balanced relationship with the host. This microbial community contributes to oral homeostasis and provides protection against pathogenic organisms (Rajasekaran et al., 2024). Disturbance of this balance, referred to as oral dysbiosis, has been associated with dental caries, gingivitis, periodontitis, peri-implant infections, and certain systemic disorders (Bourgeois et al., 2025).

Biofilm formation is a major characteristic of oral microbial colonisation. Biofilms are organised microbial communities enclosed within an extracellular matrix and attached to biological or artificial surfaces. They can develop on teeth, oral tissues, and metallic devices such as arch bars (Lv et al., 2025). Biofilms associated with arch bars are clinically important because their proximity to gingival tissues promotes the accumulation of complex microbial communities. Such biofilms can demonstrate increased tolerance to antimicrobial agents and host immune responses, potentially contributing to persistent infection, gingival inflammation, and delayed wound healing.

Microbial colonisation may also differ between the maxillary and mandibular arches because of anatomical and physiological variations. The mandibular arch is closer to the openings of the submandibular and sublingual glands and may therefore receive greater salivary cleansing. In contrast, the maxillary arch may be more susceptible to food retention and plaque accumulation. Differences in tongue movement, gravity, muscular activity, and local salivary flow may further influence microbial distribution. Thus, the effects of arch bars on oral microbiota may vary between the upper and lower arches.

Systemic factors also influence the oral microbiome in trauma patients. Maxillofacial injury can trigger inflammatory responses that modify the local environment and affect microbial colonisation. In addition, antibiotics commonly prescribed following trauma may reduce microbial diversity, disturb normal microbial balance, and encourage the

emergence of resistant organisms (Yuan et al., 2023). These alterations may increase susceptibility to opportunistic infections during the immobilisation period.

Changes in oral microbiota during IMF may have important clinical consequences, including increased gingival and periodontal inflammation, wound infection, delayed fracture healing, and, potentially, systemic complications such as bacteraemia. However, microbiological assessment is not routinely incorporated into IMF management. Therefore, investigating changes in the oral microbiome during IMF is important for improving infection prevention and patient care.

The present study therefore focuses on evaluating alterations in oral microbiota among maxillofacial trauma patients following the placement of upper and lower arch bars with IMF. Particular attention is given to microbial changes, the development of dysbiosis, and differences between the maxillary and mandibular arches, with the aim of providing evidence that may support improved clinical management and infection-control practices.

1.2. Rationale for the Present Study

Patients with maxillofacial trauma undergoing IMF with arch bars are exposed to several local and systemic factors that can alter the oral microbial environment. Although IMF is an effective, economical, and widely accepted method for stabilising maxillofacial fractures, restriction of jaw movement makes routine oral cleaning difficult. Consequently, plaque accumulation and microbial biofilm formation may increase during the fixation period (Marino et al., 2021).

The oral microbiota is essential for maintaining oral health, and disruption of its normal balance may result in dysbiosis, periodontal disease, opportunistic infections, and delayed healing. The presence of metallic arch bars further provides surfaces for microbial adhesion and biofilm persistence. These biofilms can exhibit increased resistance to antimicrobial agents and host immune mechanisms (Lv et al., 2025). Trauma-related inflammation and the frequent use of systemic antibiotics may additionally modify microbial diversity and promote antimicrobial resistance (Yuan et al., 2023).

Although previous research has examined plaque accumulation, gingival inflammation, and oral hygiene during IMF, relatively limited attention has been given to detailed changes in the oral microbiota. Furthermore, differences in microbial colonisation between the maxillary and mandibular arches remain insufficiently investigated despite variations in anatomy, salivary flow, gravity, and mechanical cleansing.

This knowledge gap is clinically relevant because identifying microbial changes and antimicrobial susceptibility patterns may assist in preventing secondary infections, periodontal complications, and delayed fracture healing. It may also support appropriate antibiotic selection and the development of effective oral hygiene measures.

Therefore, the present study evaluates changes in oral microbiota among maxillofacial trauma patients following placement of upper and lower arch bars with IMF, with particular emphasis on comparing microbial profiles between the maxillary and mandibular arches. The findings are expected to provide clinically useful information for infection control and improved management of patients undergoing IMF.

1.3. Significance of the Study

This study has important clinical and scientific relevance in oral and maxillofacial surgery, particularly for patients undergoing IMF for the management of maxillofacial fractures. Although arch-bar-assisted IMF is an established method for fracture stabilisation, its effects on the oral microbial environment have not been fully explored. Understanding these microbial changes may help reduce complications and improve patient outcomes.

The study provides a systematic assessment of alterations in oral microbiota following arch-bar placement. Identification of changes in microbial composition and the development of dysbiosis may help clarify the relationship between IMF, impaired oral hygiene, microbial accumulation, and infection risk.

From a clinical perspective, the findings may support improved oral hygiene protocols, appropriate antimicrobial use, and early identification of patients at increased risk of infection. These measures could help reduce complications such as gingival inflammation, periodontal deterioration, wound infection, and delayed fracture healing.

Another important aspect is the comparison of microbial changes between the maxillary and mandibular arches. Differences in anatomy, salivary flow, and local cleansing mechanisms may result in distinct microbial patterns. Understanding these variations could facilitate the development of more targeted, arch-specific preventive measures.

The study is also relevant to antimicrobial resistance. Since trauma patients may receive systemic antibiotics, evaluation of microbial susceptibility

patterns can provide information about emerging resistance and support rational antibiotic selection and antimicrobial stewardship.

Overall, this research addresses an important gap by integrating microbiological findings with the clinical management of maxillofacial trauma patients undergoing IMF. Its findings may contribute to evidence-based oral care, improved infection-control strategies, better treatment outcomes, and future research on oral microbiota in trauma and surgical settings.

1.4. AIMS &OBJECTIVES

Aim: To study and investigate the presence of oral pathogenic microorganisms in patients with maxillofacial fractures undergoing IMF.

Objectives:

1. To identify and characterize the oral microbiota in patients receiving maxillofacial fracture treatment with intermaxillary fixation (IMF).
2. To evaluate the antimicrobial susceptibility patterns of predominant oral microorganisms, with emphasis on emerging antibiotics.
3. To assess temporal fluctuations in microbial load and diversity across specified clinical phases (pre-operative, during IMF, and post-IMF removal)
4. To evaluate IMF-related clinical outcomes, encompassing oral hygiene, airway complications, and variations in nutritional status (body weight).
5. To systematically track dietary intake, oral hygiene habits, and related complications, and to examine their correlation with microbiological findings to ascertain determinants and risk factors for IMF-related complications

2. MATERIALS AND METHODS

2.1. Study Design and Ethical Considerations

A prospective randomized observational study was conducted in the Department of Oral and Maxillofacial Surgery, H.P. Government Dental College and Hospital, Shimla, from November 2023 to October 2025. Ethical principles were followed, written informed consent was obtained, and patient confidentiality was maintained.

2.2. Study Population and Sample Collection

The study included patients with maxillofacial fractures requiring intermaxillary fixation (IMF). Oral samples were collected at two stages: before IMF placement and after 4–6 weeks of fixation or at appliance removal. Samples were obtained from the tongue, throat, palate, dental plaque, gingival margins, and accessible periodontal sites.

2.3. Inclusion Criteria

- Patients of any age undergoing IMF for maxillofacial fractures.
- Participants of all genders.
- Patients providing informed consent.

2.4. Exclusion Criteria

- Systemic antibiotic use within 15 days before presentation.
- Immunocompromised patients or those with uncontrolled systemic diseases.
- Patients unwilling to provide consent.

2.5. Microbiological Analysis

Sterile swabs were used for sample collection under aseptic conditions. Samples were labelled and transported to a certified microbiology laboratory within 4 hours. Bacterial and fungal cultures were performed using appropriate culture media. Bacterial isolates were identified through colony morphology, Gram staining, and biochemical tests.

2.6. Bacterial Identification

Samples were cultured aerobically on 5% sheep blood agar and MacConkey agar at 37°C for 24–48 hours. Representative organisms, including *Staphylococcus aureus*, *Enterococcus spp.*, *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.*, were identified based on colony characteristics and standard laboratory methods.

2.7. Gram Staining

Gram staining was performed for preliminary bacterial classification. Gram-positive organisms appeared purple, whereas Gram-negative organisms appeared pink under oil-immersion microscopy at 100× magnification.

2.8. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was assessed using the disc diffusion method on Mueller–Hinton agar according to Clinical and Laboratory Standards Institute guidelines. Fresh bacterial cultures were tested against appropriate antibiotics and incubated at 37°C for 18–24 hours. Zones of inhibition were measured in millimetres and interpreted according to standard reference criteria.

Table 2.1: List of Antibiotics were used for Oral Pathogens with concentration and MIC Testing Range (µg/mL) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Schuetz et al., 2025).

Antibiotic	Abbreviation	Common Disk Content (µg) (Disk Diffusion)	Typical MIC Testing Range (µg/mL) (Broth Microdilution)
Ampicillin	AMP	10 µg	0.25 – 64
Gentamicin (High level)	HLG	120 µg	128 – 1024
Erythromycin	E	15 µg	0.25 – 256
Vancomycin	VA	30 µg	0.25 – 64
Linezolid	LZD	30 µg	0.12 – 32
Streptomycin (High level)	HLS	300 µg	128 – 2048
Amoxicillin	AML	25 µg	0.25 – 64
Teicoplanin	TEC	30 µg	0.12 – 32
Cefoxitin	FOX	30 µg	0.5 – 64
Penicillin	P	10 units	0.03 – 32
Gentamicin	GEN	10 µg	0.25 – 64
Cotrimoxazole	COT	1.25/23.75 µg	0.12/2.38 – 8/152
Clindamycin	CD	2 µg	0.12 – 32
Ciprofloxacin/L evofloxacin	CIP/LEV	5 µg	0.03 – 32
Doxycycline/Te tracycline	DO/TET	30 µg	0.25 – 64
Amikacin	AK	30 µg	0.5 – 128
Piperacillin/Taz obactam	PIT	100/10 µg	0.25/4 – 128/4
Ceftazidime	CAZ	30 µg	0.25 – 128
Imipenem	IPM	10 µg	0.12 – 32
Aztreonam	ATM	30 µg	0.25 – 128
Netilmicin	NET	30 µg	0.25 – 64
Tobramycin	TOB	10 µg	0.25 – 64
Levofloxacin	LEV	5 µg	0.03 – 32
Meropenem	MEM	10 µg	0.03 – 32
Cefepime	FEP	30 µg	0.25 – 128
Piperacillin	PRL	100 µg	0.5 – 256
Cefoperazone	CFP	75 µg	0.5 – 128
Cefotaxime	CTX	30 µg	0.03 – 128
Tetracycline	TET	30 µg	0.25 – 64
Trimethoprim	TMP	5 µg	0.12 – 32

2.9. Sample Size Calculation

Sample size was estimated using the standard proportion formula at a 95% confidence level. As the prevalence of IMF-associated microbial dysbiosis was uncertain, an expected prevalence of 50% and a 10% margin of error were considered. The calculated sample size was approximately 96 participants. Allowing for about 10% loss to follow-up or non-compliance, the target sample was increased to approximately 100–110 patients.

2.10. Culture Media and Reagents

The study used Blood agar, MacConkey agar, Mueller–Hinton agar, carbohydrate fermentation broth, peptone water, glucose phosphate broth, Simmons' citrate agar, Triple Sugar Iron agar, and Bile Esculin agar. Biochemical testing employed oxidase, catalase, and Kovac's reagents.

2.11. Preparation of Blood Agar

Blood agar base was dissolved in distilled water and sterilised by autoclaving at 121°C for 15 minutes. After cooling to 45–50°C, 5% sterile defibrinated sheep blood was added aseptically. The medium was then poured into sterile Petri plates and allowed to solidify.

2.12. Inoculation and Interpretation

Clinical isolates were inoculated onto blood agar using the streak-plate method and incubated aerobically at 37°C for 18–24 hours. Plates were assessed for colony morphology and haemolysis. **Beta haemolysis** indicated complete haemolysis, **alpha haemolysis** partial haemolysis with greenish discoloration, and **gamma haemolysis** absence of haemolysis.

Table 2.2: Blood agar media composition

Blood agar Ingredients	Concentration/Liter
Peptone	5 g/l
Sheep blood	15 ml
Beef extract	1.5 g
Yeast Extract	1.5 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1000 ml
Ph	6.8

2.13. Sample Size Calculation

Sample size was estimated using the standard proportion formula at a 95% confidence level. As the prevalence of IMF-associated microbial dysbiosis was uncertain, an expected prevalence of 50% and a 10% margin of error were considered. The calculated sample size was approximately 96 participants. Allowing for about 10% loss to follow-up or non-compliance, the target sample was increased to approximately 100–110 patients.

2.14. Culture Media and Reagents

The study used Blood agar, MacConkey agar, Mueller–Hinton agar, carbohydrate fermentation broth, peptone water, glucose phosphate broth, Simmons' citrate agar, Triple Sugar Iron agar, and Bile Esculin agar. Biochemical testing employed oxidase, catalase, and Kovac's reagents.

2.15. Preparation of Blood Agar

Blood agar base was dissolved in distilled water and sterilised by autoclaving at 121°C for 15 minutes. After cooling to 45–50°C, 5% sterile defibrinated sheep blood was added aseptically. The medium was then poured into sterile Petri plates and allowed to solidify.

2.16. Inoculation and Interpretation

Clinical isolates were inoculated onto blood agar using the streak-plate method and incubated aerobically at 37°C for 18–24 hours. Plates were assessed for colony morphology and haemolysis. **Beta haemolysis** indicated complete haemolysis, **alpha haemolysis** partial haemolysis with greenish discoloration, and **gamma haemolysis** absence of haemolysis.

Table 2.3: MacConkey agar composition
MacConkey agar medium
(Purpose: identifying lactos

Ingredients	Concentration /litre

Peptone	17.00 g
Sodium chloride	5.0 g
Bile salt	1.50 g
Lactose	10 g
Neutral red solution	0.03
Crystal Violet	0.001 g
Agar	13.50 g
Distilled water	1000 ml

2.17. Inoculation and Interpretation of MacConkey Agar

Bacterial isolates were streaked onto MacConkey agar using aseptic techniques and incubated aerobically at 37°C for 18–24 hours. Colonies were assessed based on lactose fermentation, with **pink/red colonies** indicating lactose fermenters and **colourless or pale colonies** indicating non-lactose fermenters.

Purpose: MacConkey agar was used as a selective and differential medium for isolating Gram-negative enteric bacteria and distinguishing them according to lactose fermentation.

Table 2.4: MHA composition

Mueller Hinton Agar Medium	
Ingredients	**Concentration/litre**
Beef extract	2.0 g
Starch	1.5 g
Agar	17 g
Distilled water	1000 ml

2.18. Preparation of Mueller–Hinton Agar

Mueller–Hinton agar (38 g/L) was dissolved in distilled water, sterilised by autoclaving at 121°C for 15 minutes, and cooled to 45–50°C. It was then poured aseptically into sterile Petri plates to obtain an approximately 4 mm depth and stored at 2–8°C until use.

2.19. Inoculation Procedure

Pure bacterial colonies were suspended in sterile saline and adjusted to 0.5 McFarland turbidity. The standardized suspension was evenly spread over Mueller–Hinton agar using a sterile swab. Appropriate antibiotic discs were placed aseptically, and plates were incubated aerobically at 37°C for 18–24 hours.

2.20. Interpretation

Following incubation, inhibition zones were measured in millimetres and classified as **Sensitive (S), Intermediate (I), or Resistant (R)** according to CLSI guidelines.

2.21. Purpose

Mueller–Hinton agar was used for antimicrobial susceptibility testing by the Kirby–Bauer disc diffusion method. It provides uniform bacterial growth and reliable antibiotic diffusion, allowing determination of the antimicrobial resistance patterns of isolated pathogens.

Table 2.5: TSI composition

Triple Sugar Iron Ingredients	Concentration/litre
Veg Peptone	20.0 g
Vegetable	6.0 g
Hydrolysate	1.0 g
Dextrose	10 g
Lactose Sucrose	10 g
Ferrous sulphate	0.2 g
Sodium chloride	5.0 g
Sodium Thiosulfate	0.30 g
Agar	12.0 g
Phenol red	0.005 g
Distilled water	1000 ml

2.22. Preparation of TSI Agar

The required quantity of Triple Sugar Iron (TSI) agar was dissolved in distilled water and sterilised by autoclaving at 121°C for 15 minutes. The medium was dispensed into sterile test tubes and allowed to solidify in a slant position, producing both a slant and butt.

2.23. Inoculation Procedure

Bacterial isolates were inoculated using a sterile needle by stabbing the butt and streaking the slant surface. The tubes were incubated aerobically at 37°C for 18–24 hours.

Interpretation

Table 2.6: Interpretation of TSI MEDIA

Reaction	Observation	Interpretation
Yellowslant/yellowbutt (A/A)	Acid throughout	Glucose and lactose/sucrose Fermentatio
Red slant/yellow butt (K/A)	Alkaline slant, acid butt	Glucose fermentation only
Red slant/red butt	No sugar Fermentatio	Non-fermenter
Black precipitate	H_2S production	Sulfur reduction
Cracks/bubbles	Gas	Carbohydrate fermentation

Purpose in Bacterial Identification

TSI agar was used for differentiation of Enterobacteriaceae based on carbohydrate fermentation, gas production, and hydrogen sulfide production.

Table 2.7: Simmons citrate agar composition

Simmons citrate agar

(Purpose:-Citrate utilization test)

Ingredients	Concentration/litre
$NH_4H_2PO_4$	1.0 g
K2HPO4	1.0 g
Sodium chloride	5.0 g
Sodium citrate	2.0 g
Magnesium sulphate	0.2 g
Bromothymol blue	0.08 g
Agar	15 g
Distilled water	1000 ml
pH	6.9

2.24. Preparation of Simmons' Citrate Agar

Simmons' citrate agar was dissolved in distilled water, sterilised by autoclaving at 121°C for 15 minutes, and dispensed into sterile tubes as slants.

2.25. Inoculation and Interpretation

Bacterial isolates were lightly streaked onto the slant and incubated aerobically at 37°C for 24–48 hours. **Blue coloration with growth** indicated a positive citrate utilisation test, whereas **no growth with a green medium** indicated a negative result.

Purpose: Simmons' citrate agar was used to assess citrate utilisation and differentiate Enterobacteriaceae, particularly *Klebsiella*, *Enterobacter*, and *Salmonella* from *Escherichia coli*.

2.26. Mannitol Salt Agar (MSA)

Preparation: Mannitol Salt Agar (111 g/L) was dissolved in distilled water, sterilised at 121°C for 15 minutes, cooled to 45–50°C, and poured aseptically into sterile Petri plates. Plates were stored at 2–8°C until use.

Inoculation: Pure bacterial isolates were streaked onto MSA plates using a sterile loop and incubated aerobically at 37°C for 18–24 hours.

Interpretation: Yellow colonies with surrounding yellow zones indicated mannitol fermentation, suggestive of *Staphylococcus aureus*. Pink/red colonies without colour change indicated non-mannitol fermenters, while absence of growth suggested inhibition by the high salt concentration.

Purpose: MSA served as a selective and differential medium for isolating *Staphylococcus* species. Its high salt content selects salt-tolerant bacteria, while mannitol fermentation helps differentiate *S. aureus* from other staphylococci.

Figure 2.1: Mannitol Salt Agar (MSA)

2.27. Preparation of Urea Agar Base

Urea agar base was dissolved in distilled water, sterilised by autoclaving at 121°C for 15 minutes, and cooled to approximately 50°C. Sterile 20% urea solution was then added aseptically. The medium was dispensed into sterile tubes and allowed to solidify as slants.

2.28. Inoculation and Interpretation

Bacterial isolates were inoculated onto the urea agar slants and incubated aerobically at 37°C for 18–24 hours. A bright pink colour indicated a positive urease reaction due to ammonia production, while no colour change indicated a negative result.

Purpose: Urea agar was used to identify urease-producing bacteria and differentiate organisms such as *Proteus* species from other Gram-negative bacilli based on their ability to hydrolyse urea.

2.29. CLED Agar

CLED agar was prepared, sterilised, and poured into Petri plates. Samples were inoculated and incubated at 37°C for 18–24 hours. Yellow colonies indicated lactose fermentation, while blue/green colonies indicated non-fermenters. The medium also prevents *Proteus* swarming and supports differentiation of urinary pathogens.

Table 2.8: CLED Agar (Cystine Lactose Electrolyte Deficient Agar) Composition of CLED Agar (w/v)

Components	Amount (g/L)
Peptone	4.0 g
Tryptone	4.0 g
Beef extract	3.0 g

Lactose	10.0 g
L-Cystine	0.128 g
Bromothymol blue	0.02 g
Agar	15.0 g
Distilled water	1000 mL
Final pH	7.3 ± 0.2

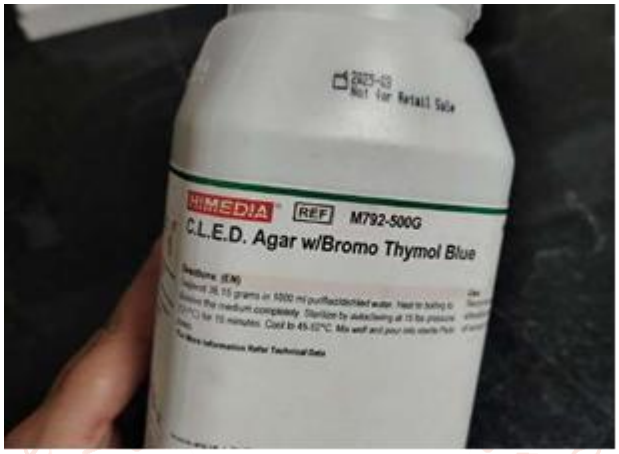
2.30. Laboratory Preparation and Isolation

Glassware and plasticware were sterilised before use. Laminar airflow surfaces were disinfected with 70% ethanol and exposed to UV light for 15 minutes. Culture media, broths, and slants were prepared according to standard procedures and stored under appropriate conditions.

Clinical swab samples were cultured on Blood agar, MacConkey, and HiChrome agar at 37°C. Isolates were assessed by colony morphology, microscopy, Gram staining, motility, and biochemical tests. Pure cultures were selected for further identification.

2.31. Gram Staining and Microscopy

Bacterial smears were prepared, heat-fixed, and stained using crystal violet, Gram's iodine, decolouriser, and safranin. Smears were examined microscopically to differentiate Gram-positive and Gram-negative organisms.



2.32. Biochemical Characterisation

Isolates were identified using standard biochemical tests, including catalase, coagulase, oxidase, indole, methyl red, citrate, urease, TSI, bile-esculin, and carbohydrate fermentation tests.

- **Catalase:** Bubble formation after adding hydrogen peroxide indicated a positive reaction.
- **Coagulase:** Clotting or visible agglutination indicated a positive reaction, supporting identification of *Staphylococcus aureus*.
- **Bile-esculin:** Growth with blackening indicated a positive result.
- **Oxidase:** Blue/purple colour development indicated oxidase activity.
- **Sugar fermentation:** Yellow colour and gas in a Durham tube indicated acid and gas production.
- **Indole:** A red ring after adding Kovac's reagent indicated a positive result.
- **Methyl red:** Bright red colour indicated a positive reaction.
- **Citrate:** Change from green to blue indicated citrate utilisation.
- **TSI:** The test differentiated Gram-negative bacteria based on carbohydrate fermentation, gas, and H_2S production.

Figure 2.2: TRIPLE SUGAR IRON TEST

2.33. Triple Sugar Iron (TSI) Test

TSI agar contains glucose, lactose, and sucrose, with phenol red for acid detection and ferric salts for H_2S detection. Pure cultures were inoculated by stabbing the butt and streaking the slant, followed by incubation at 37°C for 18–24 hours.

Interpretation:

- **K/K:** No sugar fermentation.
- **K/A:** Glucose fermentation only.
- **A/A:** Lactose and/or sucrose fermentation.
- **Gas:** Cracks or lifting of the medium.
- **H_2S:** Blackening due to ferrous sulphide formation.

2.34. Statistical Analysis

Data were analysed using Microsoft Excel and SPSS version 26.0. Descriptive statistics were expressed as frequencies, percentages, and mean ± SD. Pre- and post-IMF microbial profiles were compared using the **Chi-square/Fisher's exact test**, while body weight changes were assessed using a **paired t-test**. Logistic regression was used to identify predictors of wound infection, with adjusted odds ratios and 95% confidence intervals. Antimicrobial resistance patterns were summarised descriptively and presented using heat maps. A **p-value <0.05** was considered statistically significant.

2.35. Maintenance of Pure Cultures

Pure cultures were maintained on nutrient agar at 4°C for short-term storage. For longer preservation, cultures were stored in skimmed milk at −20°C.

Figure 2.3: Methodology steps for study on IMF in maxillo facial trauma patients

3. RESULTS

3.1. Demographic and Clinical Profile of Maxillofacial Trauma Patients

A total of **102 patients** undergoing IMF were studied. Males predominated (92.1%), with most patients aged **30–49 years (43.1%)**, and followed by 15–29 years (33.3%). A significant mean weight loss of **3.1 kg** was observed after IMF (68.9 ± 12.4 to 65.8 ± 12.1 kg; $p < 0.001$).

Falls were the leading cause of trauma (56.9%), followed by road traffic accidents (31.4%). Poor oral hygiene was present in **76.5%** of patients and was significantly associated with infection ($p = 0.0028$). Halitosis was reported in 79.4% and also showed a significant association ($p = 0.044$).

Mandibular fractures were predominant (84.3%), while midface fractures accounted for 15.7%. Arch bar fixation was the most common IMF procedure (59.8%), followed by Erich arch bars (31.4%). Overall, **44.1%** of patients had infected wounds. IMF duration was ≤4 weeks in 45.1%, 5–8 weeks in 37.3%, and >8 weeks in 17.6% of patients.

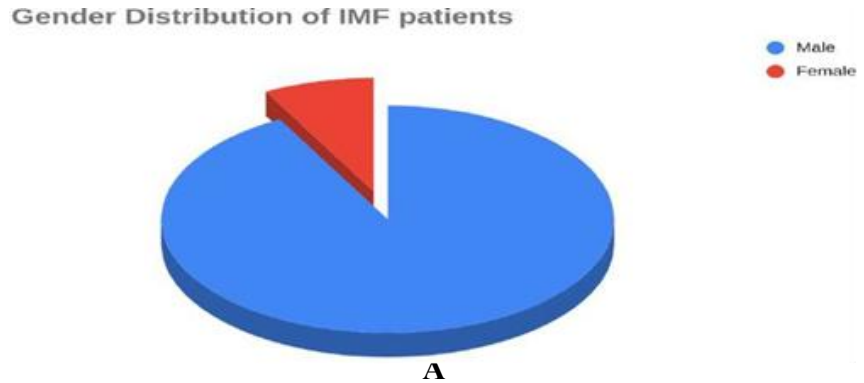
Sample collection was mainly performed from the **tooth and palate region (51.0%)**, followed by the tongue (37.3%) and throat (11.7%). Significant associations were observed for oral hygiene, halitosis, wound status, IMF duration, and body weight**,** whereas age, gender, fracture type, and sampling site were not significantly associated with clinical outcomes.

Figure 3.1: A) INTERMAXILLARY FIXATION patient with arch bar removed after 4 weeks.B) INTERMAXILLARY FIXATION patient with arch bar removed after 4 weeks C) INTERMAXILLARY FIXATION patient with arch bar during sample collection from mouth D) INTER MAXILLARY FIXATION patient with arch bar removed after 4 weeks ,Tongue with dark, furry appearance on the dorsal surface

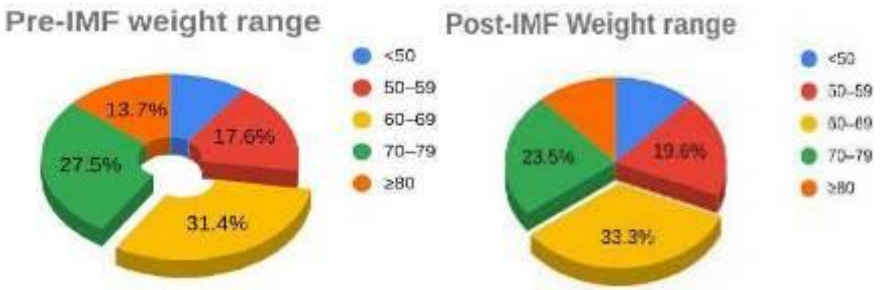
Table 3.1: Demographic Characteristics, Clinical Profile, and Statistical Analysis of IMF Patients (n = 102)

Parameters	Category	Frequency (n)/Mean± SD	Percentage (%)	Statistical Analysis
Gender	Male	94	92.1	$\chi^2 = 0.84$, p = 0.359
	Female	8	7.9	
Age Group (years)	Children (0–14)	4	3.9	$\chi^2 = 4.21$, p = 0.239
	Young adults (15–29)	34	33.3	
	Adults (30–49)	44	43.1	
	Older adults (≥50)	20	19.6	
Body Weight (kg)	Pre-IMF	68.9 ± 12.4	—	Paired t = 6.26, p <0.001*
	Post-IMF	65.8 ± 12.1	—	
	Mean difference	3.1 kg decrease	—	
Weight Distribution	<50 kg	Pre: 10 / Post: 12	9.8 / 11.8	McNemarχ^2=10.28, p= 0.036*
	50–59 kg	Pre: 18 / Post: 20	17.6 / 19.6	

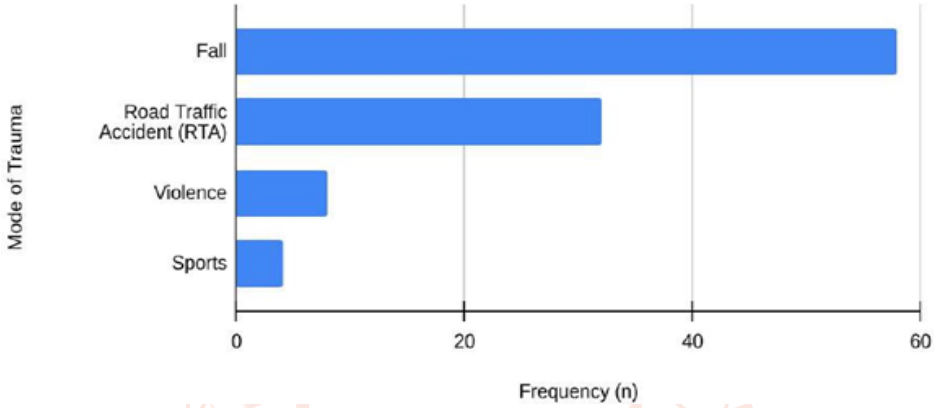
	60–69 kg	Pre: 32 / Post: 34	31.4 / 33.3	
	70–79 kg	Pre: 28 / Post: 24	27.5 / 23.5	
	≥80 kg	Pre: 14 / Post: 12	13.7/ 11.8	
Mode of Trauma	Fall	58	56.9	$\chi^2 = 7.16$, $p = 0.067$
	Road Traffic Accident (RTA)	32	31.4	
	Violence	8	7.8	
	Sports injury	4	3.9	
Oral Hygiene	Poor	78	76.5	$\chi^2 = 8.92$, $p = 0.0028$*
	Normal	18	17.6	
	Good	6	5.9	
Breath Odour	Poor	81	79.4	$\chi^2 = 4.06$, $p = 0.044$*
	Good	21	20.6	
Type of Fracture	Mandibular	86	84.3	$\chi^2 = 1.52$, $p = 0.218$
	Midface	16	15.7	
IMF Procedure Type	Arch bar	61	59.8	$\chi^2 = 3.85$, $p = 0.278$
	Erich arch bar	32	31.4	
	Archbar+ MMF	5	4.9	
	Cap splint	4	3.9	
Wound Type	Clean	57	55.9	$\chi^2 = 5.62$, $p = 0.018$*
	Infected	45	44.1	
IMF Duration	≤4 weeks	46	45.1	$\chi^2 = 6.84$, $p = 0.033$*
	5–8 weeks	38	37.3	
	>8 weeks	18	17.6	
Sample Collection Site	Tooth & palate	52	51.0	
	Tongue	38	37.3	
	Throat	12	11.7	


Gender Distribution of IMF patients
Male
Female

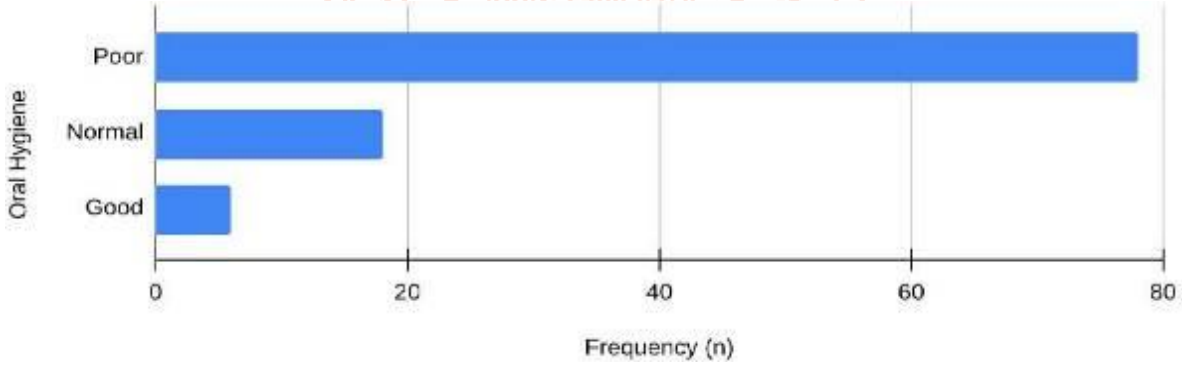
A


Pre-IMF weight range
Post-IMF Weight range
<50
50–59
60–69
70–79
≥80
13.7%
17.6%
27.5%
31.4%
23.5%
19.6%
33.3%

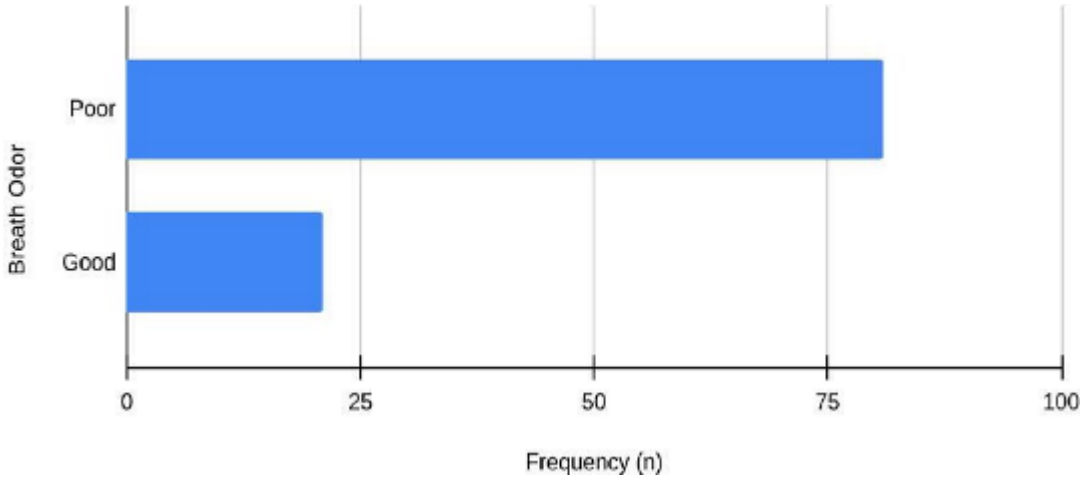
B


Mode of Trauma
Fall
Road Traffic Accident (RTA)
Violence
Sports
0
20
40
60
Frequency (n)

C


Oral Hygiene
Poor
Normal
Good
0
20
40
60
80
Frequency (n)

D


Breath Odor
Poor
Good
0
25
50
75
100
Frequency (n)

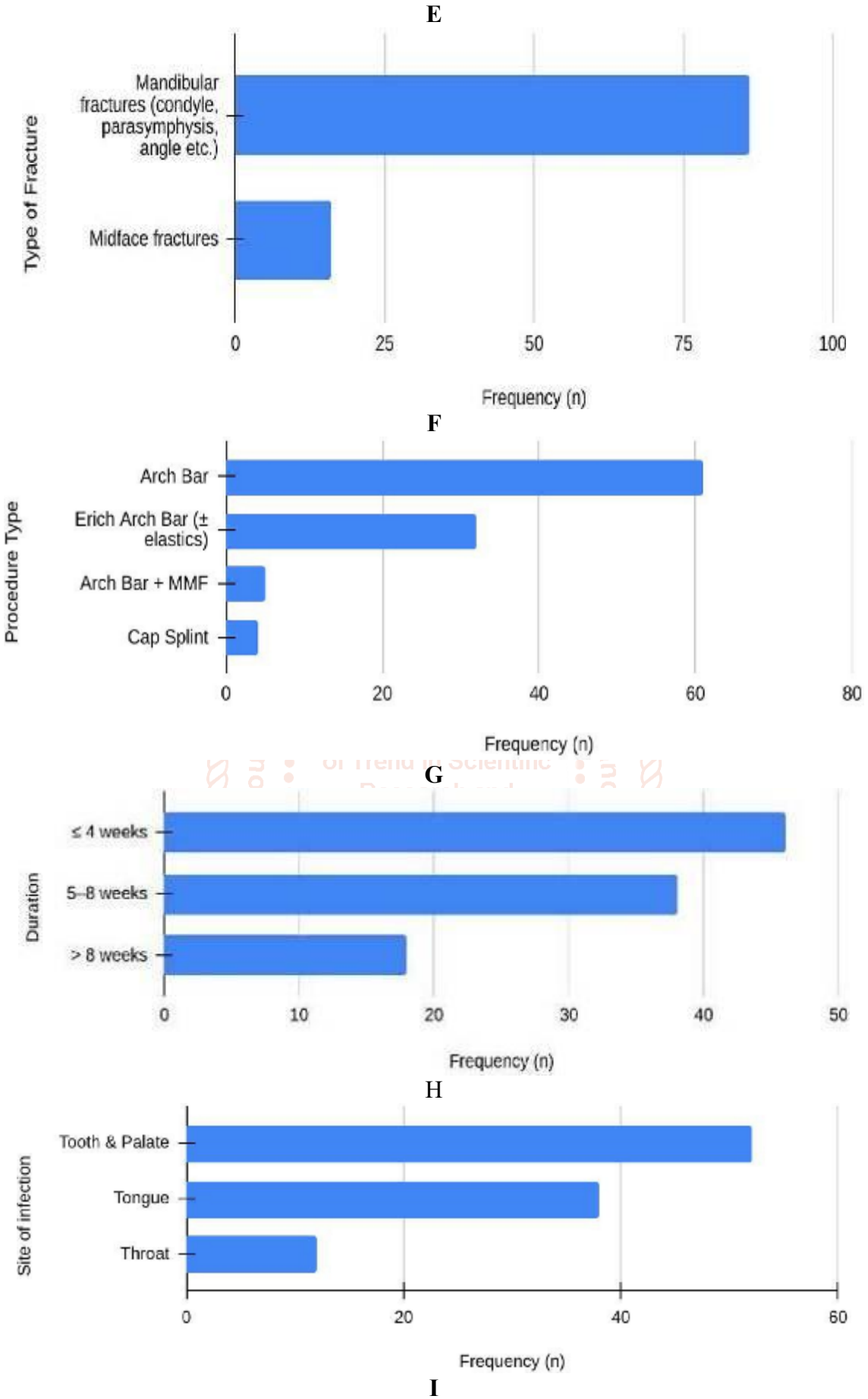


Figure 3.1 (A-I): Demographic Characteristics and Clinical Profile of IMF patients

Table 3.2: Multivariate Logistic Regression Analysis for Predictors of Wound Infection

Variable	Category	Adjusted Odds Ratio (AOR)	95% CI	p-value
Oral Hygiene	Poor vs Normal/Good	3.80	1.50 – 9.60	0.003
Breath Odor	Poor vs Good	2.45	1.02 – 5.88	0.044

IMF Duration	>4 weeks vs ≤4 weeks	2.10	0.95 – 4.65	0.067
Age Group	≥50 vs <50 years	1.60	0.70 – 3.60	0.25
Gender	Male vs Female	1.20	0.40 – 3.50	0.72
Type of Fracture	Mandible vs Midface	1.75	0.80 – 3.90	0.16

3.1.1. Multivariate Logistic Regression Analysis of Wound Infection Predictors

Multivariate analysis showed that **poor oral hygiene was the strongest independent predictor of wound infection**, increasing infection odds by 3.8-fold (p = 0.003). **Poor breath odour** was also significantly associated with infection (AOR = 2.45, p = 0.044). Longer IMF duration (>4 weeks) showed increased infection odds but was not statistically significant (p = 0.067). Age, gender, and fracture type were not significant predictors.

3.2. Microbiological Profiling: From Symbiosis to Dysbiosis

3.2.1. Colony Morphology and Biochemical Characteristics of Isolated Bacterial Strains

Microbiological examination of oral samples from maxillofacial trauma patients undergoing IMF revealed a **diverse range of Gram-positive and Gram-negative bacterial species** following arch-bar placement.

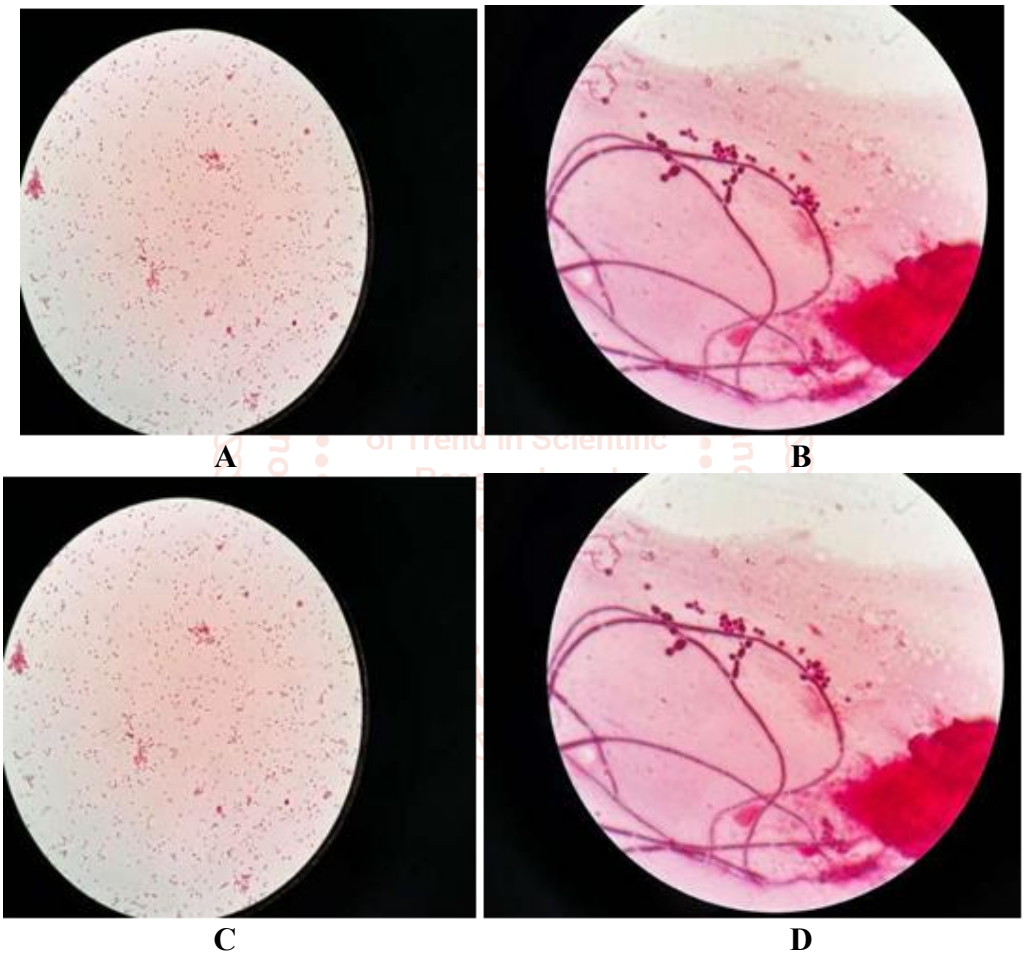


Figure 3.3: (A, B, C) Gram-negative bacilli and gram-positive cocci seen in pair, chain and clusters D) budding yeast cells seen as small, oval-shaped pseudo hyphae with long, filament-like structure (100x magnification)

3.2.2. Colony Morphology and Biochemical Characteristics of Isolated Bacterial Strains

Culture characteristics and biochemical tests identified diverse Gram-positive and Gram-negative oral microorganisms after IMF. Gram-positive isolates included *Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, E. faecium,* and *Micrococcus luteus*. *S. aureus* showed characteristic pink colonies on MacConkey agar and golden-yellow β-haemolytic colonies on blood agar, while *Enterococcus* spp. were catalase-negative.

Gram-negative isolates included *Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Enterobacter cloacae, Proteus mirabilis, Pantoea agglomerans, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Neisseria flava*. Their identification was confirmed using colony morphology, Gram staining, and biochemical tests including catalase, oxidase, urease, citrate, methyl red, Voges–Proskauer, and carbohydrate fermentation.

Most isolates showed counts ≥10^5 CFU/mL, indicating substantial microbial colonisation. Overall, the findings demonstrated increased colonisation by opportunistic and potentially resistant organisms following IMF, suggesting significant alteration of the oral microbial environment.

4. DISCUSSION

The present study evaluated the demographic, clinical, physiological, and microbiological changes associated with intermaxillary fixation (IMF) in patients with maxillofacial trauma, with particular comparison between pre-IMF and post-IMF findings. The results demonstrate that IMF is associated with important nutritional, oral health, and microbial changes.

A marked male predominance was observed (90.2%), with trauma being most common among individuals aged 30–49 years, followed by those aged 15–29 years. This pattern is consistent with previous reports showing a higher incidence of maxillofacial trauma among economically active males, largely due to occupational exposure, road travel, and risk-taking behaviour (Bither et al., 2008; Chrcanovic et al., 2004). Falls and road traffic accidents were the major causes of trauma, consistent with findings from developing regions (Shinde et al., 2024). Mandibular fractures were predominant (84.3%), supporting previous evidence that the mandible is particularly vulnerable because of its anatomical position and biomechanical characteristics (Ellis et al., 1985). Arch bars remained the most commonly used IMF technique (59.8%), reflecting their continued clinical usefulness.

A significant mean weight loss of 3.1 kg was observed after IMF ($p < 0.001$). This finding agrees with previous studies linking IMF to nutritional compromise due to restricted oral intake, altered food consistency, and patient discomfort (Pellegrini et al., 2023; Azharudeen et al., 2025). These findings highlight the importance of nutritional counselling and appropriate dietary support during fixation.

Poor oral hygiene was observed in more than three-quarters of patients and was accompanied by a high prevalence of halitosis. A significant relationship was identified between poor oral hygiene and wound infection ($\chi^2 = 8.92$, $p = 0.0028$), while 44.1% of patients developed wound infection. This supports evidence that restricted oral access during IMF promotes plaque accumulation, microbial proliferation, and wound contamination (Moharana et al., 2025).

Microbiological findings demonstrated a significant alteration in oral microbial composition following IMF. Pre-IMF samples were predominantly composed of Gram-positive commensals, particularly *Streptococcus* and *Staphylococcus aureus*, reflecting the normal oral microbial environment. In contrast, post-IMF samples showed a substantial increase in Gram-negative opportunistic organisms, including *Pseudomonas aeruginosa*, *Klebsiella*, *Acinetobacter*, and *Escherichia coli*. This shift indicates IMF-associated oral dysbiosis and suggests that prolonged fixation, impaired oral hygiene, altered diet, and reduced mechanical cleansing create favourable conditions for opportunistic microbial colonisation.

CONCLUSION AND FUTURE PROSPECTS

The present prospective randomised study demonstrated that intermaxillary fixation (IMF) with upper and lower arch bars significantly alter the oral microbial environment in maxillofacial trauma patients. Prolonged IMF was associated with a shift from predominantly commensal Gram-positive flora toward increased Gram-negative opportunistic bacterial and fungal organisms, indicating the development of oral dysbiosis. Several clinically important organisms, including *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Candida albicans*, were frequently identified following IMF. Poor oral hygiene, halitosis, and longer fixation periods were associated with increased microbial colonisation and wound infection. Antimicrobial susceptibility testing also revealed considerable resistance, particularly among Gram-negative organisms, highlighting the potential risk of multidrug-resistant infections. These findings emphasise the importance of effective oral hygiene, rational antibiotic prescribing, and appropriate microbiological monitoring during IMF to reduce complications and improve patient outcomes.

Future research should include multicentre studies with larger sample sizes and advanced molecular approaches, including 16S rRNA sequencing, metagenomic analysis, and biofilm profiling, to provide a more comprehensive understanding of IMF-associated microbial changes. Long-term studies assessing microbial recovery after removal of arch bars would help clarify the restoration of the oral microbiome. Further investigations should also evaluate preventive approaches such as probiotics, antimicrobial mouth rinses, nanotechnology-based oral hygiene methods, and personalised antimicrobial therapy. Developing evidence-based infection-control protocols that combine microbiological surveillance with clinical risk assessment may further improve the

management and outcomes of patients undergoing IMF.

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