DOI: 10.7860/JCDR/2026/89287.24217

Original Article

Microbiology Section

Phenotypic Characterisation of Resistant Profiles of the Isolates in Orthopaedic Implant Associated Infections: A Cross-sectional Study

SHAILJA GAUTAM[1], MANJULA VAGARALI[2], RAVI S JATTI[3]

ABSTRACT

Introduction: Orthopaedic Implant Associated Infections (OIAIs) are serious complications associated with prolonged morbidity, implant failure, repeated surgical interventions, and increased healthcare burden. The emergence of antimicrobial-resistant pathogens further complicates treatment outcomes.

Aim: To study the phenotypic characterisation of resistant profiles of isolates in OIAIs and to detect the bacteriological profile and antibiogram of these isolates.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College (JNMC), KAHER, Belagavi, Karnataka, India over one year (April 2024-March 2025). A total of 66 aspirated pus samples from patients with confirmed OIAIs were included. Samples were processed using standard microbiological techniques. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method according to CLSI M100, 34th edition (2024) guidelines. Phenotypic detection of resistance mechanisms including Methicillin-Resistant *Staphylococcus aureus* (MRSA), Extended-Spectrum β-Lactamase (ESBL), AmpC, and Metallo-Beta-Lactamase (MBL) production was carried out. Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 27.0, and p-value<0.05 was considered statistically significant.

Results: Out of 66 samples processed, 60/66 (90.9%) showed culture positivity. The highest proportion of patients belonged to the 31-40 years age group {18/66 (27.3%)}, and males accounted for 55/66 (83%) cases. Gram-negative organisms predominated {39/60 (65%)}, while *Staphylococcus aureus* was the most common individual isolate {21/60 (35%)}. Among *S. aureus* isolates, MRSA constituted 12/21 (57.1%). ESBL + AmpC co-production was the predominant resistance mechanism among Gram-negative isolates {23/39 (59%)}, followed by ESBL production alone {11/39 (28%)}. All *S. aureus* isolates remained susceptible to vancomycin and linezolid.

Conclusion: OIAIs predominantly affected younger males and were more commonly associated with external implants. Gram-negative organisms showed a high burden of Multidrug Resistance (MDR), including ESBL and AmpC production. Routine antimicrobial susceptibility testing, continuous resistance surveillance, and antibiogram-guided therapy are essential for effective management and antimicrobial stewardship.

Keywords: Antimicrobial resistance, Bacterial, Cross infection, Drug resistance, Orthopaedic procedures, Surgical wound infection

INTRODUCTION

The OIAIs remain a significant challenge in modern orthopaedic practice despite advances in surgical techniques, biomaterials, and perioperative infection control. These infections can lead to prolonged morbidity, implant failure, delayed union, non-union, and repeated surgical interventions, often compromising functional recovery [1]. Orthopaedic implants such as plates, screws, prosthetic joints, intramedullary nails, and external fixators provide surfaces that facilitate microbial adhesion and persistence, creating a favourable environment for infection.

The risk of OIAIs arises from complex interactions between microorganisms and implanted foreign bodies [2]. Following implantation, host immune responses are impaired as immune cells cannot effectively eliminate bacteria attached to biomaterial surfaces. In addition, the avascular nature of implant surfaces limits immune access and antibiotic penetration. These factors promote microbial survival and persistence, allowing even small bacterial inocula to establish clinically significant infections [3]. Many of these microorganisms demonstrate phenotypic resistance, contributing to treatment failure, recurrent infections, and surgical complications.

The bacteriological spectrum of OIAIs is diverse, with Gram-positive cocci-particularly *Staphylococcus aureus*-being the most common pathogens [4]. These organisms possess Microbial Surface Components Recognising Adhesive Matrix Molecules (MSCRAMMs), enabling strong adhesion to implant surfaces. In addition, Gram-negative bacilli such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter* species are increasingly reported, especially in trauma-related and hospital-acquired infections.

The growing global burden of Antimicrobial Resistance (AMR) has further complicated the management of implant-associated infections [5]. Resistant pathogens such as MRSA, Methicillin-Resistant Coagulase-Negative Staphylococci (MRCoNS), ESBL-producing Enterobacterales and carbapenem-resistant non-fermenters pose major therapeutic challenges.

Diagnosis of OIAIs relies on a combination of clinical evaluation, radiological assessment, and microbiological investigations [6]. Management of OIAIs requires both surgical and antimicrobial approaches. Treatment strategies include Debridement, Antibiotics, and Implant Retention (DAIR), staged implant removal, and prolonged antimicrobial therapy [7]. The choice of treatment depends on the infecting organism, resistance profile, duration of infection, and host factors.

Antibiogram analysis is an important tool for guiding empirical antibiotic therapy in implant-associated infections. Regular surveillance helps clinicians monitor evolving resistance trends and optimise treatment protocols [8]. A centre-specific antibiogram derived from implant-associated infections provides more clinically relevant data than general hospital antibiograms, as implant pathogens often exhibit distinct resistance patterns. Evaluating phenotypic resistance profiles also supports antimicrobial stewardship efforts aimed at minimising inappropriate antibiotic use and controlling the spread of AMR [9]. This study is unique in providing a centre-specific antibiogram exclusively for implant-associated infections and adds new data on the changing trends in pathogen profile and resistance patterns, which may help guide more appropriate empirical therapy and strengthen antimicrobial stewardship.

This study aimed to analyse the phenotypic resistant profiles of isolates obtained from OIAIs in a tertiary care centre. By evaluating the bacteriological spectrum and antibiogram patterns, the study seeks to generate institution-specific data that can guide empirical therapy, improve treatment outcomes, and strengthen antimicrobial stewardship in orthopaedic practice.

MATERIALS AND METHODS

The present study was a cross-sectional study carried out in the Department of Microbiology, Jawaharlal Nehru Medical College (JNMC), KAHER, Belagavi, Karnataka, India. The study was conducted from 1st April 2024 to 31st March 2025. . Ethical clearance was obtained prior to the study. The present research project was approved by the JNMC International Ethics Committee on Human Subjects Research (Ref No. MDC/JNMCIEC/214. Date:19/03/2024). Informed consent was obtained from patients.

Inclusion criteria: All pus samples from suspected implant infections were routed to the microbiology department as part of routine diagnostic practice. Patients with OIAIs were defined by the presence of purulent discharge, sinus tract, local inflammation, pain, or implant failure with microbiological confirmation.

Exclusion criteria: Patients with implant infections who were on antibiotics were excluded from the study.

Sample size: The study employed consecutive sampling. The sample size for the study was calculated using the standard formula for cross-sectional studies estimating proportions, taking into account the expected culture positivity rate of 72%, based on previous institutional data [9]. With total population size N = 66, expected proportion p = 0.72, allowable error d = 10% of p = 0.072, and Zα/2 = 1.96 for 95% confidence, the formula yielded a required sample size of n = 37.

The sample size was calculated as: $N (Z^2 p(1-p))/\{(d^2) (N-1) + Z^2 p(1-p)\}$, resulting in 36.56, rounded to 37. Since consecutive sampling was used, all eligible samples received during the study period were included. The sample size was used to characterise distribution of Gram-positive and Gram-negative organisms and isolates were additionally grouped by resistance mechanism: ESBL producers, MBL producers, AmpC β-lactamase producers, and MRSA. These subdivisions supported comparative evaluation of resistance distribution across organism types and helped assess prevalence patterns.

Study Procedure

Pus sample received from clinically suspected infected implant sites during the study period was included if it met the eligibility criteria. Upon receipt, samples were labelled, and processed immediately according to standard protocols. Each submitted sample underwent uniform processing including Gram staining, culture on blood agar and MacConkey agar, incubation at 37°C, and subsequent phenotypic testing. Culture, biochemical identification, and antimicrobial susceptibility testing was done using standardised microbiological procedures. The study included multiple laboratory parameters essential for characterising microbial profiles and resistance mechanisms. These parameters were culture positivity rates from implant-associated pus samples, colony morphology on blood agar and MacConkey agar, biochemical identification using standard biochemical tests such as catalase test, coagulase test, oxidase test, indole test, citrate utilisation test, urease test, Triple Sugar Iron (TSI) agar reaction, motility test, and carbohydrate fermentation tests, antimicrobial susceptibility patterns. Detection of ESBL, MBL, AmpC, and MRSA using phenotypic confirmation tests, zone diameters measured according to Clinical and Laboratory Standards Institute (CLSI) guidelines M100, 34th edition (2024) [10]. These parameters collectively enabled assessment of bacteriological diversity and resistance patterns in implant infections.

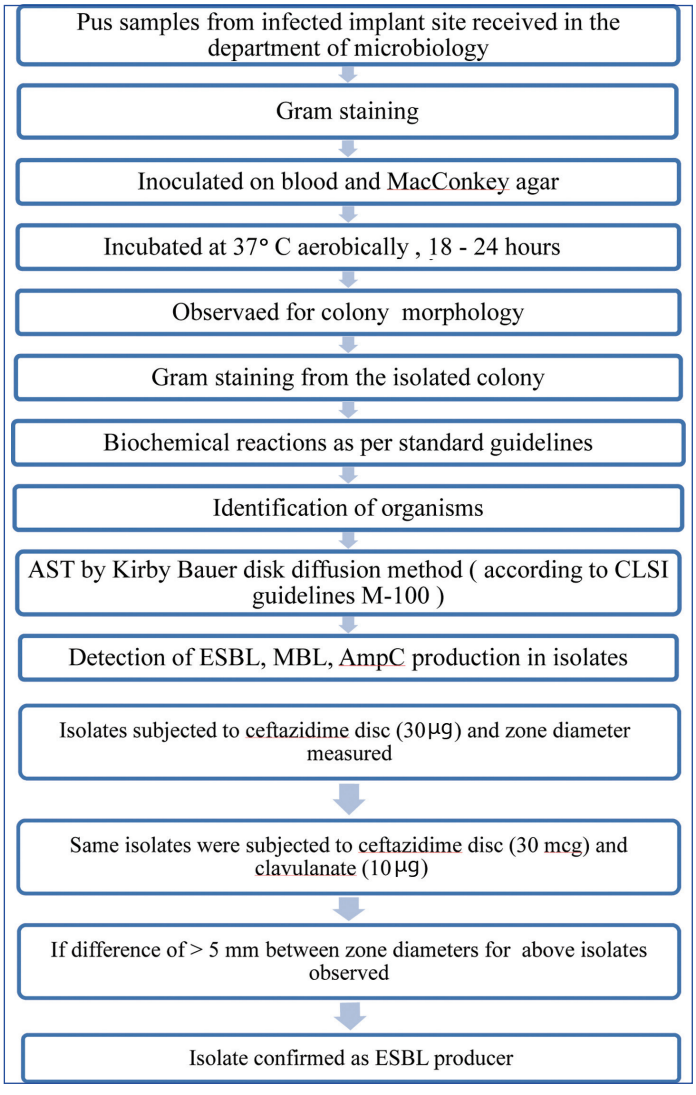
ESBL detection using ceftazidime and ceftazidime-clavulanate discs using combined disc method [Table/Fig-1a].

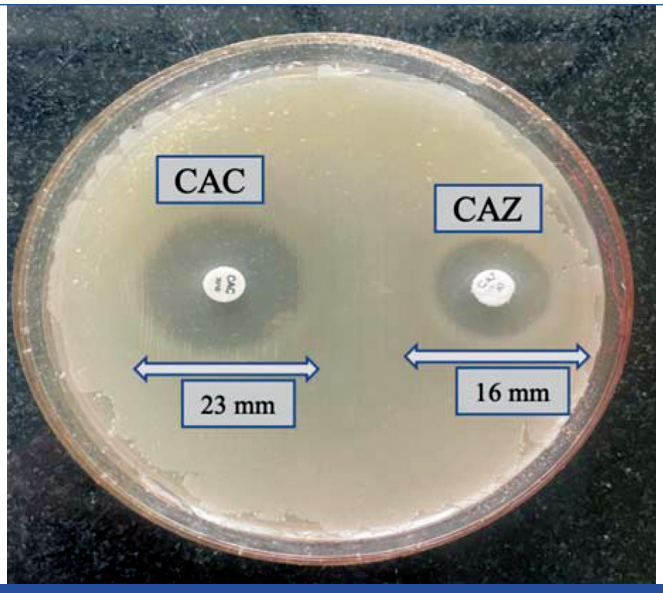
Methodologically, species-specific antibiograms were designated for descriptive epidemiological analysis. Phenotypic validation protocols adhered to CLSI interpretative guidelines (34th Edition), where ESBL expression was confirmed by a ≥ 5 mm expansion in the inhibition zone diameter of the ceftazidime-clavulanic acid combination disc relative to ceftazidime alone, while MBL expression was verified using imipenem and imipenem-EDTA discs, defined by a zone diameter increase of >7 mm in the presence of the chelating agent as demonstrated in [Table/Fig-1b].

AmpC detection using cefoxitin screening [Table/Fig-1c].

Confirmatory test for detection of AmpC - By disk approximation test [Table/Fig-1d].

MRSA detection using cefoxitin discs [Table/Fig-1e].

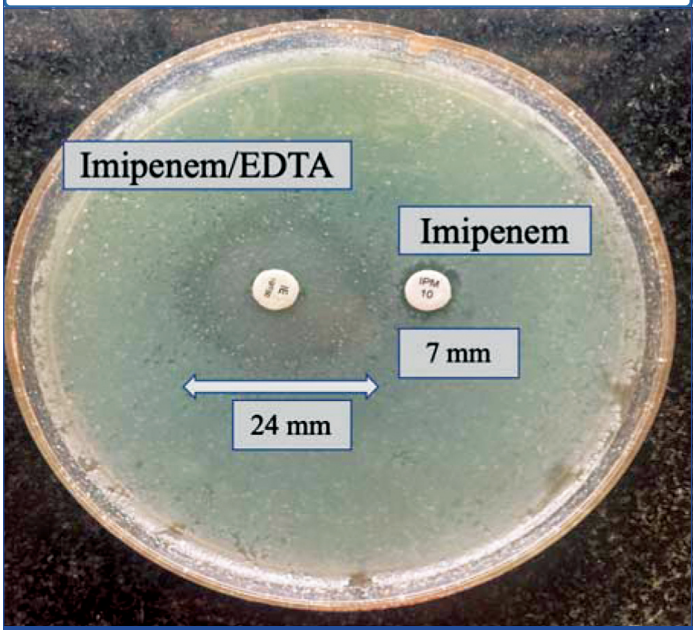




[Table/Fig-1a]: ESBL Detection - This isolate tested for production of ESBL by ceftazidime (CAZ) disc (30 µg) and Ceftazidime/Clavulanate (CAC) (30/10 µg). This is ESBL producer as difference between zone size is more than 5mm i.e., 7mm.

Isolates subjected to imipenem disc (10µg) and Imipenem/EDTA disc (10/750 µg)

If the increase in inhibition zone with the Imipenem and EDTA disc was > or = 7 mm as compared to Imipenem disc alone , the isolate considered as MBL producer



[Table/Fig-1b]: MBL Detection- This isolate tested for MBL detection by discs imipenem (10 µg) and Imipenem/EDTA (10/750 µg). Here difference in zone size is more than 7mm i.e., 17mm hence MBL producer.

STATISTICAL ANALYSIS

Data analysis was conducted using SPSS version 27.0 after verifying the data. Descriptive statistics were applied to summarise culture positivity rates, distribution of Gram-positive and Gram-negative organisms and specific resistance mechanisms. Frequencies and percentages were used to present antimicrobial susceptibility patterns and resistance profiles. Inferential statistical (Chi-square) tests were employed to determine associations between organism type and resistance patterns, p-value<0.05 taken as significant. The final output included tables and charts reflecting microbial distribution, susceptibility trends, and resistance phenotype percentages.

RESULTS

[Table/Fig-2] shows the demographic characteristics of study participants with OIAIs.

Isolate subjected to cefoxitin disc (30 µg)

Isolates < 18 mm zone

Isolate confirmed positive for AmpC production

[Table/Fig-1c]: AmpC detection - screening this isolate tested for AmpC production. As zone size around cefoxitin disc (30 µg) is less than 18 mm, so its AmpC producer.

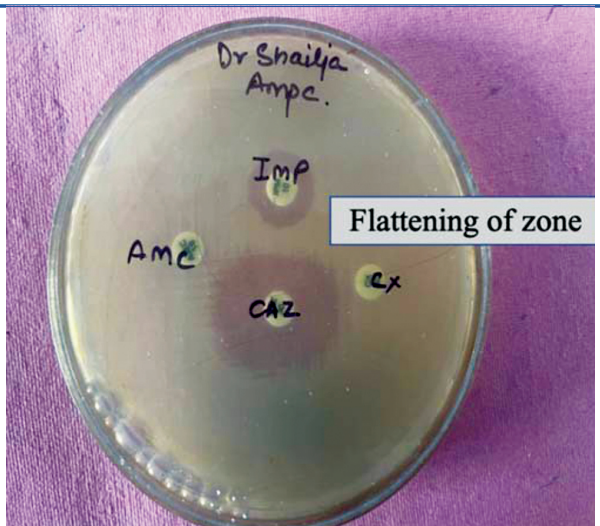
Inoculated surface of MHA plate as per standard disk diffusion method

Placed a 30 µg ceftazidime disk at the center on the plate

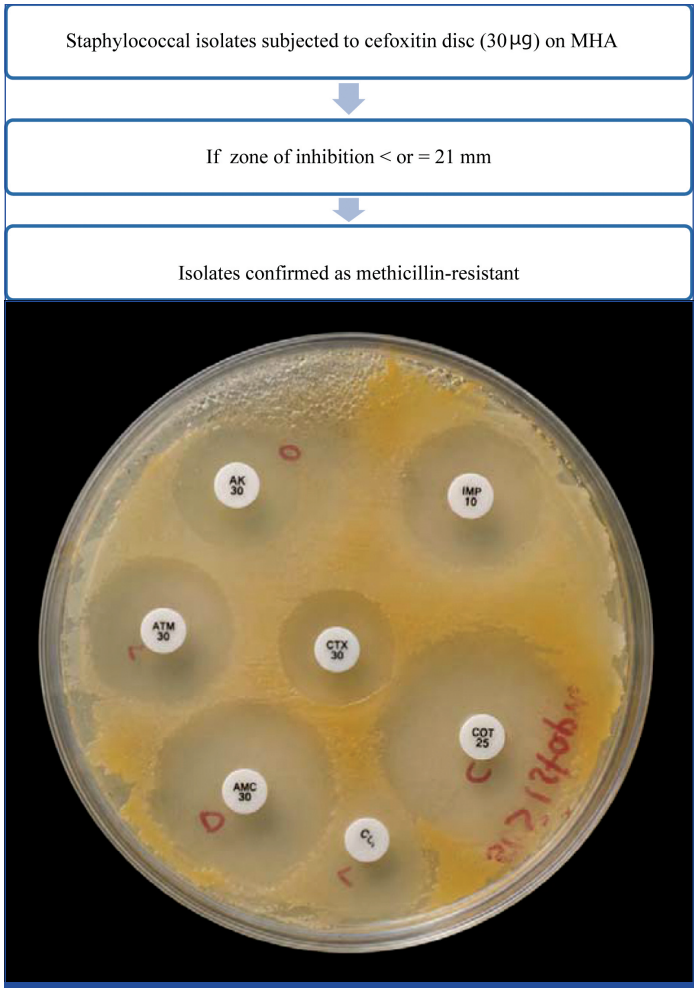
Placed 10 µg imipenem, 30 µg cefoxitin, and 20/10 µg amoxicillin-clavulanate disks at a distance of 20 mm from ceftazidime disk and incubate overnight at 37°C

Plate reading and interpretation

If any blunting or flattening of the zone of inhibition between the ceftazidime disk and the inducing substrates (imipenem, cefoxitin and amoxicillin-clavulanate disk) considered positive for *AmpC* production



[Table/Fig-1d]: Disk approximation test for detection of AmpC - Isolate tested for AmpC production. Flattening of zone of ceftazidime toward imipenem disk showing positive result. Antibiotics used were Amoxicillin-Clavulanic acid (AMC), Imipenem (IMP), Ceftazidime (CAZ), Cefoxitin (CX).



[Table/Fig-1e]: Phenotypic detection of MRSA by cefoxitin disc diffusion method. Methicillin-Resistant *Staphylococcus aureus* (MRSA) was detected using the cefoxitin (30 µg) disc diffusion method on Mueller-Hinton agar according to CLSI guidelines. The isolate showed a reduced zone of inhibition around the cefoxitin disc (≤21 mm), indicating methicillin resistance and confirming the isolate as MRSA.

A total of 66 samples were processed, of which 60 (90.9%) showed culture positivity, while six samples (9.1%) showed no bacterial growth. Although 4 (6.7%) samples demonstrated polymicrobial growth and monomicrobial was seen in 56 (93.3%), only clinically significant isolates were included in the final analysis, while commensal contaminants were excluded. The total number of 60 isolates were analysed.

Variable	Category	n (%)
Age group (years)	17-20	8 (12.1)
	21-30	15 (22.7)
	31-40	18 (27.3)
	41-50	10 (15.2)
	51-60	10 (15.2)
	61-70	5 (7.6)
Gender	Male	55 (83)
	Female	11 (17)

[Table/Fig-2]: Demographic characteristics of study participants with Orthopaedic Implant-Associated Infections (OIAI) (n = 66).

Age and gender showed no statistically significant association between demographic variables and type of bacterial isolate recovered (Chi-square test, p-value>0.05).

Among the 21 *Staphylococcus aureus* isolates, MRSA accounted for 12 (57.1%) isolates while MSSA constituted 9 (42.9%) isolates [Table/Fig-3]. MRSA isolates were more frequently associated with pin tract implants and orthopaedic plates, whereas MSSA isolates were more commonly isolated from external fixators. However, the association between implant type and MRSA prevalence was not statistically significant (Chi-square test, p-value>0.05). Culture positivity and growth pattern of samples is shown in [Table/Fig-4].

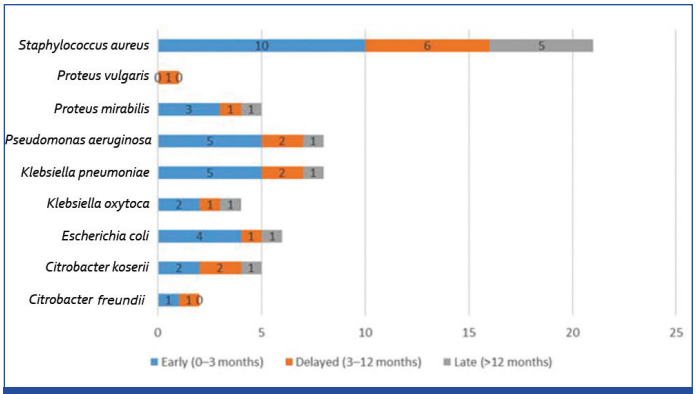
Implant type	MRSA n (%)	MSSA n (%)	Total
External fixator	4 (40)	6 (60)	10
Pin tract implant	4 (80)	1 (20)	5
Orthopaedic plate	2 (66.7)	1 (33.3)	3
Interlocking nail	1 (50)	1 (50)	2
Orthopaedic screw	1 (100)	0	1
Total	12	9	21

[Table/Fig-3]: Distribution of MRSA and MSSA among different orthopaedic implant types (n = 21).

Parameter	Category	n (%)
Culture result	Growth observed	60 (90.9)
	No growth	6 (9.1)
Growth pattern among positive cultures (n=60)	Monomicrobial	56 (93.3)
	Polymicrobial	4 (6.7)

[Table/Fig-4]: Culture positivity and growth pattern of samples (n = 66).

Distribution of bacterial isolates according to duration of OIAI is shown in [Table/Fig-5].



[Table/Fig-5]: Distribution of bacterial isolates according to duration of Orthopaedic Implant-Associated Infection (OIAI) (n = 60).

[Table/Fig-6] shows the distribution of bacterial isolates recovered from OIAIs. *Staphylococcus aureus* was the most common isolate (21/60; 35%), while Gram-negative organisms collectively predominated (39/60; 65%). Among Gram-negative bacilli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the predominant isolates (8/60; 13.3% each).

Bacterial isolate	n (%)
Staphylococcus aureus	21 (35)
Klebsiella pneumoniae	8 (13.3)
Pseudomonas aeruginosa	8 (13.3)
Escherichia coli	6 (10)
Proteus mirabilis	5 (8.3)
Citrobacter koserii	5 (8.3)
Klebsiella oxytoca	4 (6.7)
Citrobacter freundii	2 (3.3)
Proteus vulgaris	1 (1.7)
Total isolates	60 (100)

[Table/Fig-6]: Distribution of bacterial isolates from Orthopaedic Implant-Associated Infections (OIAI) (n = 60).

[Table/Fig-7] demonstrates the distribution of bacterial isolates according to implant type. External fixators (25/60) and pin tract implants (20/60) accounted for the majority of infections.

Among the 21 *Staphylococcus aureus* isolates, MRSA accounted for 12 (57.1%) isolates while MSSA constituted 9 (42.9%). Overall antibiogram analysis demonstrated high resistance to ampicillin

Implant Type	*C. freundii*	*C. koserii*	*E. coli*	*K. oxytoca*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*	*P. vulgaris*	*S. aureus*	Total
External fixator	0	3	3	1	3	3	2	0	10	25
Interlocking nail	0	0	0	1	1	0	1	0	2	5
Orthopaedic plate	1	0	0	2	1	0	0	1	3	8
Orthopaedic screw	0	0	0	0	1	0	0	0	1	2
Pin tract implant	1	2	3	0	2	5	2	0	5	20
Total	2	5	6	4	8	8	5	1	21	60

[Table/Fig-7]: Distribution of bacterial isolates according to type of orthopaedic implant (n = 60).

and amoxiclav {21 (100%) each}. Resistance to fluoroquinolones, macrolides, and aminoglycosides was also observed. All isolates remained susceptible to vancomycin and linezolid [Table/Fig-8].

Antibiotic	Resistant isolates of *Staphylococcus aureus* n (%)
Ampicillin	21 (100)
Cefoxitin	12 (57.1)
Ciprofloxacin	12 (57.1)
Levofloxacin	9 (42.9)
Cefepime	13 (61.9)
Amoxiclav	21 (100)
Erythromycin	12 (57.1)
Clindamycin	12 (57.1)
Cotrimoxazole	13 (61.9)
Gentamicin	11 (52.4)
Amikacin	10 (47.6)
Doxycycline	6 (28.6)
Vancomycin	0
Linezolid	0

[Table/Fig-8]: Antibiogram of *Staphylococcus aureus*.
*Number of isolates tested was less than 30. As per CLSI guidelines, antibiogram data generated from fewer than 30 isolates may not be statistically reliable; however, the data has been presented for epidemiological purposes and future meta-analysis.

Universal phenotypic resistance was characteristically uniform across the entire study sample, with 100% (39/39) of the isolated Gram-negative pathogens expressing at least one detectable enzymatic resistance mechanism, underscoring an extensive MDR burden complicating these OIAIs. Among these profiles, dual Beta-lactamase expression via concomitant ESBL and AmpC co-production constituted the predominant resistance isolates, driving 59% (23/39) of the Gram-negative isolates, followed sequentially by standalone ESBL production at 28% (11/39), isolated AmpC expression at 8% (3/39), and isolated MBL production at 5% (2/39) [Table/Fig-9a]. A highly significant statistical association was confirmed between distinct Gram-negative isolates and their underlying enzymatic configurations (Chi-square test, p-value<0.05). Specifically, isolated ESBL phenotypes were tightly clustered within *Escherichia coli* and *Klebsiella pneumoniae* isolates, whereas dual ESBL+AmpC co-production predominated among the non-fermenter *Pseudomonas aeruginosa* as well as *Citrobacter koserii* and *Proteus mirabilis* strains. Phenotypic MBL expression was restricted exclusively to the *Pseudomonas aeruginosa* isolates [Table/Fig-9b].

All Enterobacteriaceae isolates demonstrated 100% resistance to ampicillin, cefotaxime, ceftriaxone, and amoxiclav across all species. Resistance to gentamicin, amikacin, cefepime, and piperacillin-tazobactam was variably noted, with *Klebsiella pneumoniae* showing higher resistance rates, including 62.5% to piperacillin-tazobactam and 50% to imipenem [Table/Fig-10].

Resistance Mechanism	n (%)
ESBL + AmpC	23 (59)
ESBL	11 (28)
AmpC	3 (8)
MBL	2 (5)
Total	39 (100)

[Table/Fig-9a]: Distribution of resistance enzymes.

Organism	AmpC	ESBL+AmpC	ESBL	MBL	Total
C. freundii	0	2	0	0	2
C. koserii	0	5	0	0	5
E. coli	0	0	6	0	6
K. oxytoca	3	1	0	0	4
K. pneumoniae	0	3	5	0	8
P. aeruginosa	0	6	0	2	8
P. mirabilis	0	5	0	0	5
P. vulgaris	0	1	0	0	1
Total	3	23	11	2	39

[Table/Fig-9b]: Distribution of resistance mechanisms by Gram-negative isolates.

All *Pseudomonas aeruginosa* isolates demonstrated complete resistance to β-lactam antibiotics, aminoglycosides, and ciprofloxacin (8/8; 100%). Resistance to aztreonam was observed in 5/8 (62.5%) isolates, while meropenem resistance was detected in 3/8 (37.5%). All isolates remained susceptible to imipenem and levofloxacin (0/8 resistant) [Table/Fig-11].

A case of severe implant-associated infection showed exposed orthopaedic plate due to wound dehiscence, as depicted in [Table/Fig-12]. Among the implants studied, cases managed with Ilizarov ring fixation were also included, as shown in [Table/Fig-13], demonstrating the use of circular external fixation with transosseous pins.

DISCUSSION

Implant-related infections are associated with prolonged hospital stay, repeated surgical interventions, delayed rehabilitation, and increased healthcare costs. The emergence of resistant Gram-negative bacilli and MRSA further complicates management and underscores the need for accurate pathogen identification and resistance profiling. Phenotypic characterisation of resistance mechanisms provides crucial information for guiding targeted antimicrobial therapy and optimising antibiotic stewardship practices. The present study aimed to phenotypically characterise bacterial isolates associated with orthopaedic implant infections and evaluate their AMR patterns in a tertiary care setting.

The demographic distribution of patients in the present study demonstrated that OIAIs were more frequently observed among younger adults, particularly in the 31-40-year age group (27.3%), followed by the 21-30-year group (22.7%). Similar observations were reported by Li C et al., who noted a higher incidence of implant infections among younger and middle-aged adults [11]. Male predominance was observed with males accounting for 83% of cases. Comparable gender distribution has been reported by Li C et al., and Benazir S et al., who attributed the higher incidence among males to increased exposure to trauma, road traffic accidents, and physically demanding occupations [11,12].

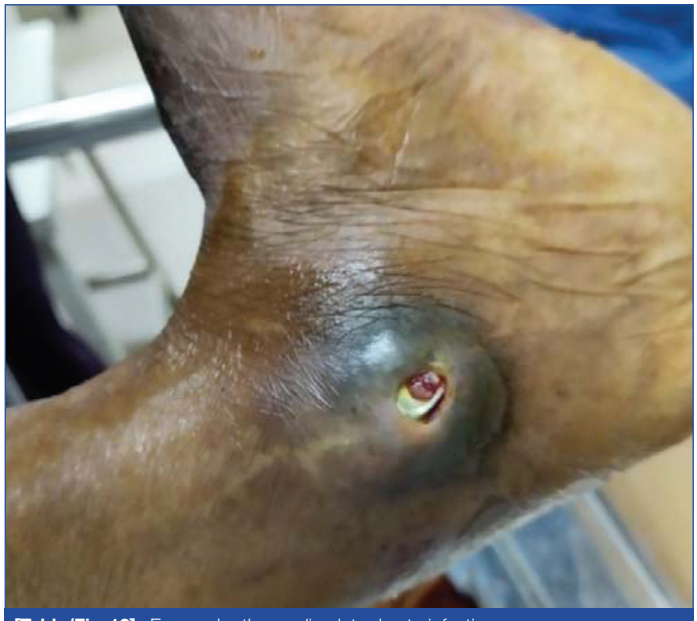
The present study demonstrated a high prevalence of MRSA (12/21; 57.1%) among implant-associated *Staphylococcus aureus* isolates. Higher MRSA occurrence was observed among

Antibiotic	*C. freundii* (n=2) (%)	*C. koserii* (n=5) (%)	*E. coli* (n=6) (%)	*K. oxytoca* (n=4) (%)	*K. pneumoniae* (n=8) (%)	*P. mirabilis* (n=5) (%)	*P. vulgaris* (n=1) (%)
Ampicillin (AMP)	2/2 (100)	5/5 (100)	6/6 (100)	4/4 (100)	8/8 (100)	5/5 (100)	1/1 (100)
Gentamicin (GEN)	0/2 (0)	0/5 (0)	0/6 (0)	1/4 (25)	3/8 (37.5)	0/5 (0)	0/1 (0)
Piperacillin-Tazobactam (PTZ)	0/2 (0)	0/5 (0)	0/6 (0)	1/4 (25)	5/8 (62.5)	0/5 (0)	0/1 (0)
Cefotaxime (CTX)	2/2 (100)	5/5 (100)	6/6 (100)	4/4 (100)	8/8 (100)	5/5 (100)	1/1 (100)
Ceftriaxone (CTR)	2/2 (100)	5/5 (100)	6/6 (100)	4/4 (100)	8/8 (100)	5/5 (100)	1/1 (100)
Amoxiclav (AMC)	2/2 (100)	5/5 (100)	6/6 (100)	4/4 (100)	8/8 (100)	5/5 (100)	1/1 (100)
Ciprofloxacin (CIP)	0/2 (0)	0/5 (0)	0/6 (0)	0/4 (0)	0/8 (0)	0/5 (0)	0/1 (0)
Levofloxacin (LEV)	0/2 (0)	0/5 (0)	0/6 (0)	0/4 (0)	4/8 (50)	0/5 (0)	0/1 (0)
Amikacin (AK)	0/2 (0)	0/5 (0)	0/6 (0)	1/4 (25)	3/8 (37.5)	0/5 (0)	0/1 (0)
Imipenem (IPM)	0/2 (0)	0/5 (0)	0/6 (0)	0/4 (0)	4/8 (50)	0/5 (0)	0/1 (0)
Cefepime (CPM)	0/2 (0)	0/5 (0)	0/6 (0)	1/4 (25)	3/8 (37.5)	0/5 (0)	0/1 (0)

[Table/Fig-10]: Antibiogram of enterobacteriaceae isolates from Orthopaedic Implant-Associated Infections (OIAI).
*Number of isolates tested was less than 30. As per CLSI guidelines, antibiogram data generated from fewer than 30 isolates may not be statistically reliable; however, the data has been presented for descriptive and epidemiological purposes.

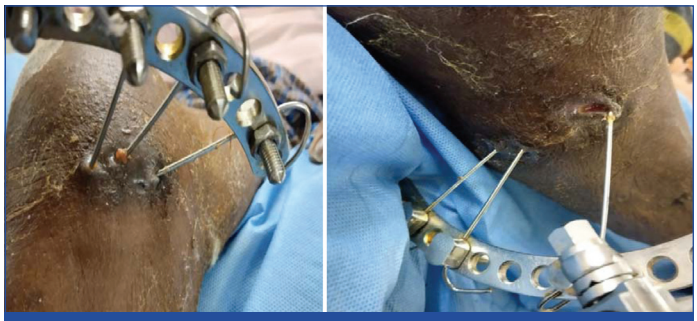
Antibiotic	Resistance n/N (%)
Piperacillin–Tazobactam (PIT)	8/8 (100)
Ceftazidime (CAZ)	8/8 (100)
Cefotaxime (CTX)	8/8 (100)
Ceftriaxone (CTR)	8/8 (100)
Cefepime (CPM)	8/8 (100)
Cefuroxime (CXM)	8/8 (100)
Ciprofloxacin (CIP)	8/8 (100)
Levofloxacin (LEV)	0/8 (0)
Gentamicin (GEN)	8/8 (100)
Amikacin (AK)	8/8 (100)
Tobramycin (TOB)	8/8 (100)
Aztreonam (AT)	5/8 (62.5)
Imipenem (IPM)	0/8 (0)
Meropenem (MRP)	3/8 (37.5)

[Table/Fig-11]: Antibiogram of *Pseudomonas aeruginosa* Isolates from Orthopaedic Implant-Associated Infections (OIAI) (n = 8).
*Number of isolates tested was less than 30. As per CLSI guidelines, antibiogram data generated from fewer than 30 isolates may not be statistically reliable; however, the data has been presented for descriptive and epidemiological purposes.



[Table/Fig-12]: Exposed orthopaedic plate due to infection.

pin tract implants and orthopaedic plates, which may be related to prolonged device exposure, repeated wound manipulation, and healthcare-associated colonisation. Similar high MRSA prevalence has been reported in orthopaedic implant infections from tertiary care centres in India. The persistence of susceptibility



[Table/Fig-13]: Ilizarov ring with pins.

to vancomycin and linezolid among all isolates is encouraging and supports their continued role in management of MRSA-associated implant infections.

This study demonstrated a high culture positivity rate of 90.9%, indicating strong correlation between clinical suspicion and microbiological confirmation. Similar culture positivity rates were reported in literature (91.8%) [11] and Benazir S et al., (86%) [12]. The small proportion of culture-negative samples may be attributed to prior antibiotic exposure, low bacterial load, or fastidious organisms not detectable by conventional culture methods.

Analysis of bacteriological growth patterns revealed that monomicrobial infections predominated (93.3%), while polymicrobial infections were relatively uncommon (6.7%). This finding was consistent with previous reports by Li C et al., and Benazir S et al., who also observed predominantly monomicrobial infections in implant-associated infections [11,12]. The predominance of monomicrobial infections allows for more targeted antimicrobial therapy and may facilitate better clinical outcomes.

In the present study, Gram-negative organisms constituted the majority of isolates 39 (65%), while Gram-positive organisms accounted for 21 (35%) isolates. Similar Gram-negative predominance was reported by Sarkar K et al., who documented Gram-negative organisms in 68% of implant infections [13]. However, Benazir S et al., observed a higher proportion of Gram-positive organisms (54.6%), indicating variability in microbial profiles across institutions [12].

Despite the predominance of Gram-negative organisms {39/60 (65%)}, *Staphylococcus aureus* remained the most frequently isolated individual pathogen {21/60 (35%)}. Similar findings were reported by other studies., identified *S. aureus* as the leading pathogen in implant-associated infections [11,14]. The persistence of *S. aureus* as a major pathogen may be related to its ability to adhere to implant surfaces. Although biofilm formation is recognised as an important factor in implant-associated infections, biofilm detection was not performed in the present study and is discussed here only as background context.

Infections were more commonly associated with external implants {46/60 (77%)} than internal implants {14/60 (23%)} in the present study. External fixators and pin-tract implants accounted for the majority of infections, reflecting the increased vulnerability of externally exposed devices to microbial colonisation. The exposed implant surface provides a favourable environment for bacterial colonisation and persistence of infection. Similar findings have been reported by Kaufman MG et al., who emphasised that exposed implant surfaces facilitate bacterial adhesion [15]. Shakthi R and Venkatesha D also documented a higher frequency of infections associated with exposed orthopaedic hardware [9].

External fixators yielded the highest number of bacterial isolates, with *Staphylococcus aureus* being the predominant organism. Pin-tract implants showed a mixed distribution of pathogens, including *Pseudomonas aeruginosa* and *S. aureus*. Coraça-Huber DC et al., demonstrated that implant surface characteristics significantly influence bacterial adhesion and biofilm architecture, particularly in externally exposed devices [16]. These findings highlight the importance of meticulous surgical technique, strict aseptic precautions, and regular monitoring of external fixation devices.

The present study demonstrated a substantial burden of AMR among both Gram-positive and Gram-negative isolates. Among *Staphylococcus aureus* isolates, MRSA accounted for 12 (57.1%) of cases. Comparable MRSA prevalence has been reported in previous studies, who reported MRSA in 40.9% of isolates [11]. Sarkar K et al., documented an even higher prevalence of 76% among implant-associated infections [13].

Gram-negative isolates in the present study demonstrated extensive resistance to β-lactam antibiotics, with universal resistance to ampicillin, cefotaxime, ceftriaxone, and amoxiclav among Enterobacteriaceae. Similar resistance patterns to third-generation cephalosporins have been reported by Benazir S et al., and Jyoti et al., [12,14]. However, complete susceptibility of *S. aureus* isolates to vancomycin and linezolid was observed, consistent with findings reported by Kaufman MG et al., and Jain S et al., [15,17].

Phenotypic detection of resistance mechanisms revealed that ESBL with AmpC co-production was the most common resistance pattern {23 (59%)}, followed by ESBL production alone {11 (28%)}. Similar ESBL prevalence has been reported by Shakthi R and Venkatesha D and Li C et al., who documented ESBL production in 20-37% of Gram-negative isolates [9,11].

Gram-negative isolates in the present study demonstrated substantial resistance to commonly used β-lactam antibiotics and third-generation cephalosporins, consistent with increasing AMR trends reported in orthopaedic implant infections.

Notably, all Gram-negative isolates evaluated in the present study demonstrated at least one detectable phenotypic resistance mechanism, highlighting the extensive burden of AMR among orthopaedic implant-associated pathogens.

A significant treatment problem is the high frequency (59%) of ESBL and AmpC co-production among Gram-negative isolates. In addition to conferring cephamycin resistance, AmpC enzymes also conceal ESBL detection, which may cause resistance to be underestimated. Clinically, this co-production severely reduces the effectiveness of routinely used empirical medicines such cephamycins, β-lactam/β-lactamase inhibitor combos, and third-generation cephalosporins. This may result in a higher chance of treatment failure in orthopaedic infections, especially those involving implants or a high bacterial burden. Although carbapenems are still the most dependable treatment option, their empirical use needs to be weighed against the possibility of encouraging carbapenem resistance.

Species-wise analysis demonstrated that ESBL and AmpC co-production was predominantly associated with *Citrobacter*, *Proteus*, and *Pseudomonas* species, whereas ESBL production alone was mainly observed among *Escherichia coli* and *Klebsiella pneumoniae*. These findings highlight the increasing complexity of AMR patterns among Gram-negative pathogens in orthopaedic implant infections.

MBL production was identified only in *Pseudomonas aeruginosa* isolates in the present study. Although only two isolates demonstrated MBL production, the finding is clinically important because carbapenem-resistant *Pseudomonas aeruginosa* is associated with limited therapeutic options, MDR, prolonged hospitalisation, and treatment failure.

Therapeutic options for such infections may include polymyxins, aminoglycosides or combination therapy guided by antimicrobial susceptibility testing. Appropriate surgical management, including debridement and possible implant removal, also remains essential in the management of implant-associated infections caused by resistant organisms.

Overall, the findings of the present study highlight the importance of early microbiological diagnosis, routine antimicrobial susceptibility testing, and strict antibiotic stewardship to optimise the management of OIAIs.

The present study provides valuable institutional data on the bacterial profile and AMR patterns associated with orthopaedic implant-related infections in a tertiary care setting. A major strength of the study was the comprehensive phenotypic characterisation of bacterial isolates, including the detection of important resistance mechanisms such as MRSA, ESBL, AmpC, and MBL production. Additionally, the study correlates microbial isolates with implant type, offering clinically relevant insights for infection control and management.

Limitation(s)

The study had certain limitations. It was conducted at a single tertiary care centre with a relatively small sample size, which may limit the generalisability of the findings. Furthermore, resistance mechanisms were identified solely using phenotypic methods and molecular characterisation was not performed. Culture-negative infections, possibly due to prior antibiotic therapy or the presence of fastidious organisms, may also have led to underestimation of certain pathogens. Organism-specific antibiograms for some bacterial species could not be comprehensively analysed because of limited isolate numbers and non-routine testing of certain antimicrobial agents during the study period. Biofilm detection and molecular characterisation of resistance genes were not performed and represent areas for future research.

CONCLUSION(S)

The OIAIs remain a significant clinical challenge due to their complex microbiological profile and rising AMR. This study found that infections predominantly affected younger males, with a high culture positivity rate supporting the importance of early microbiological diagnosis. Gram-negative organisms predominated overall; however, *Staphylococcus aureus* was the most common individual isolate, underscoring its key role in implant-related infections. External implants were associated with a higher infection burden, particularly among external fixators and pin-tract implants.

A notable finding was the high prevalence of MDR, including MRSA among staphylococci and ESBL and AmpC-producing Gram-negative bacilli, along with significant resistance to commonly used β-lactam antibiotics. Preserved susceptibility to vancomycin and linezolid among Gram-positive isolates highlights their continued therapeutic importance, though cautious use is warranted. Overall, the study emphasises the need for routine culture and susceptibility testing, continuous resistance surveillance, and antibiogram-guided therapy to improve outcomes and inform institutional antibiotic policies.

REFERENCES

[1] Vasoo S. Improving the diagnosis of orthopaedic implant-associated infections: Optimizing the use of tools already in the box. J Clin Microbiol. 2018;56(12):10-128.
[2] Cuérel C, Abrassart S, Billières J, Andrey D, Suva D, Dubois-Ferrière V, et al. Clinical and epidemiological differences between implant-associated and implant-free orthopaedic infections. Eur J Orthop Surg Traumatol. 2017;27(2):229-31.
[3] Rodríguez-Merchán EC, Davidson DJ, Liddle AD. Recent strategies to combat infections from biofilm-forming bacteria on orthopaedic implants. Int J Mol Sci. 2021;22(19):10243.
[4] Oliveira WF, Silva PM, Silva RC, Silva GM, Machado G, Coelho LC, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* infections on implants. J Hosp Infect. 2018;98(2):111-17.
[5] Lakhani A, Jindal K, Khatri K. Antimicrobial resistance (AMR) in orthopaedic surgeries: A complex issue and global threat. J Ortho Rep. 2025;4(4):100466.
[6] Depypere M, Morgenstern M, Kuehl R, Senneville E, Moriarty TF, Obremskey WT, et al. Pathogenesis and management of fracture-related infection. Clin Microbiol Infect. 2020;26(5):572-78.
[7] Vaz K, Taylor A, Kendrick B, Alvand A. A guide to debridement, antibiotics, and implant retention. Ann Joint. 2022;7:5.
[8] Abbas S, Yasmin A, Maqbool N, Shah AA, Fariq A. Insights into the microbiological and virulence characteristics of bacteria in orthopaedic implant infections: A study from Pakistan. PLoS One. 2023;18(10):e0292956.
[9] Shakthi R, Venkatesha D. Detection of biofilm production and antibiotic resistance pattern in clinical isolates from orthopaedic implants associated infections. J Med Sci Health. 2023;9(2):157-62. Doi: 10.46347/jmsh.v9i2.23.5
[10] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2024.
[11] Li C, Renz N, Trampuz A, Ojeda-Thies C. Twenty common errors in the diagnosis and treatment of periprosthetic joint infection. Int Orthop. 2020;44:3-14.
[12] Benazir S, Kakru D, Khurshid S, Bhat A, Nazir U, Nazir S, et al. Identification, antibiotic susceptibility patterns and biofilm detection of isolates in orthopaedic implant infections. J Adv Medi Med Res. 2018;25(1):1-12.
[13] Sarkar K, Mullan S, Menon H. Exploring drug resistance: Microbial profiles, antibiotic sensitivity, and biofilm development in orthopaedic implant infections. Cureus. 2024;16(7):e70938.
[14] Jyoti, Golia S, Manasa SS. A study on bacteriological spectrum of post-operative orthopaedic implant infections and their antibiotic sensitivity pattern in a tertiary care hospital. J Med Sci Clin Res. 2017;5(4):01-08.
[15] Kaufman MG, Meaike JD, Izaddoost S. Orthopaedic prosthetic infections: Diagnosis and orthopaedic salvage. Semin Plast Surg. 2016;30(2):66-72.
[16] Coraça-Huber DC, Kreidl L, Steixner S, Hinz M, Dammerer D, Fille M. Identification and morphological characterization of biofilms formed by strains causing infection in orthopaedic implants. Pathogens. 2020;9(8):649.
[17] Jain S, Chowdhury R, Datta M, Chowdhury G, Mukhopadhyay A. Characterization of the clonal profile of methicillin resistant Staphylococcus aureus isolated from patients with early post-operative orthopaedic implant based infections. Ann Clin Microbiol Antimicrob. 2019;18(1):01-09.

PARTICULARS OF CONTRIBUTORS:
1. Resident, Department of Microbiology, Jawaharlal Nehru Medical College, KAHER, Belagavi, Karnataka, India.
2. Professor, Department of Microbiology, Jawaharlal Nehru Medical College, KAHER, Belagavi, Karnataka, India.
3. Professor, Department of Orthopaedics, Jawaharlal Nehru Medical College, KAHER, Belagavi, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shailja Gautam,
Jawaharlal Nehru Medical College, KAHER, Nehru Nagar, Belagavi-590010, Karnataka, India.
E-mail: shailja.gautam2@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 04, 2026
- Manual Googling: Jul 02, 2026
- iThenticate Software: Jul 04, 2026 (5%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Mar 27, 2026**
Date of Peer Review: **Apr 22, 2026**
Date of Acceptance: **Jul 07, 2026**
Date of Publishing: **Aug 01, 2026**