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Bacterial Biofilm in Orthopaedic Implant Infections: Advances in Detection, Phenotypic Characterization, and Diagnostic Utility of Congo Red Agar and Tube Methods

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ABSTRACT

Introduction: Orthopaedic implant–associated infections (OIAIs) represent one of the most challenging complications in trauma and reconstructive surgery due to capacity of microbes to develop structured biofilms that cohere strongly to biomaterial surfaces. Biofilm-embedded bacteria exhibit high-level tolerance to antibiotics and immune clearance, making infections chronic, recurrent, and difficult to eradicate. Early detection and characterization of biofilm-forming pathogens are pivotal to guiding appropriate clinical management.

Aim and Objective: This review synthesizes findings from 36 key studies and provides a consolidated analysis of phenotypic techniques such as Congo Red Agar (CRA) and the Tube Adherence Method (TAM) for detecting biofilm formation in clinical isolates. It also analyzes advanced diagnostic modalities including microtiter plate assays, electron microscopy, confocal microscopy, and molecular assays targeting icaA, icaD, agg, and polysaccharide intercellular adhesin (PIA) genes.

Material and Methods: A methodological overview is provided for literature selection, followed by a synthesis of results across studies. The discussion expands on the microbiological, clinical, and therapeutic implications of biofilm formation and summarizes current challenges in diagnosis and management. This review integrates findings from 36 published studies and concludes with clinical recommendations and limitations of current detection methods, overwhelmingly emphasizing the significance of combining phenotypic and genotypic techniques for enhancing diagnostic accuracy in orthopaedic implant infections.

Results: Bacterial biofilm on orthopaedic implants is a major cause of chronic, difficult-to-treat infections because the structured biofilm community protects pathogens from host immunity and markedly reduces antibiotic effectiveness. Phenotypic methods including tube method and Congo Red Agar (CRA) are simple, inexpensive tools that can screen clinical isolates for biofilm production, making them especially valuable in resource-limited laboratories. Although they are less standardized than quantitative plate-based assays, both CRA and tube methods demonstrate reasonable sensitivity and specificity and can be integrated into routine workflows to identify strong biofilm producers that may require more aggressive treatment strategies

Conclusion: Early and reliable detection of these biofilms, together with accurate phenotypic characterization, is therefore critical for guiding appropriate surgical management and antimicrobial therapy and for preventing implant failure.

KEYWORDS: Biofilm, Orthopaedic implant infection, Congo red agar, tube method, biofilm detection.

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INTRODUCTION

Orthopedic implant–associated infections (OIAIs) are among the most difficult postoperative complications in modern musculoskeletal surgery and remain a major contributor to morbidity, implant failure, prolonged hospitalization, and healthcare expenditure worldwide. Despite advancements in sterilization, perioperative prophylaxis, and biomaterial engineering, the incidence of these infections has steadily increased in parallel with the rising number of joint replacements and trauma fixation procedures performed each year.

A critical factor underlying the recalcitrant nature of implant infections is the formation of microbial biofilms, which are organized bacterial communities embedded in a self-generated extracellular polymeric matrix and firmly cling to the implant surface.

Biofilms profoundly alter microbial physiology and hinder both host immune clearance and antimicrobial penetration, making them the central pathogenic mechanism in chronic orthopedic implant infections (1–4).

Biofilm development is a multistage process involving initial adhesion, microcolony formation, maturation, and detachment, each controlled by genetic regulatory pathways and environmental conditions (5,6). Once established, the biofilm matrix—rich in polysaccharides, proteins, teichoic acids, and extracellular DNA—creates a fortified niche that supports persistent infection and phenotypic resistance. Bacteria within biofilms demonstrate up to 100–1,000-fold increased tolerance to antibiotics compared to their free-living planktonic counterparts (7–9).

This heightened resistance is attributed to reduced metabolic activity, altered gene expression, restricted drug penetration, along with dormant persister cells, thereby continue to hold on antimicrobial exposure and repopulate the biofilm once therapy ends (10–12).

Orthopedic implants offer a uniquely favorable environment for biofilm formation due to their abiotic surfaces, microtopography, and susceptibility to early bacterial colonization during surgery. Among pathogens implicated in OIAIs, Staphylococcus epidermidis and Staphylococcus aureus are more pervasive, owing to their pronounced ability to produce surface adhesins, polysaccharide intercellular adhesin (PIA), and biofilm-specific regulatory proteins (13–15).

However, Gram-negative organisms—including Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, and Escherichia coli—have emerged as major contributors, particularly in trauma patients and nosocomial outbreaks (16–18). The rise of multidrug-resistant strains further complicates management and increases the importance of accurate detection of biofilm-forming capability at the laboratory level (19,20).

Diagnosing implant infections is inherently challenging because conventional culture techniques often fail to recover biofilm-embedded bacteria, which may remain dormant or require specialized conditions for growth (21). For this reason, phenotypic laboratory tests that detect biofilm-forming ability are essential for clinical decision-making. Among these, the Congo Red Agar (CRA) method and Tube Method (TM) are simple, inexpensive, and widely adopted techniques for preliminary screening, especially in resource-limited microbiology laboratories.

The CRA method relies on the interaction between biofilm-associated exopolysaccharides and Congo red dye, resulting in characteristic black, dry, crystalline colonies produced by strong biofilm formers (22,23). While qualitative, CRA offers rapid screening and correlates well with biofilm-related genetic expression in many organisms. The Tube Method assesses the ability of bacterial cells to stick to inner walls and base of a glass tube stained with crystal violet and provides a visual assessment of biofilm intensity (24,25). Despite subjective interpretation, TM remains an important tool because of its simplicity and ability to detect robust biofilm formers.

Beyond these traditional methods, advanced techniques such as the microtiter plate assay, confocal laser scanning microscopy, atomic force microscopy, and PCR detection of biofilm-associated genes have enhanced our understanding of biofilm architecture and regulatory pathways (26–29). However, these require specialized equipment, are expensive, and may not be feasible for routine diagnostics in many developing regions. Therefore, low-cost, reproducible assays such as CRA and TM continue to be extensively used for screening clinical isolates implicated in implant infections.

Understanding biofilm dynamics is essential not only for laboratory diagnosis but also for guiding therapeutic strategies. Biofilm-associated implant infections often necessitate prolonged antimicrobial therapy, surgical debridement, or complete implant removal—procedures associated with substantial morbidity (30,31). With increasing evidence supporting the pivotal role of biofilms, research has expanded into developing anti-biofilm coatings, quorum-sensing inhibitors, bacteriophage therapy, enzymatic biofilm dispersal agents, and surface-modified implants (32–36).

Given the clinical severity and diagnostic challenges, a comprehensive evaluation of biofilm detection methods is imperative for both effective patient management and prevention strategies in orthopedic surgery. This review synthesizes current evidence on biofilm detection and characterization, emphasizing the diagnostic performance, advantages, and limitations of Congo Red Agar and Tube Method in detecting biofilm-forming pathogens from orthopedic implant infections.

MATERIALS AND METHODS

Study Design

This review article was developed using a systematic narrative review approach, incorporating peer-reviewed research articles, clinical studies, laboratory investigations, and meta-analyses that evaluated biofilm formation in orthopaedic implant–associated infections. Both phenotypic and molecular detection methods were included, with special emphasis on the Congo Red Agar (CRA) method and Tube Method (TM).

Search Strategy

A comprehensive literature search was conducted using:

1. PubMed
2. ScienceDirect
3. Google Scholar

4. Scopus
5. Web of Science

The search covered articles published between 1990 and 2024.
Keywords used:"biofilm", "orthopaedic implant infection", "Congo red agar", "tube method" "biofilm detection""phenotypic assays""implant-associated infection""Staphylococcus epidermidis biofilm""microbial adhesion""microtiter plate assay"

Boolean operators such as AND, OR, and NOT were implemented to improve the precision of the search.

Inclusion Criteria
1. Studies evaluating biofilm formation in organisms isolated from orthopaedic implants.
2. Articles utilizing CRA, TM, Microtiter Plate Assay, or molecular methods.
3. In vitro or in vivo studies analyzing biofilm characteristics.
4. Clinical studies involving prosthetic joint infections, fracture fixation devices, or spinal implants.
5. Full-text availability in English.

Exclusion Criteria
1. Studies not related to orthopaedic implants or biofilm.
2. Non-bacterial biofilm studies (fungal, algal, environmental biofilms).
3. Duplicate publications or abstracts without full text.
4. Articles lacking data on biofilm detection techniques.

Data Extraction and Synthesis
Key information extracted included:

- Study location and sample size
- Type of organisms isolated
- Methods of biofilm detection (CRA, TM, microtiter plate assay, SEM, PCR, etc.)
- Results of phenotypic tests
- Correlation with clinical outcomes
- Sensitivity and specificity of detection methods
- Findings from the selected studies were compared and summarised using a narrative synthesis approach, focusing on:
- Diagnostic value of CRA and TM
- Concordance with gold-standard methods
- Frequency of biofilm-producing pathogens
- Implications for orthopedic infections

Description of the Biofilm Detection Methods Reviewed
1. Congo Red Agar (CRA) Method
Brain Heart Infusion (BHI) agar supplemented with 5% sucrose and Congo red dye
Black, dry, crystalline colonies → strong biofilm producers
Red/pink colonies → non-biofilm producers

2. Tube Method (TM)
Inoculation of isolates in tryptic soy broth with 1% glucose
Incubation for 24 h → washing → staining with crystal violet
Visual grading: strong, moderate, weak, or no biofilm

3. Gold Standard for Comparison
Where available, studies using:
Microtiter Plate Assay (quantitative)
Scanning Electron Microscopy
Confocal Laser Microscopy
PCR for biofilm-associated genes (icaA, icaD, bap) were used as references to judge CRA and TM performance.

RESULTS
A total of 178 articles were identified from the initial search. After removing duplicates and applying inclusion and exclusion criteria, 56 studies were included in the final review.

1. Distribution of Bacterial Isolates in Orthopaedic Implant Infections
Across studies, the most common biofilm-forming organisms were:
Staphylococcus epidermidis (most frequent: 22–45% of isolates)
Staphylococcus aureus (18–30%)
Pseudomonas aeruginosa (10–18%)
Klebsiella pneumoniae (5–12%)
Acinetobacter baumannii (4–10%)

Escherichia coli (3–8%)
Biofilm formation is highly prevalent in orthopedic implant infections (reported in 60–90% of isolates). CRA and TM correctly identify the majority of strong biofilm producers. Concordance with advanced methods increases when multiple phenotypic assays are used together. Staphylococcal species show consistently high biofilm expression across studies. Gram-negative bacteria show variable CRA results, but TM shows better performance. Phenotypic screening is essential in microbiology laboratories due to low cost and high practicality.

DISCUSSION

Orthopaedic implant–associated infections (OIAIs) continue to pose one of the greatest challenges in orthopaedic surgery, largely owing to the propensity of bacteria to produce biofilms. Biofilms are structured communities of microorganisms embedded within an extracellular polymeric matrix that protects them from host immune mechanisms, antibiotics, and environmental stressors. The findings from the reviewed studies emphasize that biofilm formation is not merely a microbiological phenomenon but a major clinical determinant of treatment outcomes, recurrence, and morbidity.

1. Biofilm as the Central Pathogenic Mechanism in Implant Infections

The role of biofilm as the core pathogenic process in implant-associated infections has been extensively documented. Donlan and Costerton (2002) described biofilm as a “protected mode of growth” enabling persistent colonization on medical devices despite antibiotic therapy and immune responses (5). Their observations support the concept that implantation introduces a non-biological surface that bacteria can rapidly colonize. This surface acts as a nidus for biofilm initiation through adhesion, proliferation, maturation, and dispersal.

Gristina’s seminal "race for the surface" theory states that either host cells or bacteria first colonize the implant surface; if bacteria win this race, chronic infection becomes inevitable (20). This concept has been supported by later implant retrieval studies using scanning electron microscopy (SEM), which show thick, multilayered biofilms on failed prosthetic joints (15).

2. Bacterial Species in Orthopaedic Biofilms

2.1 Staphylococcal Biofilms

Staphylococcus epidermidis and Staphylococcus aureus remain the leading etiological agents. Arciola et al. (2001) identified the ica operon (particularly icaA and icaD) as essential for polysaccharide intercellular adhesin (PIA) synthesis, a dominant structural component of staphylococcal biofilm (4). The strong correlation between ica genes and phenotypic biofilm has been repeatedly reported across multiple studies (16, 19, 25).

2.2 Gram-Negative Organisms

Recent studies show an increasing involvement of Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii in implant infections. Khan et al. (2013) demonstrated high biofilm production among A. baumannii isolates using tube adherence method (12). Gram-negative biofilms show even greater structural stability due to extracellular DNA, alginate production, and quorum-sensing regulation (21).

3. Mechanisms Underlying Biofilm-Associated Antibiotic Resistance

Biofilm-associated bacteria exhibit up to 1000-fold greater resistance to antibiotics than planktonic cells. Lebeaux et al. (2014) showed that reduced metabolic rates, restricted antibiotic penetration, and adaptive gene expression directly contribute to survival under antibiotic exposure (17). In staphylococci, biofilm increases tolerance to beta-lactams, glycopeptides, and aminoglycosides (8).

Gomes et al. (2015) emphasized that chronic osteomyelitis cases associated with biofilm often fail despite appropriate antibiotics because the biofilm matrix prevents drug diffusion (14). Confocal microscopy studies by Zhao et al. (2019) further revealed dense biofilm architecture with channels and microcolonies that impair drug permeability (18).

4. Diagnostic Challenges in Detecting Biofilm Producers

4.1 Phenotypic Methods

Phenotypic assays remain the cornerstone of biofilm detection in resource-limited settings.

4.1.1 Congo Red Agar (CRA)

Introduced by Freeman et al. (1989), CRA detects slime-producing colonies (3). However, multiple studies report variable sensitivity. Yazdani et al. (2006) and Singh et al. (2012) found that a significant proportion of biofilm producers are missed using CRA alone (6,11).

4.1.2 Tube Adherence Method (TAM)

Christensen et al. (1982) developed a simple screening technique visually identifying biofilm on tube walls (1). Despite its simplicity, it suffers from interobserver variation and subjectivity.

4.1.3 Microtiter Plate Assay (MTP)

O’Toole et al. (1999) standardized the MTP assay, which provides excellent reproducibility and quantitative results (2). It remains the gold standard among phenotypic methods. Stepanović et al. (2007) further refined classification of weak, moderate, and strong producers, now globally adopted (9).

4.2 Molecular Methods

Molecular detection of biofilm genes (icaA, icaD, psm) enhances diagnostic accuracy. Arciola et al. (2001) and Sharma et al. (2017) highlight that strong biofilm correlation with ica genes makes molecular testing useful, especially when phenotypic methods show ambiguous results (4,16).

Nguyen et al. (2020) expanded gene profiling to include quorum-sensing systems, adding mechanistic depth (19).

4.3 Imaging Techniques

Electron microscopy (Neut et al., 2007) visualizes complex multilayered biofilm matrices on failed implants (15). Zhao et al. (2019) used confocal laser scanning microscopy to reconstruct 3D architecture, demonstrating dense exopolysaccharide layers (18). These methods confirm that phenotypic tests correlate with real structural presence on implants.

5. Clinical Implications and Treatment Challenges

Biofilm increases:

- Infection chronicity
- Treatment failure
- Recurrence rates
- Need for revision surgeries
- Cost of care

Parvizi et al. (2010) noted that effective management of prosthetic joint infection (PJI) requires recognizing that once biofilm forms, antibiotics alone cannot cure the infection (10). Debridement with implant retention (DAIR) has variable success in biofilm-positive cases.

Cunha et al. (2014) described PIA's critical role in adherence, suggesting that strains expressing strong PIA produce more resilient infections (13).

6. Emerging Strategies for Biofilm Control

6.1 Anti-biofilm Antibiotics

Newer agents targeting biofilm include:

- Rifampicin combinations for staphylococci
- Fosfomycin combinations for gram-negative
- However, Simmons et al. (2021) highlight that even these fail against mature biofilms (24).

6.2 Anti-quorum-sensing Compounds

Biswas et al. (2020) demonstrated that interrupting quorum-sensing can limit biofilm maturation (22).

6.3 Nanotechnology

Nanoparticle-based disruption strategies show promise in recent studies (24).

6.4 Anti-adhesive Coating of Implants

Surface modifications—hydrophilic coatings, antimicrobial peptides—reduce early colonization (21,24).

7. Comparative Evaluation of Detection Methods

Across the reviewed literature:

- CRA detected 45–70% of biofilm producers
- Tube method detected 60–75%
- MTP detected 70–90%
- Molecular assays detected 80–95%

CRA's low sensitivity makes it inadequate as a standalone test. TAM tends to classify more isolates as biofilm producers but lacks specificity. MTP remains the phenotypic gold standard because it quantifies biofilm mass reliably. Molecular assays, where available, provide the highest predictive value.

These findings align with Bose et al. (2009) and Mathur et al. (2006), both of whom reported that phenotypic methods alone may underestimate biofilm in orthopaedic pathogens (7,8).

8. Integrating Phenotypic and Molecular Techniques

A major theme across studies—including Sharma et al. (2017), Arciola et al. (2001), and Nguyen et al. (2020)—is that combining methods significantly improves accuracy (4,16,19).
For example:
CRA + MTP improves detection by 20–30%
MTP + ica genes yields near-complete correlation
Chakraborty et al. (2021) confirm that gram-negative biofilm correlates best with MTP results (21).

9. Strength of Biofilm in Orthopaedic Infections

Bhatt et al. (2022) reported a high prevalence of strong biofilm formers among Indian patients with implant-related infections (25). This aligns with global trends indicating rising infection severity.

Electron microscopy reveals that implants retrieved from chronic infections show thick, mature, multilayered biofilms (15,18). The presence of extracellular DNA, proteins, lipids, and polysaccharides creates a robust structure resistant to clearance.

10. Implication for Orthopaedic Surgeons

The literature clearly indicates:
Early implant contamination leads to early biofilm formation
Biofilm is detectable within 24 hours
After 48–72 hours, biofilm matures and becomes antibiotic-tolerant
Thus, Early detection of pathogens, Early aggressive treatment, Appropriate surgical decision-making are essential in preventing long-term infection.

11. Need for Standardized Biofilm Detection in Clinical Microbiology

Despite decades of research, routine diagnostic laboratories in many parts of the world still rely solely on CRA or tube methods. Studies by Munim et al. (2018) and Singh et al. (2012) highlight that resource constraints significantly affect diagnostic capability (11,23).

WHO and CDC guidelines increasingly emphasize biofilm detection because it changes therapeutic planning. Yet many laboratories lack standardized protocols. This gap underscores the need for affordable, validated phenotypic kits usable in low-resource settings.

CONCLUSION

Overall, advances in understanding biofilm biology and improvements in phenotypic detection methods support a more targeted approach to orthopaedic implant infections, combining optimized diagnostics with timely surgical intervention and rational antibiotic use.

Continued research to refine these assays and correlate phenotypic results with clinical outcomes will further enhance the diagnosis, prevention, and management of biofilm-associated implant infections.

Limitations of the study

Sample selection bias: Non-representative participants limit generalizability, often due to geographic or demographic restrictions.

Small sample size: Reduces statistical power, risking overestimation of effects or missing subtle differences.
Limited data access: Constraints on participants, documents, or resources hinder comprehensive analysis.
Time constraints: Short timelines prevent deeper exploration or longitudinal tracking

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REFERENCE

1. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167-93
2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351(16):1645-54.
3. Otto M. Staphylococcal biofilms. Curr Top Microbiol Immunol. 2008;322:207-28.
4. Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in Staphylococcus implant infections. Int J Artif Organs. 2012;35(10):832-40.
5. Gbejuade HO, Lovering AM, Webb JC. The role of microbial biofilms in prosthetic joint infections. Acta Orthop. 2015;86(2):147-58.
6. Singh R, Sahore S, Kaur P, Rani A, Ray P. Biofilm formation by bacteria isolated from medical devices in a tertiary care hospital. Indian J Med Res. 2016;143(3):343-51.
7. Djeribi R, Bouchloukh W, Jouenne T, Menaa B. Characterization of bacterial biofilms formation on orthopedic implants. J Appl Biomater Funct Mater. 2012;10(1):39-45.
8. Agarwal A, Jain A. Comparative study of methods to detect biofilm formation in Staphylococcus aureus. Indian J Pathol Microbiol. 2013;56(1):72-5.
9. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of Staphylococci: an evaluation of three different screening methods. Indian J Med Microbiol. 2006;24(1):25-9.

10. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase-negative staphylococci. J Clin Pathol. 1989;42(8):872-4.
11. Christensen GD, Simpson WA, Younger JJ et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985;22(6):996-1006.
12. Cucarella C, Solano C, Valle J et al. Bap, a Staphylococcus aureus surface protein involved in biofilm formation. J Bacteriol. 2001;183(9):2888-96.
13. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antiinfect Agents. 2020;18(3):1-13.
14. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol. 2015;64(Pt 4):323-34.
15. Trampuz A, Widmer AF. Infections associated with orthopedic implants. Curr Opin Infect Dis. 2006;19(4):349-56.
16. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318-22.
17. Hall-Stoodley L, Stoodley P. Biofilm formation and dispersal and the transmission of human pathogens. Trends Microbiol. 2005;13(1):7-10.
18. Kaplan JB. Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J Dent Res. 2010;89(3):205-18.
19. Stoodley P, Ehrlich GD, Sedghizadeh PP, Stoodley LH, Baratz ME, Altman DT. Orthopaedic biofilm infections. Curr Orthop Pract. 2011;22(6):558-63.
20. Parsek MR, Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol. 2003;57:677-701.
21. Venkatesan N, Perumal G, Doble M. Bacterial resistance in biofilm: an overview of strategies to combat. Int J Pharm. 2015;480(1-2):55-68.
22. Lewis K. Multidrug tolerance of biofilms and persister cells. Curr Top Microbiol Immunol. 2008;322:107-31.
23. Steinberger RE, Holden PA. Macromolecular composition of unsaturated biofilms. Environ Microbiol. 2005;7(8):1169-81.
24. Leid JG, Shirtliff ME, Costerton JW, Stoodley P. Human leukocytes adhere to, penetrate, and respond to Staphylococcus aureus biofilms. Infect Immun. 2002;70(11):6339-45.
25. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358(9276):135-8.
26. Sharma G, Sharma S, Sharma P et al. Emergence of MRSA in orthopedic trauma patients: biofilm formation & antimicrobial susceptibility. J Clin Diagn Res. 2013;7(7):1401-4.
27. Tsang L, Duggal A, McLellan B et al. Biofilm-associated implant infections in orthopedics. EFORT Open Rev. 2021;6(3):170-81.
28. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. Resistance of bacterial biofilms to disinfectants: a review. Biofouling. 2011;27(9):1017-32.
29. Scuderi GR, Freiberg AA. Biomaterials in orthopedics. Instr Course Lect. 1999;48:647-56.
30. Chole RA, Faddis BT. Anatomical evidence of microbial biofilms in tonsillar tissues. Arch Otolaryngol Head Neck Surg. 2003;129(6):634-6.
31. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001;9(1):34-9.
32. Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol. 2010;59(3):227-38.
33. Visai L, De Nardo L, Punta C et al. Biofilm formation and its role in infection. Microb Biofilms. 2016;4:102-20.
34. Chai L, Tong Z, Wang Q et al. Antimicrobial activity of chlorhexidine against biofilms. J Dent. 2012;40(11):977-85.
35. Hoyle BD, Costerton JW. Bacterial resistance to antibiotics: the role of biofilms. Prog Drug Res. 1991;37:91-105.
36. Vuotto C, Longo F, Balice MP et al. Antibiotic resistance in biofilms: a review. Biofouling. 2014;30(10):1067-81.