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Study the relationship between antibiotic resistance and the biofilm-forming ability of bacteria isolated from cases of osteomyelitis after surgery

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ABSTRACT

Background: Biofilms play an important role in protecting bacteria from antibiotics, which makes it difficult to treat and heal, in addition to the occurrence of bacterial resistance to antibiotics due to the long treatment period, in the case of bone inflammation after surgical operations. **Objectives:** One of the most important objectives of this study is to investigate the correlation between antibiotic resistance and the ability to form biofilms of bacteria isolated from cases of osteomyelitis. **Methods:** Sixty osteomyelitis samples have been collected, the ages of patients included in this study are between (7-70) years old, among whom 11(18.34%) were females and 49(81.66%) were males. Three types of culture media were used for primary isolation (nutrient agar, MacConkey agar, and blood agar). **Results:** The results of the initial isolation showed that 56 samples out of 60 gave positive results for the bacterial infection, with 67 bacterial isolates. The ability of the isolates to form biofilms was evaluated in several ways, the first method on medium Congo Red Agar, the second by Microtiter Plate Method, and the third by Scanning Electron Microscope technology. The percentages were for Congo Red Agar (62.5% strong, 25% moderate, and 12.5% mild) and by the Microtiter Plate Method (50% strong, 25% moderate, 25% mild). By Scanning Electron Microscope technology, biofilm formation steps were proven by examining the biofilm of *Escherichia coli*. The aggregation of bacteria forming biofilms was confirmed by staining the bacterial swabs. Biofilm-specific gene (ica_C) and (Fim_H) for *S. aureus* and *E. coli* respectively. Azithromycin resistance gene sequences (msr_A) for *S. aureus* and (mph_A) for *E. coli*. The results showed that there was a 100% correlation between the two types of genes in both types of bacteria. **Conclusion:** Osteomyelitis is considered a multifaceted infection, because the bacterial species that make up the biofilms are diverse, which leads to their resistance to a large number of antibiotics.

Introduction

In chronic infections, bacterial biofilms pose a particularly serious problem. The biofilm matrix is one of the things antibiotics cannot penetrate making it difficult to deal with these types of infections; thus, higher dosages and extended periods are given for treatment. Device-related infections are associated with biofilms too, such as

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those caused by catheters, prosthetic joints, and heart valves, Abebe states that biofilms cause 80% of all microbial infections in the body thereby emphasizing their clinical significance [1].

The formation of biofilms often accompanies osteomyelitis a severe bone infection. This condition can be brought about by bacteria entering the bone tissue through the bloodstream, from an adjacent infection, or directly through an open fracture or surgical procedure. Consequently, bacteria can build up within bones to become biofilms hence rendering such an infection very difficult in treatment terms. In this regard, biofilm provides a way for microorganisms to evade our immune system reactions and also counter drug actions; thus, resulting in continuous inflammation and the persistent presence of bacteria [2].

Developmental stages in the occurrence of biofilms in osteomyelitis begin by affixing the bacteria initially on the bone tissue or implanted medical devices. Later, they multiply and form an Extracellular Polymeric Substances (EPS) matrix that covers bacterial cells holding them onto the surface. With time, biofilms develop into elaborate structures having nutrient channels for bacterial growth and communication. Consequently, this maturation increases resistance and virulence of the microbial population complicating treatment interventions. The understanding of biofilm development dynamics and its relevance to disease chronicity is important in fostering a better treatment for infections caused by biofilms such as osteomyelitis [3]. Ongoing research in this area is mainly focused on devising new strategies to prevent the formation of biofilms as well as improving the performance of already available therapies

Material and methods

Several methods have been adopted to verify the ability of bacterial isolates to form biofilms, including, to prove the aggregation of bacterial cells using the differential Gram stain, was done based on the method [4], the Congo Red Method was done based on the method the Microtiter Plate Biofilm Production Assay [5]. Scanning Electron Microscope (SEM) to detect the steps of bacterial biofilm formation and a genetic study was conducted to prove the relationship between biofilm formation and antibiotic resistance. To achieve this, two osteomyelitis isolates were used: *E. coli* and *S. aureus*. Biofilm-specific gene (ica_C) and (Fim_H) for *S. aureus* and *E. coli* respectively. Azithromycin antibiotic resistance gene (msr_A) for *S. aureus* and (mph_A) for *E. coli* as shown in **Tables 1 & 2**.

Results

The results showed that out of 60 cases of osteomyelitis, 56(93.34%) samples gave positive results for the bacterial infection, out of 56 positive osteomyelitis samples, 67 bacterial strains were isolated that were diagnosed with the Vitek 2 compact, as in **Figure 1**.

The results of the initial isolation showed in **Figure 1** that the highest percentage of isolation was for *Staphylococcus aureus* 22 isolates (32.8%); followed by *Pseudomonas aeruginosa* 13 isolates (19.4%) and then *Enterobacter cloacae* 5 isolates (7.5%) *Klebsiella pneumoniae* 4 isolates (5.9%); *Escherichia coli* 4 isolates (5.9%); *Proteus mirabilis* 3 isolates (4.5%); *Streptococcus viridans* 2 isolates (3%); *Corynebacterium* spp 2 isolates (3%), as for the following bacterial isolates, each of them constituted one isolate out of a total of 67 isolates, at a rate of 1.5% for each. The diagnosis was confirmed by molecular diagnosis by sequencing the 16S rRNA gene for seven isolates, and 6 isolates were registered with the National Center for Biotechnology Information (NCBI) because the percentage identity was between (99.83% - 100%) with the following names: *Morganella morganii* PP508217.1 (AST1); two isolates of *Corynebacterium striatum* PP508222.1 (AST2) and PP508248.1 (AST7); *Pseudomonas aeruginosa* PP508238.1 (AST3) ; *Escherichia coli* PP508239.1 (AST4) ; *Staphylococcus aureus*PP508249.1(AST6).

The number and percentage of Gram-negative and positive isolates out of the total isolates were 38 (56.71%) and 29 (43.28%), respectively.

Biofilm formation is a process where bacteria bind to surfaces and produce sticky (EPS) **(Figure 2)** that ease their attachment and create a shielding matrix.

The ability of the isolates to form biofilms was evaluated in several methods.

The first method is on medium Congo Red Agar the second by Microtiter Plate Method **(Figure 3)**.

The results showed that all isolates could form biofilms, but with different strengths, classified as (strong, moderate, and mild). The percentages were for Congo Red Agar (62.5% strong, 25% moderate, and 12.5% mild) and by the

Microtiter Plate Method (50% strong, 25% moderate, 25% mild).

Finally, the steps for biofilm formation were determined using Scanning Electron Microscopy (SEM) technology by selecting *Escherichia coli* isolated from cases of osteomyelitis, which gave strong positive results for the formation of biofilms using the Microtiter plate method and biofilm formation on Congo red agar. The SEM pictures, showed the steps for biofilm formation for these bacteria.

Detection of fim_H and mph_A genes in *E. coli*. The fim_H gene is one of the genes responsible for the adhesion of *E. coli* to epithelial cells in the human body and plays an important role in increasing its ability to colonize and invade host tissues, and the mph_A gene is responsible for resistance to azithromycin. The results showed that the bacteria under study possess the fim_H gene at 504 bp while for the mph_A gene, we found it at 500 bp. as shown in **(Figure 4)**.

Detection of mrc_A and ica_C Genes in *S. aurues*. The results showed that the bacteria under study possess the ica_C gene (for biofilm) at 198 bp. and, the mrc_A gene (for resistance to azithromycin) at 202 bp. **(Figure 5)**.

Table 1. Primers of (Fim_H and mph_A) genes used for amplification in *Escherichia coli.*

Primers	Function	Primer sequence	Size of product (bp)	Reference
FimH-F	**Biofilm formation**	**GAGAAGAGGTTTGATTTAACTTATTG**	**559**	[6]
FimH-R		**AGAGCCGCTGTAGAACTGAGG**		
mphA-F	**Azithromycin resistance**	**GTGAGGAGGAGCTTCGCGAG**	**403**	[6,7]
mphA-R		**TGCCGCAGGACTCGGAGGTC**		

Table 2. Primers of (ica_C and msr_A) used for amplification in *Staphylococcus aureus.*

Primers	Function	Primer sequence	Size of product (bp)	Reference
icaC-F	**Biofilm formation**	**CTTGGGTATTTGCACGCATT**	**209**	[8]
icaC-R		**GCAATATCATGCCGACACCT**		
msrA-F	**Azithromycin resistance**	**TCCAATCATTGCACAAAATC**	**163**	[9]

Figure 1. Percentages and numbers of bacteria isolated from osteomyelitis cases.

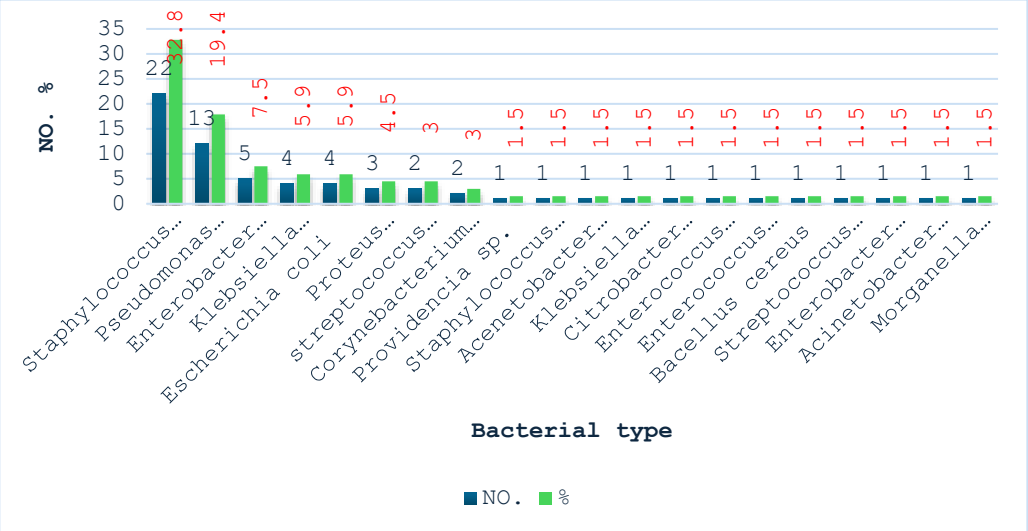


Figure 2. The picture shows the Hypermucoviscous (HMV) of *Escherichia coli*. HMV strains can be pulled into a strand more than 5mm from the MacConkey agar plate.

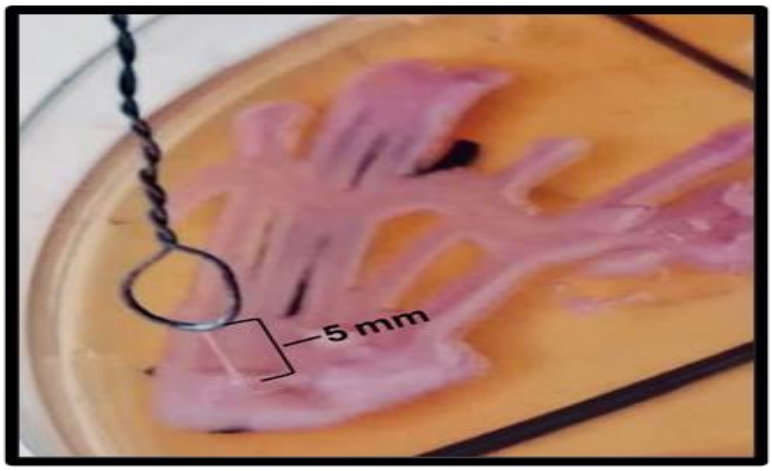


Figure 3. Biofilm assay for some bacterial isolates from osteomyelitis cases using 96-well microtiter plate Method.

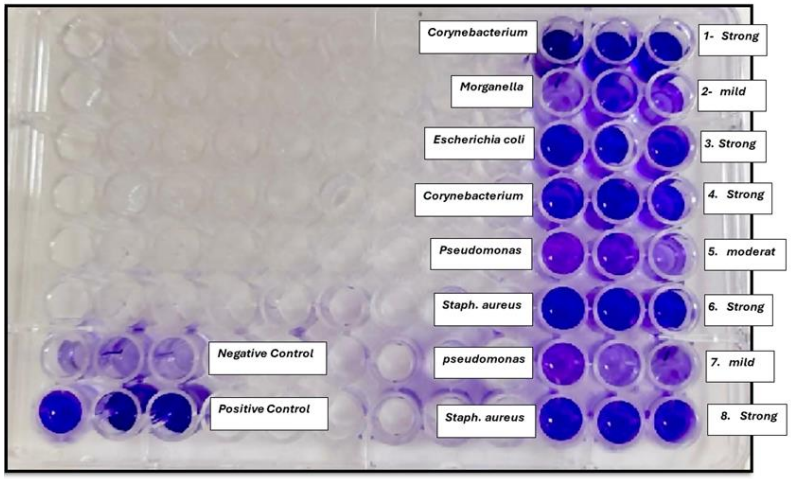


Figure 4. *mph*$_A$ 500 bp. and *Fim*$_H$ 504 bp. genes in *E. coli*

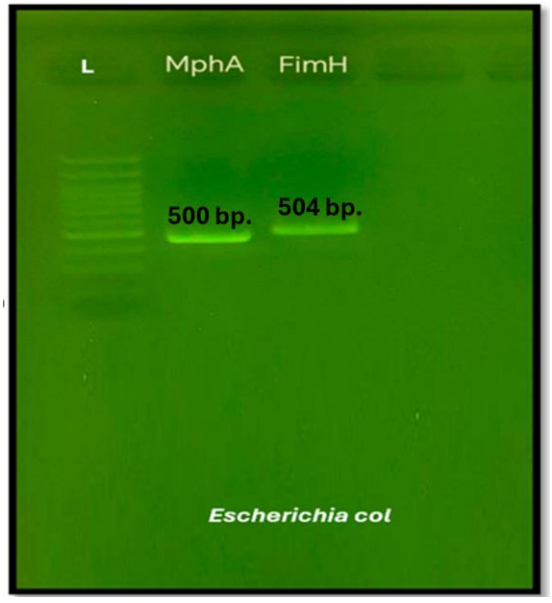
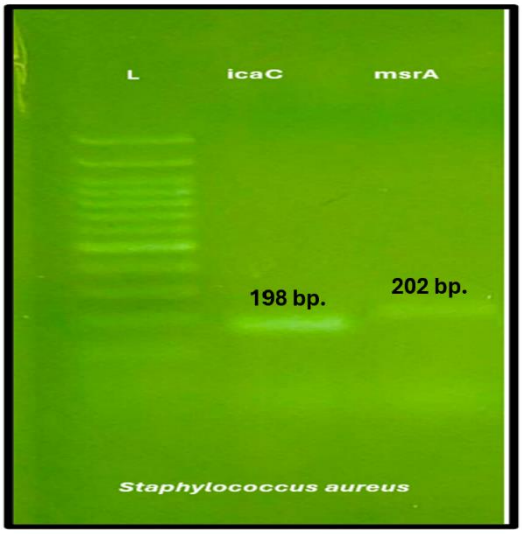


Figure 5. *mrs*$_A$ 202 bp. and *ica*$_C$ 198 bp. genes in *S. aureus*.



Discussion

Biofilm formation by bacteria is a crucial factor in the pathogenesis of many chronic and recurrent infections, including osteomyelitis. Biofilms are structured communities of bacteria encapsulated within a self-produced extracellular polymeric substance (EPS) matrix and nucleic acids. It protects from environmental threats, such as antibiotics and the host immune system, and facilitates bacterial survival and persistence [10, 11].

Osteomyelitis, an infection of the bone, can be caused by various types of bacteria. The specific pathogens involved can vary based on the patient's age, underlying health conditions, the route of infection, and the site of infection.

This was almost similar to the results of some studies, as several types of bacteria were isolated from cases of osteomyelitis in different proportions [12].

Osteomyelitis is mainly caused by *S. aureus* among the very many types of bacteria that can cause it because of its adhesion to bone and implants. If this occurs, *S. aureus* will then adhere to the bone tissue and medical implants like prosthetics through its surface adhesins with proteins such as MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules), which allow it to bind itself to certain bone matrix and implant components. *S. aureus* generally resides in human nasal passages and on human skin. Its normal flora presence increases the likelihood of it entering the bloodstream or wounds, causing infections like osteomyelitis [13].

Biofilm-related osteomyelitis, a bacterial infection, also upregulates particular virulence factors that further enhance the severity of the disease. For instance, *S. aureus* produces different adhesins like fibronectin-binding proteins that help in the initial attachment to bone tissue and medical devices; Besides, it makes toxins such as alpha-toxin and phenol-soluble modules which directly damage bone cells, alter the host's immune response, and worsen the infection while enabling its spread into tissues and hiding from immunity [14]. It can produce molecules that hamper phagocytosis, scavenge oxygen free radicals, and undermine normal immune processes thus allowing its colonization in osteoblasts –bone-producing cells – where it remains protected from drugs or the host's body defense mechanisms particularly *MRSA* which is the commonest form of resistance to several antibiotics resulting in protracted infections that are difficult to treat. This intracellular survival contributes to chronic and recurrent infections [15].

Unlike what was previously thought, the instances of Gram-negative infections turned out to be higher, majority of contiguous chronic osteomyelitis lesions were polymicrobial in nature but solitary organisms predominantly Gram-negative with *P.aeruginosa*. Although less frequently than *S. aureus*, *P. aeruginosa* is a causative agent of osteomyelitis. The pathogenicity of *P. aeruginosa* osteomyelitis is characterized by virulence factors such as exotoxin A, enzymes (such

as elastase and alkaline protease), or other molecules that disrupt host tissues and defenses. And like *S. aureus, P. aeruginosa* can generate biofilms too [16]. Intrinsic resistance to many antibiotics and the ability to pick up additional resistance mechanisms are characteristics of the *P. aeruginosa* species. This resistance makes treatment of *pseudomonas* osteomyelitis particularly challenging, making it a chronic infection and often necessitates combination antibiotic therapy [17].

To investigate and evaluate the biofilm formation, biofilm swabs were made and stained with differential Gram stain, Congo Red Agar, microtiter plate Method, and scanning electron microscopy. All methods showed effectiveness in diagnosing biofilm formation, as in Figures (2, 3, 4&5). This study revealed that a greater percentage of clinical isolates from individuals with osteomyelitis produced biofilm. The microtiter plates revealed better sensitivity and specificity values than other phenotypic methods, such as Congo Red Agar; these findings resemble those of other studies [18,19]. Static observation of dynamic processes at vast microscale resolution is the most important aspect of Scanning Electron Microscopy (SEM), which has a high resolution. It allows for the examination of three-dimensional structures [20]. In our research, we focused on examining the *E. coli* formed biofilms, where different magnification powers were used in taking numerous pictures. These images depict various stages during *E. coli* development and maturation of its biofilm.

These steps cause antibiotic resistance, immune evasion, the occurrence of chronic infection, and also recurrence of infection as a whole.

Physical Barriers: It is an extracellular polymeric substance (EPS) matrix that acts as a physical barrier to antibiotics in the biofilm. Altered Microenvironment; Biofilms generate nutrient and oxygen gradients that create a non-uniform environment where some cells grow slowly or become dormant. These are called “persisters” which are typically less sensitive than bacteria actively replicating targeted by antibiotics [21].

The formation of biofilm by *E. coli* in bacterial infections is highly significant due to different reasons: Antibiotic resistance, immune evasion, increased virulence, and diagnostic and treatment challenges [22]. At last, all these bring about chronic infections whose essence is how hazardous they are to any bacterial infection because it can be hard to handle but even if managed, recurrence of infection may arise [23].

Moreover, type 1 fimbriae and P fimbriae, which aid in its initial binding on human host tissues and biofilm making, are among the adhesins that these bacteria synthesize [24,25]. In addition to this, enterotoxigenic *E. coli* secretes siderophores that bind iron from the host; thus, depriving it of essential nutrients for bacterial multiplication and biofilm production [26]. This will make the infection worse by direct tissue destruction and inflammation due to the release of exotoxins like hemolysins [27,28].

Thus, for instance, in osteomyelitis infections caused by *E. coli,* a kind of biological defense mechanism known as biofilm formation has led to the emergence of drug-resistant strains. This is because typical antibiotic therapies usually fail in such cases where drugs are aimed at killing bacteria within a slime matrix [29]. These strategies include developing biofilm-disrupting agents; employing combination antimicrobial therapy targeting different groups of microbes residing within a slime matrix, as well as bacteriophages widely known to infiltrate into biofilms and destroy bacterial cells through the lysis process [30].

Biofilm formation by bacteria has been shown in many studies, and this study plays an important role in bacterial infections for several reasons, such as antibiotic resistance, immune evasion, increased virulence diagnostics, and treatment challenges [22,23].

For this reason, genetic tests were done to prove the relationship between biofilm formation and antibiotic resistance. To achieve this goal, gene sequencing was performed on the bacterium *S. aureus,* which is a representative of Gram-positive bacteria, and *E. coli,* which is a representative of Gram-negative bacteria. Ica_{C} and fim_{H} genes are specific for biofilms in *S. aureus* and *E. coli,* respectively. Azithromycin-resistant gene sequences, msr_{A} for *S. aureus* and phm_{A} for *E. coli,* the findings demonstrated that there was a 100% correlation between the gene types in these two types of bacteria [31].

The highly adherent *E. coli* isolate, as seen in SEM photographs, explains why it possesses the fim_{H} gene, thereby demonstrating type 1 fimbriae functions on colonization and infection by other

adhesions and virulence agents in uropathogenic *E. coli* [32].

Our research discovered two genes, *mph*$_A$ and *mph*$_B$, in *E. coli* that mediate macrolide resistance, with the former playing a role in its expression of resistance to azithromycin [33]. Our results were also close to those of Kumar and his group **Q4**, who found out that among their thirty isolates obtained during a years period from 2016-2018, twenty-six isolates could resist azithromycin [34].

Methicillin-resistant *S. aureus* (*MRSA*) is important in public health, as it has high virulence and antibiotic-resistance properties thus causing death among humans through hospital infections [35]. The *S. aureus* carries three genes that code for methionine sulfoxide reductase A specific for *msr*$_A$. resistant strains of antimicrobial factors are increasing worldwide due to the rising incidence of nosocomial bacteria. *Staphylococci* have become one of the common causes of nosocomial infections. MDR *Staphylococci* – a growing problem for human health [19].

Staphylococcus spp. biofilm formation process is driven by polysaccharides intracellular adhesins (PIA) which are all under the genetic control of operon ica ABCD [36].

According to studies, all three *ica* genes (*ica*$_A$, *ica*$_B$, and *ica*$_D$) were found in *S. aureus* with the capability to form biofilm while variation in the ability of bacteria to produce biofilm was observed in the case of the gene known as *ica*$_C$ whereby such got from about 56% samples. The various reasons accounted for differences in these genes' possession among bacteria encompassing factors like changes in physiological conditions affecting biofilm formation while the smallest proportion of this gene responsible for creating an adhesive substance was recorded as a percentage that reached as low as half or *icac*50.7% [37]. Out of 16 clinical *Staphylococcus* spp. Isolates have the *ica*$_A$ gene at 188 bp, and six isolates have the *ica*$_D$ gene at 198 bp. [38].

Conclusion

In conclusion, Osteomyelitis is a multifaceted infection with several causes and numerous related issues that complicate its treatment. This is because bacteria can form biofilms, which leads to multiple resistance against antibiotics. Genetic research has indicated that there is a link between antibiotic resistance and the development of biofilms. It follows that this calls for further investigations to devise therapeutic protocols to achieve optimal outcomes in the management of this intractable disease.

Conflict of interest

The authors declare that there is no conflict of interest.

Financial disclosures

This research has not received any funding.

Data availability

All data generated or analyzed during this study are included in this puplished article.

Authors' contribution

All authors made significant contributions to the work presented, including study design, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

References

1- Abebe GM. The Role of Bacterial Biofilm in Antibiotic Resistance and Food Contamination. Int. J. Micro. 2020. https://doi.org/10.1155/2020/1705814

2- Singh S, Tan CL, Ahmad AR. Explaining osteomyelitis and prosthetic joint infections (Pji) in terms of biofilm – a review. M.Ortho. J. 2021; 15(2): 1–8. https://doi.org/10.5704/MOJ.2107.001

3- Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. F.Cel.Infect. Microb. 2023.

4- Pratiwi SUT, & Hamzah H. Inhibition and degradation activity of (Sapindus rarak seeds) ethanol extract against polymicrobial biofilm. R. J. Pharm. Tec. 2020; 13(11): 5425–5430. https://doi.org/10.5958/0974-360X.2020.00947.6

5- Borah M, Maheswari D, Dutta HS. Fabrication of microtiter plate on paper using 96-well plates for wax stamping. Microfluidics and Nanofluidics 2022; 26(12): 1–9. https://doi.org/10.1007/s10404-022-02606-3

6- Johura FT, Tasnim J, Barman I, Biswas SR, Jubyda FT, Sultana M, et al. Colistin-resistant Escherichia coli carrying mcr-1 in food, water, hand rinse, and healthy human gut in Bangladesh. Gut Pathogens 2020; 12(1): 1–8. https://doi.org/10.1186/s13099-020-0345-2

7- Atshan SS, Hamat RA, Coolen MJL, Dykes G, Sekawi Z, Mullins BJ, et al. The role of subinhibitory concentrations of daptomycin and tigecycline in modulating virulence in Staphylococcus aureus. Antibiotics 2021; 10(1): 1–16. https://doi.org/10.3390/antibiotics10010039

8- Abd El-Razik KA, Soliman YA, Fouad EA, Arafa AA, Syame SM, Soror AH. Macrolides-resistant Staphylococcus aureus Associated with Clinical Mastitis in Cattle and Buffalo in Egypt. J. Ad.Vet. R. 2023; 13(3): 461–468.

9- Wang X, Liu M, Yu C, Li J, Zhou X. Biofilm formation: mechanistic insights and therapeutic targets. Mol. Bio. 2023; 4(1). https://doi.org/10.1186/s43556-023-00164-w

10- Kang HJ, Lee SH, Kim HS, Jung YW, Park HD. Rapid and sensitive detection of gram-negative bacteria using surface-immobilized polymyxin B. PLoS ONE 2023; 18(8 August): 1–13. https://doi.org/10.1371/journal.pone.0290579

11- Zhang X, Lu Q, Liu T, Li Z, Cai W. Bacterial resistance trends among intraoperative bone culture of chronic osteomyelitis in an affiliated hospital of South China for twelve years. BMC Infectious Diseases 2019; 19(1): 1–8. https://doi.org/10.1186/s12879-019-4460-y

12- Burke Ó, Zeden MS, O'Gara JP. The pathogenicity and virulence of the opportunistic pathogen Staphylococcus epidermidis. Virulence 2024; 15(1). https://doi.org/10.1080/21505594.2024.2359483

13- Schlievert PM, Kilgore SH, Beck LA, Yoshida T, Klingelhutz AJ, Leung DYM. Host Cationic Antimicrobial Molecules Inhibit S. aureus Exotoxin Production. MSphere 2023; 8(1): 1–10. https://doi.org/10.1128/msphere.00576-22.

14- Vestergaard M, Frees D, Ingmer H. Antibiotic resistance and the MRSA problem. Gram-Positive Pathogens 2019; 747–765. https://doi.org/10.1128/9781683670131.ch47.

15- Mulcahy LR, Isabella VM, Lewis K. Pseudomonas aeruginosa Biofilms in Disease. Microb. Eco. 2014; 68(1): 1–12. https://doi.org/10.1007/s00248-013-0297-x

16- Høiby N, Ciofu O, Bjarnsholt T. Pseudomonas aeruginosa biofilms in cystic fibrosis. F. Microb. 2010; 5(11): 1663–1674. https://doi.org/10.2217/fmb.10.125

17- Basnet A, Tamang B, Shrestha MR, Shrestha LB, Rai JR, Maharjan R, et al. Assessment of four in vitro phenotypic biofilm detection methods in relation to antimicrobial resistance in aerobic clinical bacterial isolates. PLoS ONE 2023; 18(11 November): 1–15.

18- Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. B. J. Infect. Dis. 2011; 15(4): 305–311. https://doi.org/10.1590/S1413-86702011000400002

19- Huang Y, Chakraborty S, Liang H. Methods to probe the formation of biofilms: Applications in foods and related surfaces. Analytical Methods 2020; 12(4): 416–432. https://doi.org/10.1039/c9ay02214g

20- Zou J, Peng B, Qu J, Zheng J. Are Bacterial Persisters Dormant Cells Only? F. Microb. 2022; 12(February): 1–10. https://doi.org/10.3389/fmicb.2021.708580

21- Cavallo I, Sivori F, Mastrofrancesco A, Abril E, Pontone M, Di Domenico EG, et al.

Bacterial Biofilm in Chronic Wounds and Possible Therapeutic Approaches. Biology 2024; 13(2): 1–19. https://doi.org/10.3390/biology13020109

22- Saaed A, & Sulaiman A. Identification of blaOxa23 Gene in Acinetobacter baumannii Isolated From Clinical and Hospitals Environment in Mosul City-Iraq. E. J. Vet. Sci. 2025; 56(7): 1457-1465. DOI: 10.21608/EJVS.2024.282669.2003

23- Yamanaka T, Yamane K, Mashimo C, Nambu T, Maruyama H, Leung KP, et al. Exopolysaccharide Productivity and Biofilm Phenotype on Oral Commensal Bacteria as Pathogenesis of Chronic Periodontitis. Patho. Treat. Peri. 2012. https://doi.org/10.5772/31034

24- Whelan S, Lucey B, Finn K. Uropathogenic Escherichia coli (UPEC)-Associated Urinary Tract Infections: The Molecular Basis for Challenges to Effective Treatment. Microorganisms 2023; 11(9). https://doi.org/10.3390/microorganisms11092169

25- Tsylents U, Burmistrz M, Wojciechowska M, Stępień J, Maj P, Trylska J. Iron uptake pathway of Escherichia coli as an entry route for peptide nucleic acids conjugated with a siderophore mimic. F. Microb. 2024; 15(January): 1–16. https://doi.org/10.3389/fmicb.2024.1331021

26- Ristow LC, & Welch RA. Hemolysin of uropathogenic Escherichia coli: A cloak or a dagger? Biochimica et Biophysica Acta – Biomembranes 2016; 1858(3): 538–545. https://doi.org/10.1016/j.bbamem.2015.08.015.

27- Pokharel P, Dhakal S, Dozois CM. The Diversity of Escherichia coli Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. Microorganisms 2023; 11(2). https://doi.org/10.3390/microorganisms11020344

28- Zafer MM, Mohamed GA, Ibrahim SRM, Ghosh S, Bornman C, Elfaky MA. Biofilm-mediated infections by multidrug-resistant microbes: a comprehensive exploration and forward perspectives. Archives of Microbiology 2024; 206(3): 1–23. https://doi.org/10.1007/s00203-023-03826-z

29- Gordon M, & Ramirez P. Efficacy and Experience of Bacteriophages in Biofilm-Related Infections. Antibiotics 2024; 13(2). https://doi.org/10.3390/antibiotics13020125

30- Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. In Microorganisms 2023;11(6). https://doi.org/10.3390/microorganisms11061614

31- Ali OA, & Taha RQ. Distribution of fimh gene in local isolates of adhesive uropathogenic Escherichia coli. International Journal of Drug Delivery Technology 2019; 9(3): 1–5. https://doi.org/10.25258/ijddt.v9i3.9

32- Jost C, Bidet P, Carrère T, Mariani-Kurkdjian P, Bonacorsi S. Susceptibility of enterohaemorrhagic Escherichia coli to azithromycin in France and analysis of resistance mechanisms. J. Anti. Chemo. 2016; 71(5): 1183–1187. https://doi.org/10.1093/jac/dkv477

33- Sulaiman AI, & Fadhil A. Effect of alcoholic extract of strawberries and green tea on Enterococcus faecalis isolated from urinary tract infections. Scientific Journal of King Faisal University: Basic and Applied Sciences

2024; 25(1): 69–73. DOI: 10.37575/b/sci/240007

34- Samuel P, Kumar YS, Suthakar BJ, Karawita J, Sunil Kumar D, Vedha V, et al. Methicillin-Resistant Staphylococcus aureus Colonization in Intensive Care and Burn Units: A Narrative Review. Cureus 2023; 15(10): e47139. https://doi.org/10.7759/cureus.47139

35- Ghaioumy R, Tabatabaeifar F, Mozafarinia K, Mianroodi AA, Isaei E, Morones-Ramírez J, et al. Biofilm formation and molecular analysis of intercellular adhesion gene cluster (Icaabcd) among Staphylococcus aureus strains isolated from children with adenoiditis. Irn. J. Microb. 2021; 13(4): 458–463. https://doi.org/10.18502/ijm.v13i4.6969

36- Abdelmoneim A, Ali R, Abdelhafeez A, Khallaf A, Molham FM, Tohamy Abdel-Aziz MAB. Detection of ica C gene involved in biofilm formation in methicillin-resistant Staphylococcus aureus (MRSA) isolates. E. J. Med. Res. 2023; 4(4): 63–77. https://doi.org/10.21608/ejmr.2023.218352.1408

37- Abdrabaa MK, & Aburesha RA. Assessment of ica A and D Genes in Biofilm producers' methicillin-resistant Staphylococcus aureus isolates. Iraqi Journal of Science 2023; 64(6): 2696–2706. https://doi.org/10.24996/ijs.2023.64.6.4

38- Zou J, Peng B, Qu J, Zheng J. Are Bacterial Persisters Dormant Cells Only? Frontiers in Microbiology 2022; 12(February): 1–10. https://doi.org/10.3389/fmicb.2021.708580

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Tariq TZ, Al-Sultan SAH, Sulaiman AI. Study the relationship between antibiotic resistance and the biofilm-forming ability of bacteria isolated from cases of osteomyelitis after surgery. Microbes Infect Dis 2025; 6(4): 6699-6708.