https://doi.org/10.48047/AFJBS.6.12.2024.899-914

African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com
Research Paper Open Access

EVALUATION OF BIOFILM DETECTION AND ANTIBIOTIC RESISTANCE PATTERN IN CLINICAL ISOLATES

NIDHI SINGH[1] Dr. NOOR JAHAN[2*]NEHA TIWARI[2]SNEHI SINGH[3] NIKITA MISHRA[4]

[1,2,3,4]PhD Scholar, Department of Microbiology, Integral Institute of Medical Science & Research,Lucknow, UP,India
Email: ns76278@gmail.com
[2*]Professor, Department of Microbiology,Integral Institute of Medical Science & Research, Lucknow, UP, India
Main Author- Miss Nidhi singh
Corresponding author- Nidhi singh
Other Authors- Dr. Noor Jahan, Neha Tiwari, SNehil Singh, Nikita Mishra

Article History
Volume 6 Issue 12, 2024
Received: 25 May 2024
Accepted : 25 June 2024
doi:
10.48047/AFJBS.6.12.2024.899-914

ABSTRACT:
BACKGROUND: Microorganisms adhere to surfaces, creating biofilms with polysaccharides, fostering drug-resistant pathogens. These biofilms on surfaces and implants lead to persistent infections. Our study aims to detect biofilm formation and their antibiotic susceptibility pattern, shedding light on the development of chronic and challenging infections.
MATERIAL METHODS: This cross-sectional study is carried out in the department of Microbiology Integral Institute of Medical Science & Research Lucknow. Totalsamples collected were 300 from various clinical samples and processed as per standard protocolsand identification of biofilm production was done using Congo Red Agar and Tube Method.
RESULTS: Out of 300 positive samples 142 were gram positive and 158 were gram negative. In this study most frequently isolated organism wasE.coli followed by CONS, Staphylococcus aureus, Klebsiella sp., Enterococcus sp. and Pseudomonas sp. Biofilm detection by CRA method and Tube Method shows the result 47.7% and 50.3% respectively.
Key Words:BP, CRA, NBP,Spp., TM

INTRODUCTION:

Biofilm constitute microbial communities wherein cells adhere to surfaces and are enveloped within a self-produced matrix commonly known as extra cellular polymeric matrix.[1]They poses major challenges in various fields both in medical settings such as persistent infection, recurring infection as well as medical device related infection and in non-medical settings like industrial environments, causing issues like biofouling in water systems and contamination in food processing.

Bacteria present in biofilm are stationary and drive most activities within the biofilm environment.[2] The stationary bacterial biofilm groups undergo various changes in growth, gene expression, and rates of transcription and translation. These traits develop as they adapt to environments with limited nutrients, increased cell density and higher osmolarity. As a result, the biofilm structure becomes highly elastic, displaying a rubbery behaviour.[3]Most common bacteria found in biofilm includeStaphylococcus epidermidis, Staphylococcus aureus,Streptococcus viridans,Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiellapneumoniae,and Proteus mirabilis.[4]Staphylococci are diverse group of Gram-positive bacteria commonly present on mucosa of mammals and their skin. These bacteria, making up about 80% of infections from implanted devices in humans, play a significant role.[5,6,7,8,9,10,11,12,13]According to a recent NIH study, 70% of human microbial infections arise from biofilms, contributing to various diseases like chronic wounds, osteomyelitis, periodontitis, meningitis, cystic rhinosinusitis,endocarditis, kidney infections, fibrosis, and infections related to prostheses and implantable devices.[5,14,15,16,17,18,19,20]Although great care is taken to ensure the sterility of implantable devices during manufacturing, contamination during or after implantation can lead to severe device-related infections. Such infections may necessitate device removal and can even be fatal.

PATHOGENIC MECHANISM:

Various ways in which biofilms can cause harm have been suggested. These include:[21]

1. **Attachment to Surfaces:** Biofilms let microorganisms stick to different surfaces.
2. **Evading Host Defenses:**They can escape the body's defense mechanisms, like phagocytosis.
3. **High Microorganism Density:** Biofilms help in gathering a lot of microorganisms in one place.
4. **Gene Exchange:**Biofilms can facilitate the exchange of genes, leading to the creation of more harmful microorganism strains.
5. **Toxin Concentration:**Biofilms have the capability to generate significant quantities of toxins.
6. **Shielding Against Antimicrobial Agents:**Biofilms provide protection against substances that kill microorganisms.
7. **Transmission to Other Sites:**Microbial groups within biofilms can break away and spread to other locations.

In simple terms, biofilms make it easier for harmful microorganisms to stick to surfaces, avoid our body's defenses, gather in large numbers, exchange harmful genes, produce lots of toxins, resist antimicrobial substances, and move to other areas.

Biofilms resist antibiotics in a few ways:

1. **Trapping Antibiotics:** The slimy substance in biofilms acts like a barrier, preventing antibiotics from reaching the bacteria inside. This makes the antibiotics less effective because their concentration gets diluted before reaching individual bacterial cells.[22,23]

2. **Immune System Escape:**Bacteria in biofilms can escape the immune system's antibodies. Biofilm-producing bacteria avoid the damage caused by the host's immune response.[24]

3. **Quorum Sensing and Genotyping Adaptations:**Bacteria in biofilms communicate with each other through chemical signaling, known as quorum sensing. They release molecules that induce specific gene transcription in the bacterial population. This communication decreases the bacteria's growth rate, making them less susceptible to antibiotics.

In simple terms, biofilms resist antibiotics by creating a barrier, escaping the immune system, and using communication systems that slow down bacterial growth and make treatment less effective.

MATERIAL AND METHODS:

The investigation was undertaken at the Department of Microbiology, Integral Institute of Medical Science & Research, Lucknow, India from January 2022 to 2023.The test group comprised patients from various departments of the hospital. A total of 300 positive samples were collected without regard to age, gender, occupation, religion, or ethnicity.

SAMPLE COLLECTION:

Over the course of one year, 300 positive samples were collected from various hospital wards. These samples varied and could include sputum, urine, blood, endotracheal tips and secretions, pus and swabs, suction tips, stents and valves, and various bodily fluids. Each sample was collected carefully to maintain cleanliness and prevent contamination, then promptly transported to the laboratory under ideal conditions for analysis.

PHENOTYPIC ASSAY OF BIOFILM DETECTION:

The isolates underwent biofilm detection using two distinct methods:

- Congo Red Agar Method
- Tube Method

CONGO RED AGAR METHOD:

Congo Red (ori):

A special solid medium called Brain Heart Infusion broth (BHI) was prepared supplemented with sucrose 5% and Congo red dye wasprepared. Preperation of congo red is done separately from the other medium and then autoclave at 121℃ for 15 min it is prepared as concentrated solution and add slowly when the agar had properly cooled to 55℃.

Following the preparation of the plates, they were subsequently inoculated with the desired microorganisms and allowed to incubate for a duration of 24 hours at 37°C under aerobic conditions.This whole process was done twice to ensure consistency and accuracy in the results.

A quintuple-color reference scale was employed for precise discernment of all color variations exhibited by the colonies. Isolates demonstrating dual shades of black—bright black (BB) and opaque black (OB)—were categorized as affirmatively indicative of biofilm production, whereas colonies displaying hues of pink, red, and Bordeaux were classified as negative.[26]

Congo Red (mod):

A modified version of Congo red agar (CRAmod) was developed to address limitations observed in the original CRAori, particularly regarding inconsistencies in black pigment formation, which affected the accuracy of biofilm identification. This modification involved several adjustments to the agar constituents to enhance the reliability of the method.

Firstly, Congo red dye concentration was reduced to 0.4 g/L. Secondly, sucrose was replaced with glucose at a concentration of 10 g/L. Finally, instead of using BHI and agar no. 1, an alternative agar, blood agar base-2 (BAB-2), was employed at a concentration of 40 g/L.

These modifications were aimed at optimizing the conditions for biofilm identification by minimizing variability in pigment formation and providing a more consistent and reliable medium for the detection of biofilms.[27 28]

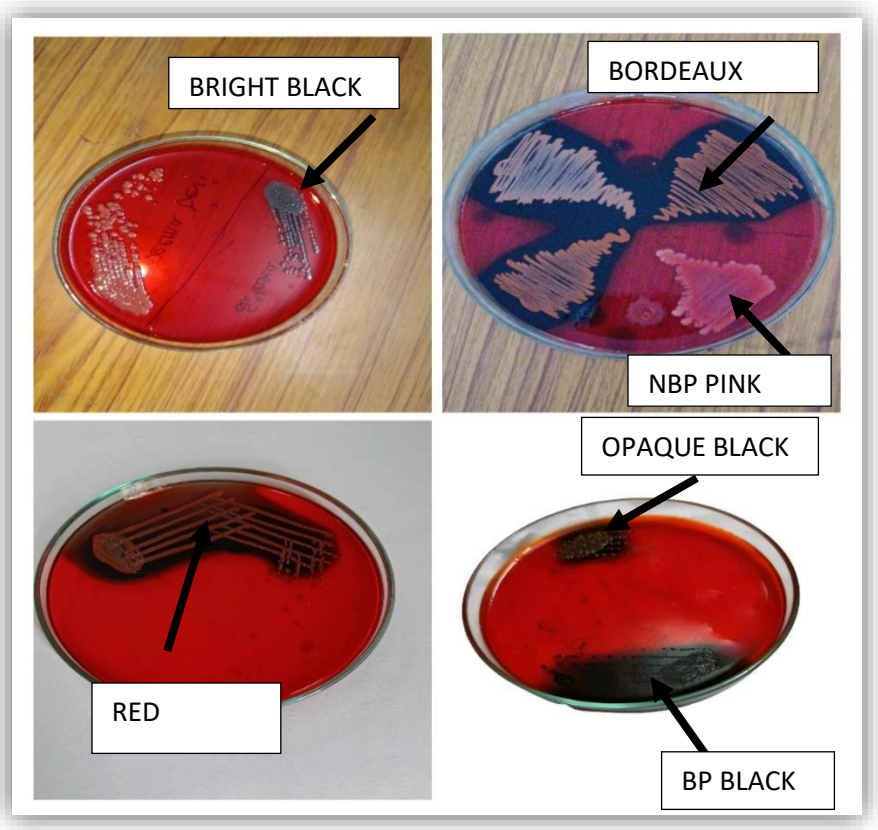

Figure 1:Variability in Biofilm-Producing Isolates Evidenced by Congo Red Agar Method

TUBE METHOD:

The Tube Method (TM) described by Christensen et al. is a qualitative assay used to detect microorganisms that produce biofilms. In this method, isolates are introduced into polystyrene test tubes with 2ml Tryptic Soy Broth (TSB) and then incubated at 37°C for a period of 24-48 hours. After incubation, removal of any planktonic cells by rinsing the test tubes twice with phosphate-buffered saline (PBS), pH 7.2. Next, the sessile isolates, which have developed biofilms on the walls of the polystyrene tubes, are stained with safranin/crystal violetfor a duration of 1 hour. The safranin-stained test tube is rinsed twice with PBS to remove excess stain. Once the rinsing process is complete, the test tube is allowed to air dry. The presence of a visible film lining the walls and bottom of the tube indicates the production of biofilm by the microorganisms being tested.[25]Two observers independently scored biofilm formation in tubes on a scale of 0 to 3 (0 = absent, 1 = weak, 2 = moderate, 3 = strong). The experiment was conducted in duplicates and repeated twice for reliability.

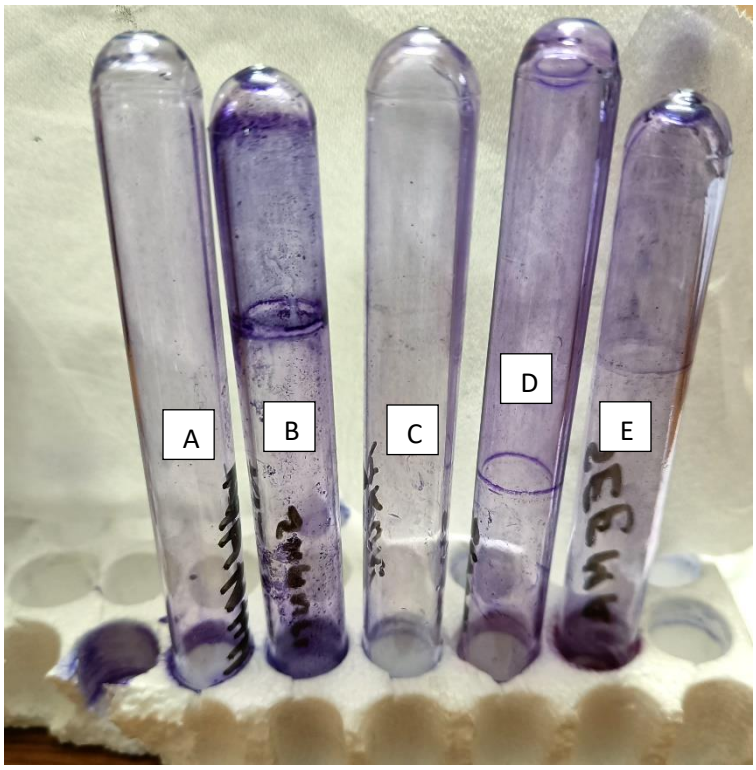

Figure: 2Tube Adherence method. A & C non-adherent, E weakly adherent,D moderately adherent, and B strongly adherent

RESULT:

Throughout the study period, a total of 300 samples exhibiting substantial bacterial growth were collected.Out of which 142 sample showed growth of Gram-positive and 158 samples showed growth of Gram-negative.(Figure.3)Among 300 isolated organisms, 143 were BP by CRA method and 151 by TM.

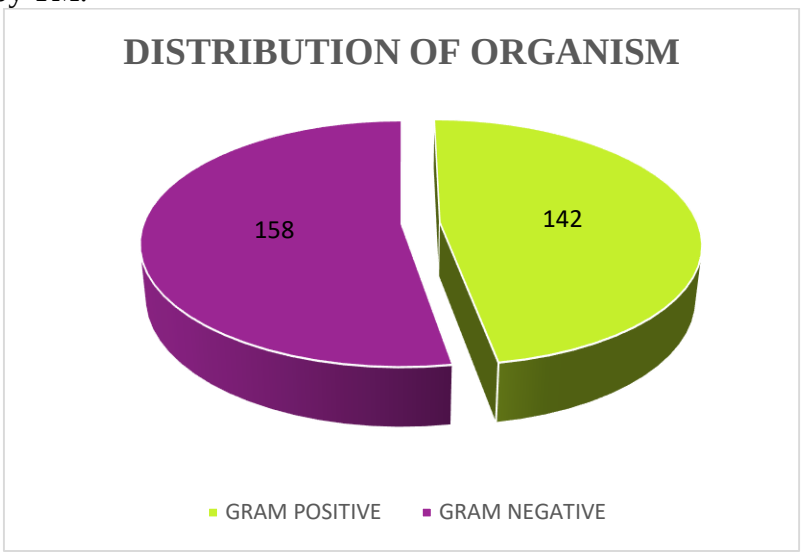

Figure 3

Various sample received during the study included blood (14 samples), pus (86 samples), urine (152 samples), respiratory secretions such as sputum (22 sample) and others (15 samples) included fluid, swab, ET tube. Among these the highest growth of gram negative bacilli was

observed in urine sample, while gram positive cocci were predominantly isolated from pussample.(Figure.4)

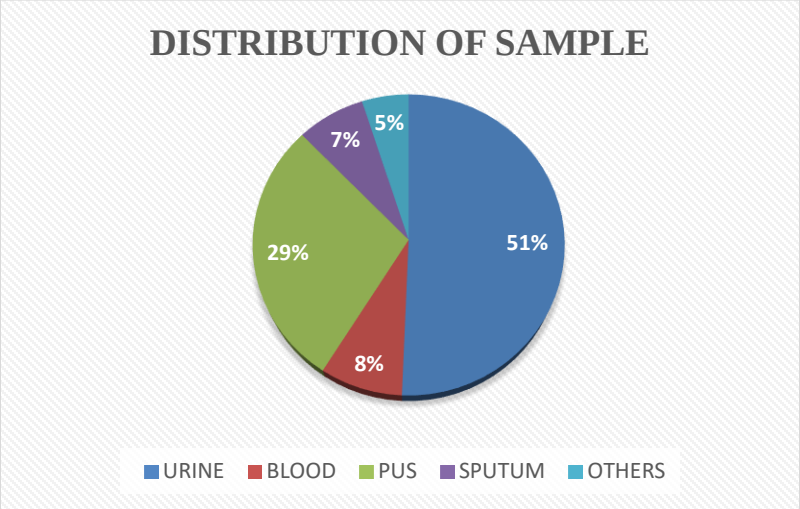

Figure 4

In this study, two methods were employed to assess biofilm production:The Tube method identified 151 (50.3%) isolates as biofilm producers and 149 (49.7%) as non-producers.(Table.1) On the other hand, the CRA method, utilizing two variants (CRAori and CRAmod), showed variations in identifying biofilm producers. CRAori classified 135 (45%) isolates as biofilm producers and 165 (55%) as non-producers, while CRAmod identified 143 (47.7%) isolates as biofilm-producers and 157 (52.3%) as non-producers.

Comparing the performance of these methods, the Tube method demonstrated higheraccuracy, sensitivity and specificity compared to the CRA method. Overall, the Tube method appears to be more effective in detecting biofilm production.

TABLE 1: Distribution of Biofilm Formation PercentagesUsing Different Detection Methods

Phenotypic Method in %	**BP: Biofilm detected in %**	**NBP: No Biofilm detected**
CRA	47.7	52.3
TM	50.3	49.7

The Table.2 shows biofilm production among various bacterial isolates by Gram staining and screening methods. Around 58.45% ,61.27% and 64.79% of Gram-positive isolates tested positive for biofilm production in CRA original, modified and Tube Method screenings, respectively. In Gram-negative isolates, approximately 32.91%,64.56% and 37.34% exhibited biofilm formation in CRA original and modified as well as Tube Method screenings, respectively.These findings highlight the diverse biofilm-forming abilities and genetic traits across bacterial species.

TABLE 2: Distinctive Breakdown of Biofilm Producers (BP) and Non-Biofilm Producers (NBP) by Organism

BIOFILM PRODUCTION							
ISOLATES	**TOTAL**	**CRA ori.**		**CRA mod.**		**TM**	
		+	-	+	-	+	-
GRAM-POSITIVE	**142**	**83**	**59**	**87**	**55**	**92**	**50**
CONS	68	58	10	60	8	60	8

S. AUREUS	41	23	18	25	16	27	14
ENTEROCOCCI	33	02	31	02	31	05	28
GRAM-NEGATIVE	**158**	**52**	**106**	**56**	**102**	**59**	**99**
E.COLI	91	35	56	37	54	42	49
KLEBSIELLA	40	0	40	02	38	0	40
PSEUDOMONAS	27	17	10	17	10	17	10
TOTAL	**300**	**135**	**165**	**143**	**157**	**151**	**149**

In the present investigation it has been noted that among the Gram-negative isolates, Escherichia coli(91) emerges as the predominant organism, succeeded by Klebsiella (40) and Pseudomonas (27). (Figure.5) Conversely, among the Gram-positive counterparts, coagulase-negative Staphylococcus (68) emerges as the predominant species, trailed by Staphylococcus aureus (41) and Enterococcus (33). (Figure.6)

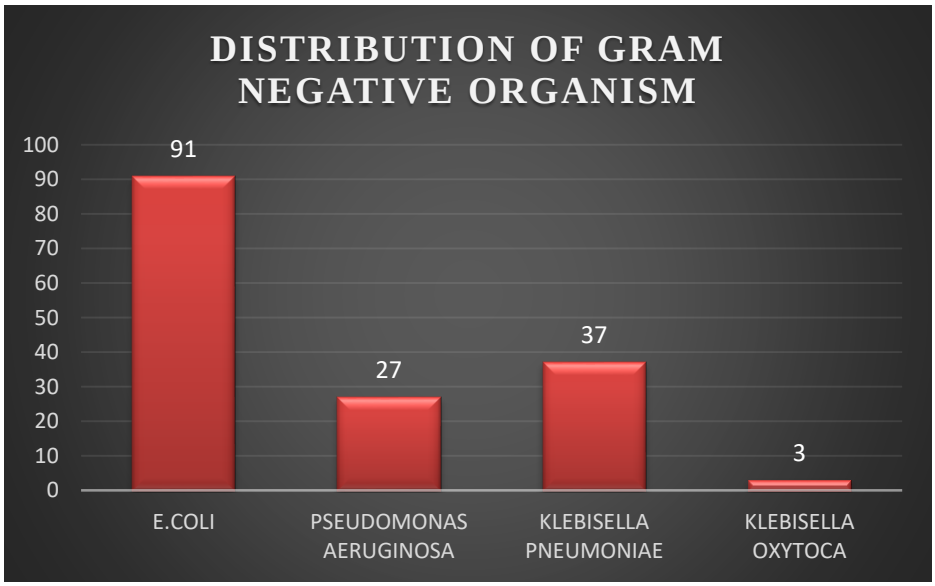

Figure 5 Distribution of gram- negative organisms

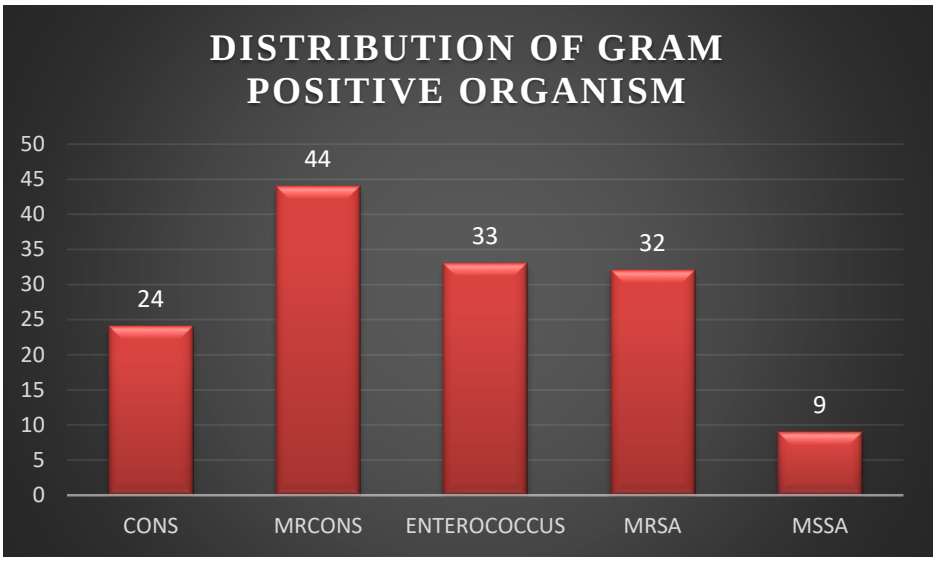

Figure 6 Distribution of gram-positive organisms

In this study the antibiotic susceptibility test outcomes of both slime-producing and non-slime-producing Klebisella strains, gathered from diverse clinical specimens, are delineated in (Figure.7) most of the strains shows higher resistance towards ciprofloxacin, followed by levofloxacin, ceftriaxone, ampicillin/sulbactam whereas higher sensitivity towards tigecycline.

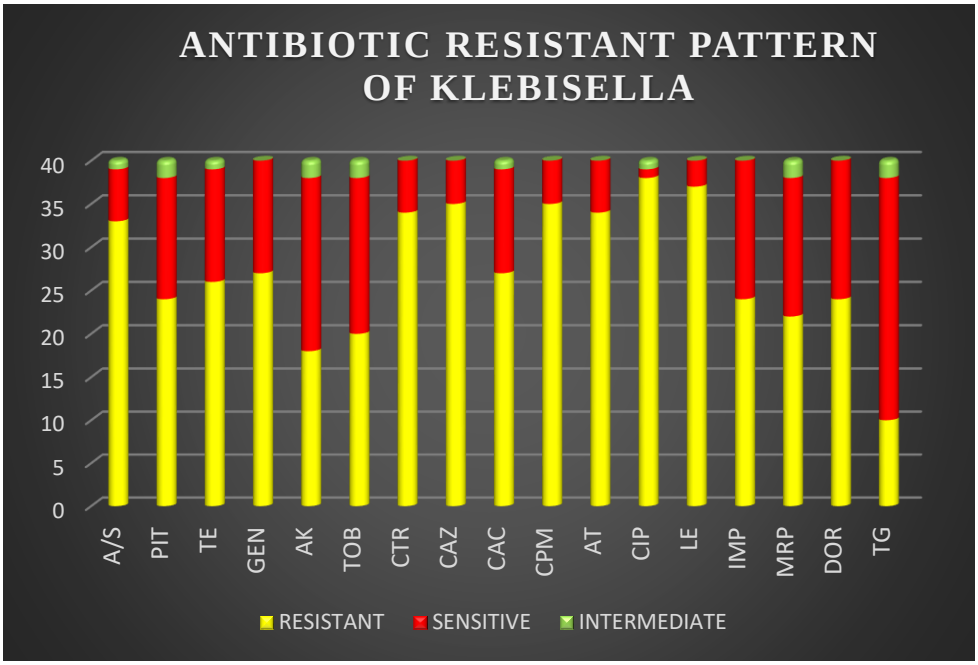

Figure 7

This study shows the antibiotic susceptibility test outcomes of both biofilm-producing as well as non-biofilm-producing e.coli strains, collected from various clinical specimens, are shown in (Figure.8) E.coli is the most commonest organism found in this study both biofilm producing and non producinge.coliisolates shows higher resistance towards ciprofloxacin, followed by levofloxacin, ciprofloxacin, cefepime, ceftazidime, ceftriaxone, ampicillin/sulbactam whereas higher sensitivity towards tigecycline.

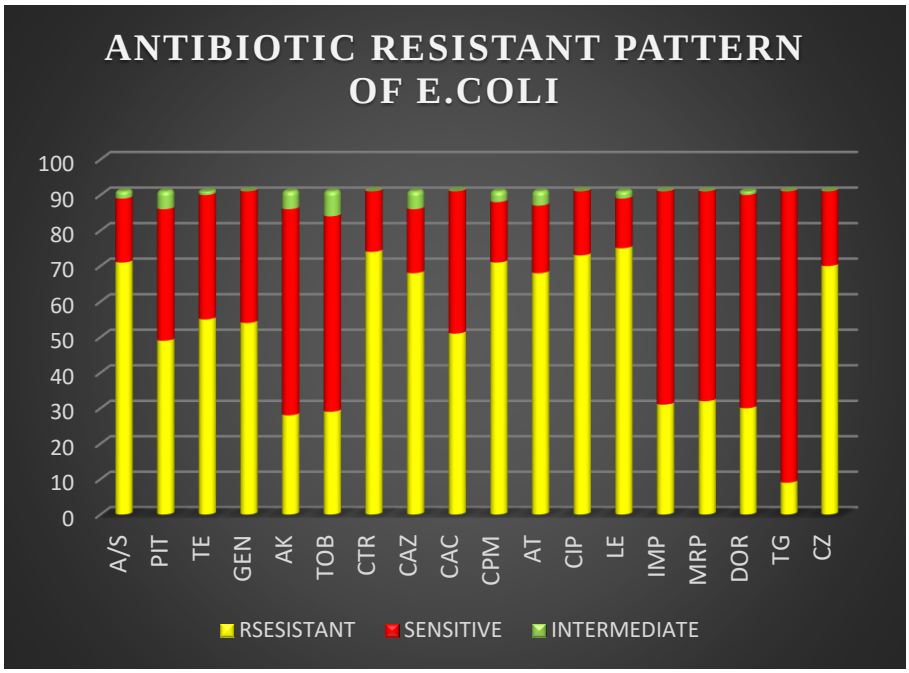

Figure 8

This study shows the higher sensitivity rate of pseudomonas towards colistin, polymixin-B whereas highest resistance shown among biofilm-producer as well non biofilm-producer levofloxacin, followed by ceftazidime, ciprofloxacin, imipinem.(Figure.9)

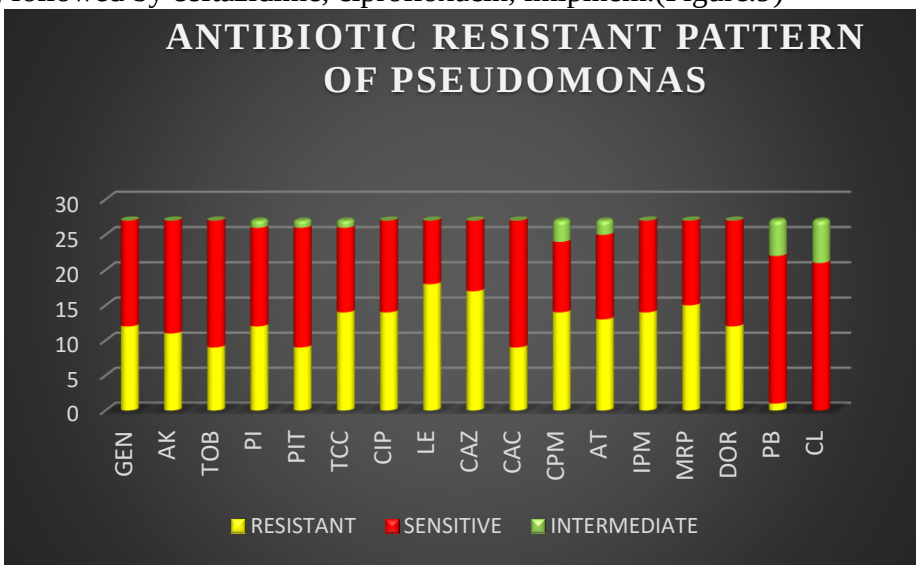

Figure 9

The antibiotic susceptibility test outcomes of both slime-producing and non-slime-producing Enterococcus strains, gathered from diverse clinical specimens, are delineated in (Figure.10) A notable trend of heightened resistance is evident in th group of the biofilm-producing and non-biofilm producing strain, the highest resistance is directed towards ciprofloxacin succeeded by, levofloxacin, high level gentamycin and tetracycline. Conversely, all strains exhibited 100% susceptibility tolienzolidfollowed by vancomycin and teicoplanin.

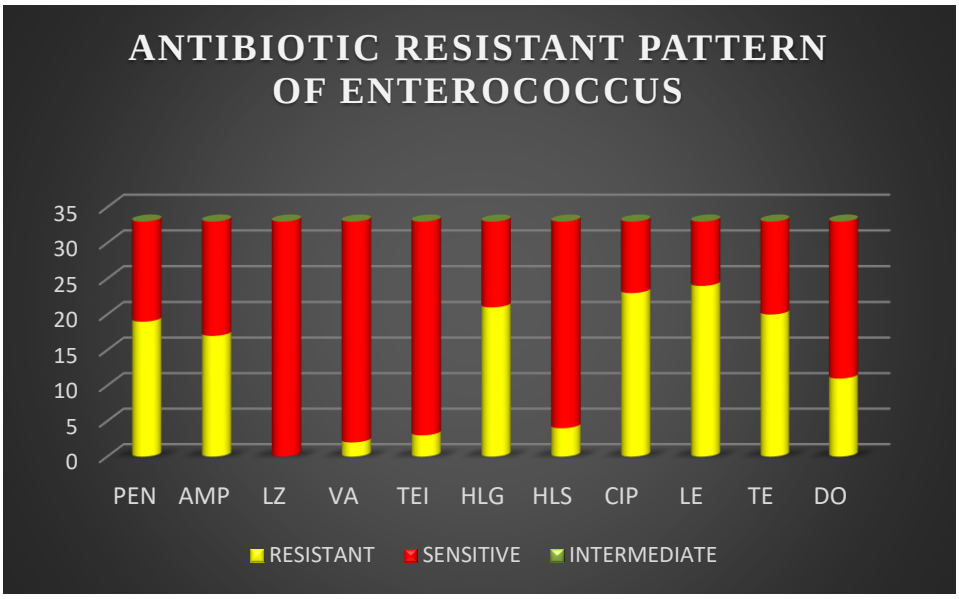

Figure 10

The antibiotic susceptibility test outcomes of both biofilm-producing and non-biofilm-producing CONS strains, gathered from diverse clinical specimens, are shown in (Figure.11) A notable trend of heightened resistance is evident among biofilm-producing isolatescompared to their non-biofilm-producing counterparts. Predominantly, the highest resistance is directed towards ciprofloxacin succeeded by, levofloxacin, cefoxitin, erythromycin and clindamycin. Conversely, Moststrains exhibited susceptibility to vancomycin, linezolid and teicoplanin.

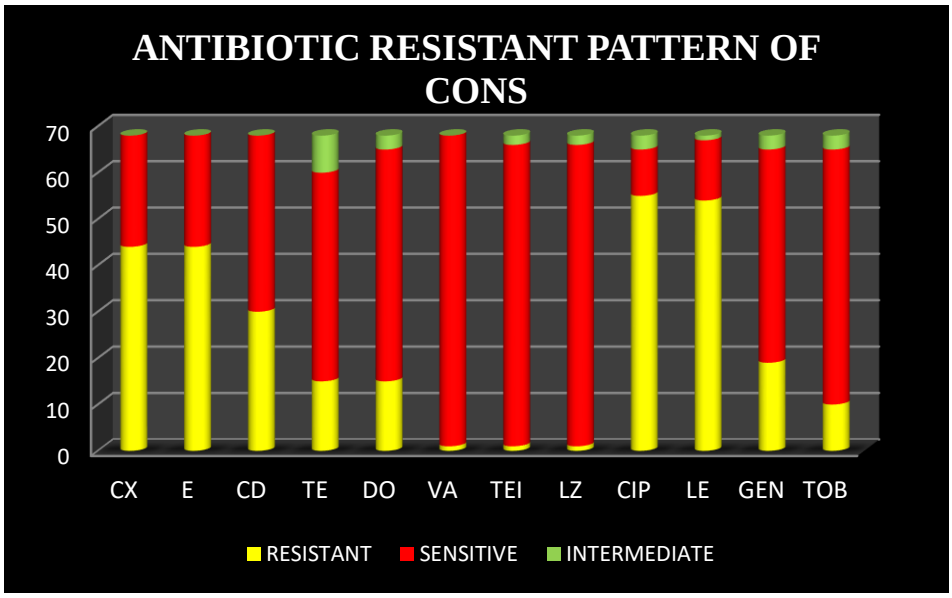

Figure 11

The antibiotic susceptibility test outcomes of both slime-producing and non-slime-producing S. aureus strains, gathered from diverse clinical specimens, are delineated in (Figure.12) A notable trend of heightened resistance is evident among biofilm-producing staph.strains compared to their non-biofilm-producing counterparts. Predominantly, the highest resistance is directed towards ciprofloxacin succeeded by, levofloxacin, cefoxitin, erythromycin, clindamycin and

tetracycline. Conversely, most of the strain shows sensitivity towards vancomycin, teicoplanin and exhibited 100% susceptibility to linezolid.

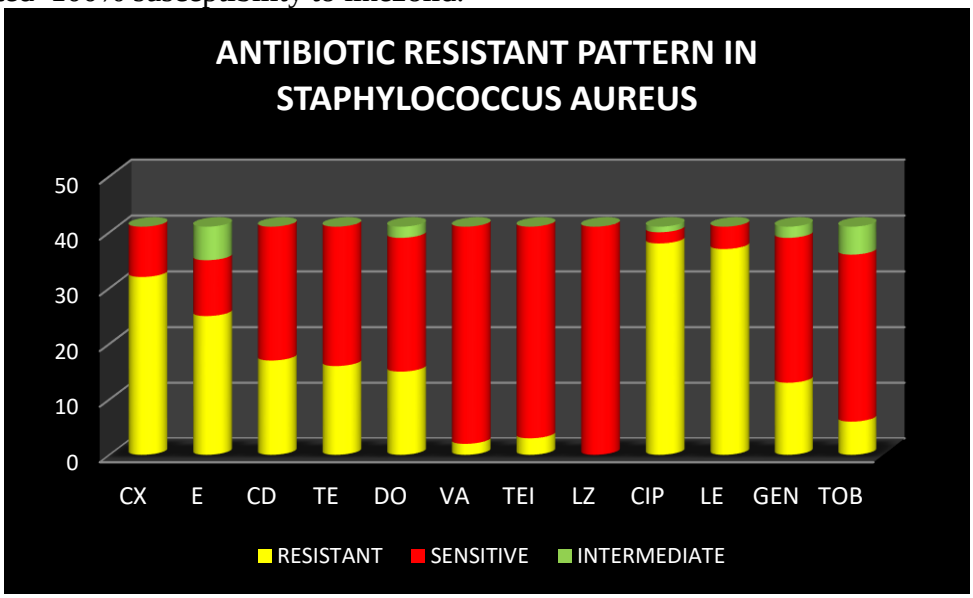

Figure12

DISCUSSION:

Biofilm-producing bacteria pose a significant challenge in combating infections, exhibiting notable resilience and resistance to eradication attempts. Their robust defense mechanisms include hindering antibiotic penetration into the biofilm matrix, reducing growth rates, and enhancing expression of resistance genes. These sophisticated strategies collectively bolster the bacterial community within the biofilm, rendering conventional antibiotic therapies ineffective and underscoring the imperative for innovative therapeutic modalities.[29]

In our investigation, among the isolates with positive cultures, the majority were GNB (52.7%), outnumbering GPC (47.3%). These findings mirror those of previous studies by Suneet T, et al. (60% and 38.80%) and Khosravi, et al. (64.5% and 33.5%).[30,31]

Gram-positive isolates demonstrated an augmented proclivity towards biofilm formation, a phenomenon corroborated by Sarangi et al.[32]Pseudomonas aeruginosa, Klebsiella pneumoniae, and E. coli are identified as the principal biofilm producers within gram-negative isolates, aligning with findings from the research conducted by Harika et al.[33] This underscores the critical imperative of addressing infections associated with biofilms.

In our research, we noted a notable increase in resistance levels among bacterial isolates capable of forming biofilms compared to those that cannot. This resistance was particularly evident against commonly prescribed drugs at our institution, including penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and tetracyclines. Additionally, biofilm-producing gram-negative isolates exhibited a higher prevalence of resistance to carbapenems such as Meropenem and Imipenem. While Gram positive isolates shows higher sensitivity towards vancomycin, linezolid.[41,42]Dumaru et al.'s study also revealed a comparable resistance pattern among gram-negative isolates capable of producing biofilms.[34]Cepas et al.[35] and Asati et al.[36] similarly identified a parallel resistance pattern in gram-negative isolates that produce biofilms. Additionally, Harika et al.'s[33] study demonstrated that biofilm producers exhibited a highest prevalence of resistance to commonly used antibiotics.

In numerous articles, a strong association is observed between biofilm formation, resistance patterns and pathogenicity especially with strong or moderate biofilms. This suggests a pivotal role for biofilm formation strength in influencing resistance.[37,38,39,40]DevangaRugupathi et al.[39] revealed a stronger correlation between robust biofilm formation and carbapenem resistance compared to moderate or weak biofilm formation, implying that an organism's susceptibility pattern may hinge on the biofilm's strength it forms. Several scientific inquiries have postulated that biofilm formation impedes the effective diffusion of antibiotics, leading to a notable reduction in bacterial exposure to antimicrobial agents and subsequent antibiotic efficacy.

Our observed high antimicrobial resistance pattern may be attributed to the tertiary care nature of our center, as we receive numerous referrals from primary centers where patients are already undergoing extensive antibiotic treatment. The injudicious use of antibiotics further exacerbates this issue, leading to selection pressure that favors the acquisition of resistance among microorganisms, including the formation of biofilms.

CONCLUSION:

This study underscores that microorganisms possess a propensity to form biofilms on various clinical sites. This biofilm formation process is linked with heightened resistance to antimicrobial agents, potentially attributable to their role as persistent sources of infection. However, routine administration of antimicrobials often proves insufficient for treating such infections due to poor drug penetration. Therefore, there is a pressing need to develop methods for both preventing biofilm formation and removing existing biofilms. Additionally, there is a necessity to routinely identify strains that produce biofilms. This would enable healthcare providers to devise effective patient management strategies.

ACKNOWLEDGMENT:

This study was funded by Seed Money Grant for Research 2022 (project sanction no.IUL/IIRC/SMP/2022/009)

Manuscript no.- IU/R&D/2024-MCN0002799

CONFLICT OF INTEREST:

The author(s) declare that there is no conflict of interest

REFERENCE:

1. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*. 2002;15:167–193. [PMC free article] [PubMed] [Google Scholar] [Ref list]
2. Costerton J.W., Lewandowski Z., Caldwell D.E., Korber D.R., Lappin-Scott H.M. Microbial biofilms. *Annu. Rev. Microbiol*. 1995;49:711–745. doi: 10.1146/annurev.mi.49.100195.003431. [PubMed] [CrossRef] [Google Scholar] [Ref list]
3. Stoodley P., Lewandowski Z., Boyle J.D., Lappin-Scott H.M. Oscillation characteristics of biofilm streamers in turbulent flowing water as related to drag and pressure drop. *Biotechnol. Bioeng*. 1998;57:536–544. doi: 10.1002/(SICI)1097-0290(19980305)57:5<536::AID-BIT5>3.0.CO;2-H. [PubMed] [CrossRef] [Google Scholar] [Ref list]
4. Chen M., Yu Q., Sun H. Novel strategies for the prevention and treatment of biofilm related infections. *Int. J. Mol. Sci*. 2013;14:18488–18501. doi: 10.3390/ijms140918488. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

5. Paharik A.E., Horswill A.R. The Staphylococcal Biofilm: Adhesins, Regulation, and Host Response. *Microbiol. Spectr.* 2016;4 doi: 10.1128/microbiolspec.VMBF-0022-2015. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
6. Zaborowska M., Tillander J., Branemark R., Hagberg L., Thomsen P., Trobos M. Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants. *J. Biomed. Mater. Res. B Appl. Biomater.* 2017;105:2630–2640. doi: 10.1002/jbm.b.33803. [PubMed] [CrossRef] [Google Scholar] [Ref list]
7. Khan M.S., ur Rehman S., Ali M.A., Sultan B., Sultan S. Infection in orthopedic implant surgery, its risk factors and outcome. *J. Ayub Med. Coll. Abbottabad.* 2008;20:23–25. [PubMed] [Google Scholar] [Ref list]
8. Cerca N., Pier G.B., Vilanova M., Oliveira R., Azeredo J. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of Staphylococcus epidermidis. *Res. Microbiol.* 2005;156:506–514. doi: 10.1016/j.resmic.2005.01.007. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
9. Rahim M.I., Rohde M., Rais B., Seitz J.M., Mueller P.P. Susceptibility of metallic magnesium implants to bacterial biofilm infections. *J. Biomed. Mater. Res. A.* 2016;104:1489–1499. doi: 10.1002/jbm.a.35680. [PubMed] [CrossRef] [Google Scholar] [Ref list]
10. Koseki H., Yonekura A., Shida T., Yoda I., Horiuchi H., Morinaga Y., Yanagihara K., Sakoda H., Osaki M., Tomita M. Early staphylococcal biofilm formation on solid orthopaedic implant materials: In vitro study. *PLoS ONE.* 2014;9:e107588. doi: 10.1371/journal.pone.0107588. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
11. Glage S., Paret S., Winkel A., Stiesch M., Bleich A., Krauss J.K., Schwabe K. A new model for biofilm formation and inflammatory tissue reaction: Intraoperative infection of a cranial implant with Staphylococcus aureus in rats. *Acta Neurochir.* 2017;159:1747–1756. doi: 10.1007/s00701-017-3244-7. [PubMed] [CrossRef] [Google Scholar] [Ref list]
12. Oliveira W.F., Silva P.M.S., Silva R.C.S., Silva G.M.M., Machado G., Coelho L., Correia M.T.S. Staphylococcus aureus and Staphylococcus epidermidis infections on implants. *J. Hosp. Infect.* 2018;98:111–117. doi: 10.1016/j.jhin.2017.11.008. [PubMed] [CrossRef] [Google Scholar] [Ref list]
13. Zheng Y., He L., Asiamah T.K., Otto M. Colonization of medical devices by staphylococci. *Environ. Microbiol.* 2018;20:3141–3153. doi: 10.1111/1462-2920.14129. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
14. Irie Y., Borlee B.R., O'Connor J.R., Hill P.J., Harwood C.S., Wozniak D.J., Parsek M.R. Self-produced exopolysaccharide is a signal that stimulates biofilm formation in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA.* 2012;109:20632–20636. doi: 10.1073/pnas.1217993109. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
15. Gupta P., Sarkar S., Das B., Bhattacharjee S., Tribedi P. Biofilm, pathogenesis and prevention--a journey to break the wall: A review. *Arch. Microbiol.* 2016;198:1–15. doi: 10.1007/s00203-015-1148-6. [PubMed] [CrossRef] [Google Scholar] [Ref list]

16. Bjarnsholt T. The role of bacterial biofilms in chronic infections. *APMIS Suppl.* 2013;121:1–51. doi: 10.1111/apm.12099. [PubMed] [CrossRef] [Google Scholar] [Ref list]
17. Joo H.S., Otto M. Molecular basis of in vivo biofilm formation by bacterial pathogens. *Chem. Biol.* 2012;19:1503–1513. doi: 10.1016/j.chembiol.2012.10.022. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
18. Veerachamy S., Yarlagadda T., Manivasagam G., Yarlagadda P.K. Bacterial adherence and biofilm formation on medical implants: A review. *Proc. Inst. Mech. Eng. H.* 2014;228:1083–1099. doi: 10.1177/0954411914556137. [PubMed] [CrossRef] [Google Scholar] [Ref list]
19. Chao Y., Marks L.R., Pettigrew M.M., Hakansson A.P. Streptococcus pneumoniae biofilm formation and dispersion during colonization and disease. *Front. Cell. Infect. Microbiol.* 2014;4:194. doi: 10.3389/fcimb.2014.00194. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
20. Buttner H., Mack D., Rohde H. Structural basis of Staphylococcus epidermidis biofilm formation: Mechanisms and molecular interactions. *Front. Cell. Infect. Microbiol.* 2015;5:14. doi: 10.3389/fcimb.2015.00014. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
21. Aparna MS, Yadav S. Biofilms: microbes and disease. Braz J Infect Dis 2008 Dec;12(6):526-530.
22. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000;54:49-79.
23. Danhorn T, Fuqua C. Biofilm formation by plant associated bacteria. Annu Rev Microbiol 2007;61:401-422.
24. Davies DG, Parsek MR, Pearson JP,Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell to cell signals in the development of a bacterial biofilm. Science 1998 Apr 10;280(5361):295-298.
25. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. Journal of Clinical Microbiology. 1985;22:996-1006
26. Oliveira A, Cunha Mde L (2010) Comparison of methods for the detection of biofilm production in coagulase-negative Staphylococci. BMC Res Notes 3:260. doi:10.1186/1756-0500-3-260
27. Kaiser TD, Pereira EM, Dos Santos KR, Maciel EL, Schuenck RP, Nunes AP (2013) Modification of the Congo red agar method to detect biofilm production by Staphylococcus epidermidis. Diagn Microbiol Infect Dis 75(3):235–239. doi:10.1016/j.diag microbio.2012.11.014
28. Mariana NS, Salman SA, Neela V, Zamberi S (2009) Evaluation of modified Congo red agar for detection of biofilm produced by clinical isolates of methicillin–resistance Staphylococcus aureus. Afr J Microbiol Res 3(6):330–338
29. Kim L. Riddle of biofilm resistance. Antimic Ag Chemother 2001; 45(4):999-1007
30. Tandon S, Pathak A, Mishra SK, Vijayvargiya M. Incidence and Risk Factors for Early Surgical Site Infection in Elective Orthopaedic Implant Surgeries: A Prospective Study. *Journal of Evolution of Medical and Dental Sciences*. 2015;4(15):2525–2531. Available from: https://doi.org/10.14260/jemds/2015/364

31. Khosravi AD, Ahmadi F, Salmanzade S, Dashtbozor A, Montazeri EA. Study of Bacteria Isolated from Orthopedic Implant Infections and their Antimicrobial Susceptibility Pattern. *Research Journal of Microbiology*. 2009;4(4):158–163. Available from: https://doi.org/10.3923/jm.2009.158.163
32. Sarangi S, Padhi S: Bacteriological profile of post-operative orthopedic implant infections and their antibiotic sensitivity pattern in a tertiary care hospital of southern Odisha. J Dr NTR Univ Heal Sci. 2019, 8:114. doi: 10.4103/JDRNTRUHS.JDRNTRUHS_48_19
33. Harika K, Shenoy V, Narasimhaswamy N, et al.: Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. J Glob Infect Dis. 2020, 12:129-34. doi: 10.4103%2Fjgid.jgid_150_19
34. Dumaru R, Baral R, Shrestha LB: Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dharan. BMC Res Notes. 2019, 12:1-6. doi: 10.1186/s13104-019-4084-8
35. Cepas V, López Y, Muñoz E, et al.: Relationship between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. Microb Drug Resist. 2019, 25:72-9. doi: 10.1089/mdr.2018.0027
36. Asati S, Chaudhary U: Comparison of tissue culture plate and modified tissue culture plate method for biofilm detection in members of family enterobacteriaceae. J Clin Diagnostic Res. 2018, 12:20-3. doi: 10.7860/JCDR/2018/34795.11827
37. Shadkam S, Goli HR, Mirzaei B et al. Correlation between antibiotic resistance and biofilm formation capacity among Klebsiella Pneumoniae strains isolated from hospitalized patents in Iran. Ann Clin Microbial Antimicrob 20, 13(2021) https://doi.org/10.1186/s12941-021-00418-X.
38. Karimi K, Zarei O, Sedighi P, Taherei M, Doosti-Irani A, Shokoohizadeh. Investigation of antibiotics resistance and biofilm formation in clinical isolates of *Klebsiella Pneumoniae*. International Journal of Microbiology, Vol.2021, Article ID 5573388, 6 pages, 2021. https://doi.org/10.1155/2021/5573388.
39. DevangaRugupathi NK, MuthuirulandiSethuvel DP, TriplicaneDwarakanathan H, Murugan D, Urnashankar Y, Monk PN Karunakaran E, Veeraraghavan B. The Influence of Biofilms on Carbapenem Susceptibility and patient's outcome in Device Associated *Klebsiella pneumoniae* Insights into Phenotype vs Genome – wide Analysis and Correction. Front. Microbiol. 11:591679. https://doi.10.3389/fmicb.2020.591679. CrossRef
40. Lihua Q, Hao L, Chuanfu Z, Beibei L, Jie Li, Ligui W, et al. Relationship between Antibiotic Resistance, Biofilm Formation and Biofilm-Specific Resistance in Acinetobacter baumanni. Front Microbiol. 2016;7:483. https://doi:10.33889/Fmicb.2016.00483. PMID: 27148178.
41. Nikita Mishra1, Shilpi Srivastava, Namita Srivastava. Detection of Pyogenic Infections among Clinical Samples Received in Department of Microbiology in Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh Volume 10 Issue 2, February 2021.DOI: 10.21275/SR21215014547
42. Namita Srivastava , Shilpi Srivastava, et al. Detection of Antimicrobial Susceptibility Pattern of Staphylococcus aureus among Clinical Samples Received in Department of

Microbiology in Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh.Volume 10 Issue 7, July 2021 DOI: 10.21275/SR21727193038