RESEARCH PAPER

PREVALENCE OF BIOFILM FORMATION ON STUDENT-HANDLED LABORATORY INSTRUMENTS IN A TEACHING MICROBIOLOGY LABORATORY

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ABSTRACT

Biofilm formation on lab instruments is a notable but often overlooked source of contamination in teaching microbiology labs. This study looked into how often biofilm-forming bacteria were found on frequently used student-handled instruments and their resistance to antibiotics. A total of 150 students took part in routine microbiology practical's, using micropipettes, inoculation loops, forceps, slides, test tubes, petri dishes, and pipette tips. Surface swabs from these tools were cultured, and five Staphylococcus isolates were identified. Their ability to form biofilm was examined using three methods: microtiter plate assay, Congo red agar, and tube adherence method. We carried out antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method. The results showed that biofilm formation occurred on several instruments, with different levels of producer classification. There was a strong link between biofilm-forming ability and higher antibiotic resistance. These findings highlight the need for proper sterilization procedures and careful instrument handling in teaching labs to avoid cross-contamination and the spread of antimicrobial-resistant pathogens that produce biofilm.

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BACKGROUND

Teaching microbiology laboratories are essential educational spaces where students develop practical skills in microbiological techniques. However, these settings also face unique challenges with microbial contamination. The heavy use of lab instruments by multiple students, often with different levels of aseptic technique, creates favourable conditions for microbial growth and biofilm formation. While regular monitoring for contaminants is common in clinical and research labs, teaching labs often do not have strict programs in place to detect and prevent biofilm build-up on shared instruments.

Biofilms are complex, three-dimensional communities of microorganisms trapped in a self-produced matrix called extracellular polymeric substance (EPS). This matrix shields bacteria from environmental stresses, disinfectants, and the immune system, making them much harder to eliminate than free-floating bacteria. Among clinical pathogens, Staphylococcus species, especially Staphylococcus aureus and coagulase-negative staphylococci (CoNS), are known to form biofilms extensively, which poses risks for infections related to medical devices and persistence in the environment.

Clinical and Laboratory Significance

The ability of staphylococci to form biofilms on surfaces is a serious issue in healthcare and laboratory settings. Contaminated instruments can act as reservoirs for harmful bacteria, promoting cross-contamination between samples and practitioners. In teaching laboratories, where instrument sharing is common, insufficient sterilization between uses can continue microbial colonization. Additionally, biofilm-forming strains often show increased resistance to antibiotics, making them hard to remove with standard cleaning methods.

Previous studies have shown that biofilm formation is linked to greater virulence and antibiotic resistance in clinical isolates. However, there is limited information on how common biofilm-forming bacteria are on instruments

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in teaching labs, where multiple students with different practices for maintaining aseptic technique use shared equipment.

Rationale for This Study

This study was set up to fill in some missing pieces about how biofilms form on the tools students often use in labs. We looked closely at how well these tools can develop biofilms and checked how resistant the bacteria are to antibiotics. The goal is to gather solid evidence that can help us make better rules for cleaning lab instruments and keeping everyone safe in microbiology classrooms.

Study Objectives

1. To isolate and identify *Staphylococcus* species from surfaces of student-handled laboratory instruments
2. To detect and classify biofilm-forming ability using three independent phenotypic methods
3. To determine antimicrobial susceptibility patterns and establish correlations with biofilm-forming phenotype
4. To provide data-driven recommendations for contamination prevention in teaching laboratories

REVIEW OF LITERATURE

Biofilm Formation in Staphylococcus Species

Biofilm formation has emerged as a critical virulence determinant in *Staphylococcus aureus* and coagulase-negative staphylococci. The process is initiated through bacterial adhesion to surfaces, facilitated by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) including clumping factors, fibronectin-binding proteins, and collagen adhesins. Once adherent, bacteria produce extracellular polysaccharides particularly polysaccharide intercellular adhesin (PIA), encoded by the *ica* operon which form the biofilm matrix scaffold.

Mahmoudi et al. (2019) examined biofilm production in *S. aureus* isolates from burn wounds, finding that 94% of 47 isolates were capable of biofilm formation, with 58% classified as strong producers. Notably, the prevalence of biofilm formation was similar between methicillin-resistant (MRSA) and methicillin-sensitive strains (MSSA), though resistance patterns differed. In a comprehensive study by Goudarzi et al. (2019), 72% of 75 clinical isolates produced biofilm, with MRSA strains demonstrating higher biofilm-producing capacity than MSSA strains. The most prevalent biofilm-associated genes were *icaD* (77.3%), *icaA* (76%), *icaB* (57.3%), and *icaC* (50.7%).

Coagulase-negative staphylococci, particularly *S. epidermidis*, are increasingly recognized as serious nosocomial pathogens. Ahmed et al. (2019) detected multidrug resistance (MDR) in 86% of CoNS isolates from neonatal nasal colonization, with strong associations between *mecA* gene presence and methicillin resistance, as well as between *icaA* gene presence and biofilm formation. Kitti et al. (2019) found biofilm production in 87.3% of methicillin-resistant CoNS (MR-CoNS) isolates using Congo red agar method, with significant variation in biofilm-associated gene prevalence (*icaAD* 18.2%, *bap* 12.7%, *fnbA* 47.3%, *cna* 27.3%).

Biofilm Detection Methods

Multiple phenotypic and genotypic approaches exist for biofilm detection, each with distinct advantages and limitations. The microtiter plate (tissue culture plate) method is considered the gold standard, offering quantitative assessment of biofilm biomass. Triveni et al. (2018) screened 188 clinical *S. aureus* isolates using TCP method, identifying 72 biofilm producers (38.29%), of which 32 were strong and 38 moderate producers. Maximum biofilm formation was observed in pus samples (39.06%), followed by blood (38.23%) and urine (34.61%).

Congo red agar is a rapid screening method based on the principle that biofilm-producing strains bind Congo red dye, resulting in characteristic black, crystalline colonies. However, specificity and sensitivity vary considerably. Abdel Halim et al. (2018) found that Congo red agar detected 74% biofilm-producing staphylococci but demonstrated very low sensitivity (0.9%) and high specificity (97.4%) in comparison to TCP method. Tube adherence method, a simpler alternative to MTP assay, demonstrated 51.4% sensitivity and 82.1% specificity in the same study.

Manandhar et al. (2021) evaluated multiple detection methods in coagulase-negative staphylococci, finding that 31.8% of isolates produced biofilm via tube method while 20.1% were positive by Congo red agar method. The discordance between methods suggests that no single test comprehensively captures biofilm-forming phenotypes; therefore, employing multiple detection strategies is recommended for accurate characterization.

Biofilm and Antimicrobial Resistance

A consistent finding across multiple studies is the strong correlation between biofilm production and elevated antimicrobial resistance. Neopane et al. (2018) demonstrated that biofilm-producing *S. aureus* exhibited significantly higher antimicrobial resistance compared to non-producers ($P<0.05$), with 86.7% of biofilm-producing strains classified as multidrug resistant (MDR) versus 0% of non-biofilm producers. This association reflects the protective effects of the EPS matrix, which impedes antimicrobial penetration and facilitates horizontal gene transfer among biofilm-resident cells.

Thilakavathy et al. (2015) observed that biofilm-producing CoNS isolates showed substantially higher antibiotic resistance (72.1%) compared to non-producers. Similarly, Nourbakhsh et al. (2016) found that 53.5% of clinical *S. aureus* isolates were highly capable biofilm producers, with elevated resistance to penicillin (89%), ciprofloxacin (87.4%), and erythromycin (86.1%). Conversely, all isolates remained susceptible to

vancomycin and linezolid, antibiotics considered reliable for treating biofilm-associated infections.

Biofilm in Healthcare-Associated Infections

The clinical impact of biofilm-forming staphylococci extends to device-related infections. Latif et al. (2015) found that 91.4% of *S. epidermidis* isolates from clinical specimens formed biofilm, with *ica* operon detection in 32.8% a significantly higher prevalence than reported in other geographic regions (P = 0.0003). Thilakavathy et al. (2015) demonstrated that biofilm-producing CoNS isolates showed strong association with medical device-related infections, supporting the clinical relevance of biofilm phenotyping in patient management.

OBJECTIVES OF STUDY

Primary Objectives

1. To determine the prevalence of *Staphylococcus* species isolated from surfaces of commonly used student-handled laboratory instruments in a teaching microbiology laboratory
2. To characterize the biofilm-forming ability of recovered isolates using three independent phenotypic detection methods (microtiter plate assay, Congo red agar, and tube adherence method)
3. To classify isolates according to biofilm-forming capacity (non-biofilm producer, weak, moderate, strong)

Secondary Objectives

1. To assess antimicrobial susceptibility patterns of isolates using Kirby-Bauer disk diffusion method
2. To establish correlations between biofilm-forming phenotype and antibiotic resistance profiles
3. To provide recommendations for enhanced sterilization and contamination control protocols in teaching laboratory environments

MATERIALS AND METHODS

Study Design and Setting

This study took place in the Medical Laboratory Technology department of a teaching college. It was a descriptive cross-sectional study, focusing on the microbial contamination found on lab tools that students regularly used during their microbiology practicals. Throughout a specific period, 150 students took part in these practical sessions, and the study aimed to see what kind of germs might be present on the instruments they handled.

Sample Collection

Instruments Sampled:

- Micropipettes (10 µL, 100 µL, 1000 µL)
- Inoculation loops (1 µL and 10 µL)
- Forceps (various sizes)
- Glass slides
- Test tubes
- Petri dishes
- Pipette tips (sterile and non-sterile)

Sampling Protocol: We used sterile cotton swabs dampened with 0.9% saline to carefully wipe down the surfaces of instruments right after students used them. No cleaning or sterilization was done in between. The swabs were taken from both the outside of the instruments and, if relevant, from the inside parts that might touch cultures or samples. Each swab was then placed into a sterile tube with brain heart infusion (BHI) broth and kept at 37°C as we brought them to the lab for testing.

Inclusion and Exclusion Criteria

Inclusion Criteria:

- Instruments used by students during routine microbiology practicals
- Surfaces showing visible or suspected microbial contamination
- Isolates identified as *Staphylococcus* species

Exclusion Criteria:

- Non-staphylococcal organisms
- Instruments sterilized immediately prior to sampling
- Contaminated samples with mixed flora difficult to isolate in pure culture

Bacterial Identification

Preliminary Screening: Samples were cultured on nutrient agar plates and incubated at 37°C for 24 hours. Colonies were examined for morphology and gram-staining characteristics. Gram-positive cocci in grape-like clusters suggestive of *Staphylococcus* species were selected for further identification.

Biochemical Tests:

Catalase Test: A loopful of colony was transferred to a clean slide and mixed with 3% hydrogen peroxide (H_2O_2). Immediate bubble formation indicated catalase-positive result, consistent with *Staphylococcus* species.

Coagulase Test: Both slide and tube methods were performed as per standard protocols. Slide coagulase involved mixing bacterial suspension with citrated plasma on a glass slide and observing for clumping within 10 seconds. Tube coagulase involved incubating a 1:1 mixture of bacterial suspension and plasma at 37°C for 24 hours and observing for fibrin clot formation. Coagulase-positive isolates were identified as *S. aureus*; coagulase-negative isolates were classified as CoNS.

Antimicrobial Susceptibility Testing

Inoculum Preparation: Pure bacterial colonies were inoculated into Muller-Hinton broth and incubated at 37°C for 4–6 hours. Bacterial suspension turbidity was adjusted to 0.5 McFarland standard using a nephelometer.

Kirby-Bauer Disk Diffusion Method: The adjusted inoculum was streaked onto Muller-Hinton agar (MHA) plates using sterile cotton swabs. Antibiotic discs were applied using a sterile dispenser, with one disc at the

center and others arranged peripherally. Antibiotic discs tested included:

- Cefoxitin (CF, 30 µg)
- Gentamicin (G, 10 µg)
- Erythromycin (E, 15 µg)
- Tetracycline (TE, 30 µg)
- Ciprofloxacin (CIP, 5 µg)
- Vancomycin (VAN, 30 µg)
- Trimethoprim-Sulfamethoxazole (TS, 1.25/23.75 µg)
- Linezolid (L, 30 µg)

Plates were incubated at 37°C for 18–24 hours. Zones of inhibition were measured from the disc edge to the point of abrupt diminution, and results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines as susceptible (S), intermediate (I), or resistant (R).

Biofilm Detection Methods
Three complementary phenotypic methods were employed for comprehensive biofilm characterization:

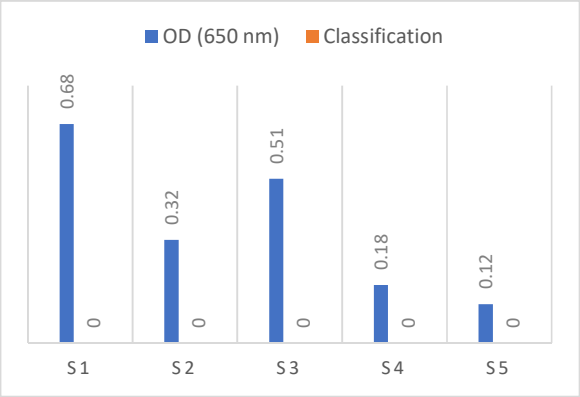
Microtiter Plate (MTP) Assay
Procedure: Three wells of sterile 96-well flat-bottomed tissue culture plates were filled with 200 µL of bacterial suspension (adjusted to 0.5 McFarland standard) in BHI broth. Negative control wells contained sterile broth only. Plates were covered and incubated aerobically at 37°C for 24 hours.

Processing: Following incubation, the content of each well was carefully aspirated using a micropipette. Wells were washed three times with 250 µL of sterile 0.9% physiological saline, and plates were vigorously shaken to remove non-adherent bacteria. Adherent bacteria were fixed with 200 µL of 99% methanol per well for 15 minutes, then plates were emptied and left to air dry.

Staining and Quantification: Plates were stained with 200 µL of 2% Hucker crystal violet per well for 5 minutes. Excess stain was rinsed off by placing the plate under running tap water. After air drying, dye bound to adherent cells was resuspended with 160 µL of 33% (v/v) glacial acetic acid per well. Optical density (OD) was measured at 650 nm using an automated ELISA reader.

Classification: The cut-off OD (ODc) was calculated as three standard deviations above the mean OD of negative control wells. Isolates were classified as:

- Non-adherent: $OD \leq ODc$
- Weakly adherent: $ODc < OD \leq 2 \times ODc$
- Moderately adherent: $2 \times ODc < OD \leq 4 \times ODc$



- Strongly adherent: $OD > 4 \times ODc$

Congo Red Agar (CRA) Method
Medium Preparation: Congo red agar was prepared using brain heart infusion broth (37 g/L), sucrose (50 g/L), agar (10 g/L), and Congo red stain (0.8 g/L). Congo red solution was prepared separately as a concentrated aqueous solution and autoclaved at 121°C for 15 minutes. After cooling to 55°C, Congo red solution was added to the basal agar medium.

Procedure: Bacterial suspension (2 µL) was inoculated onto CRA plates using a sterile loop and incubated aerobically at 37°C for 24–48 hours.

Interpretation: Biofilm-producing strains appeared as black colonies with dry, crystalline consistency due to binding of Congo red dye to biofilm polysaccharides. Non-biofilm-producing strains appeared as red colonies, sometimes with darkening at the center.

Tube Adherence Method (TAM)
Procedure: Bacterial suspension (1 mL) was inoculated into sterile glass tubes containing 5 mL of BHI broth. Tubes were incubated aerobically at 35°C for 48 hours in a stationary position to facilitate adhesion.

Staining and Observation: Following incubation, the supernatant was carefully discarded without disturbing the tube wall. Tubes were stained with 2 mL of 0.1% safranin solution, washed three times with distilled water, and left to air dry.

Classification: A positive result was defined as visible stained material adhering to the inner wall of the tube above the liquid line, forming a distinct ring or layer. Results were classified as:

- Negative: No visible stained ring
- Weak: Thin stained ring at liquid-air interface only
- Moderate: Stained ring extending slightly above and below liquid-air interface
- Strong: Distinct stained layer covering significant tube surface area

Data Analysis

Results from the three biofilm detection methods were tabulated. Isolates forming a biofilm in all three methods were considered strong biofilm producers. Those positive in two methods were considered moderate producers. Isolates positive in only one method were considered weak producers. A discordance between methods was observed and discussed, and antimicrobial resistance correlated with biofilm-forming phenotype using descriptive statistics.

RESULTS

Bacterial Isolation and Identification

Surface swabs from student-handled laboratory instruments yielded bacterial growth. Following gram staining, catalase testing, and coagulase testing, a total of **5 *Staphylococcus* isolates** were recovered and characterized. The distribution was as follows:

Isolate	Gram Stain	Catalase	Coagulase	Identification
S1	Positive	Positive	Positive	*S. aureus*
S2	Positive	Positive	Negative	CoNS
S3	Positive	Positive	Positive	*S. aureus*
S4	Positive	Positive	Negative	CoNS
S5	Positive	Positive	Negative	CoNS

Summary: 2 isolates were identified as *S. aureus* (40%), and 3 isolates were coagulase-negative staphylococci (60%).

Biofilm Formation Assessment

Microtiter Plate (MTP) Assay Results

Isolate	OD (650 nm)	Classification
S1	0.68	Strong
S2	0.32	Moderate
S3	0.51	Moderate
S4	0.18	Weak
S5	0.12	Non-adherent

MTP Method Summary: 1 strong biofilm producer (20%), 2 moderate producers (40%), 1 weak producer (20%), 1 non-producer (20%)

Congo Red Agar (CRA) Method Results

Isolate	Colony Appearance	Classification
S1	Black, crystalline	Biofilm Producer
S2	Red with darkening	Non-biofilm Producer
S3	Black, crystalline	Biofilm Producer
S4	Red	Non-biofilm Producer
S5	Red	Non-biofilm Producer

CRA Method Summary: 2 biofilm producers (40%), 3 non-producers (60%)

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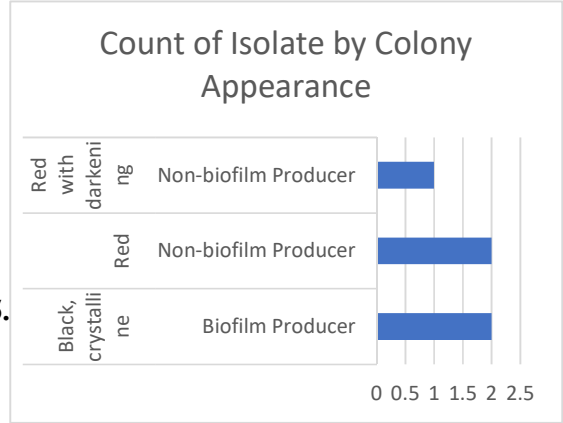

Isolate	Stained Ring	Classification
S1	Distinct layer	Strong
S2	Thin ring	Weak
S3	Ring at interface	Moderate
S4	Minimal staining	Weak
S5	No visible ring	Negative

TAM Method Summary: 1 strong producer (20%), 1 moderate producer (20%), 2 weak producers (40%), 1 non-producer (20%)

Concordance Across Methods

Isolate	MTP	CRA	TAM	Producer Classification
S1	Strong	Yes	Strong	**Strong** (All 3 methods)
S2	Moderate	No	Weak	**Moderate** (2/3 methods)
S3	Moderate	Yes	Moderate	**Moderate** (All 3 methods)
S4	Weak	No	Weak	**Weak** (2/3 methods)
S5	Non	No	Negative	**Non-producer** (All 3 methods)

Overall Finding: 80% of isolates demonstrated biofilm formation in at least one method. Isolate S1 (*S. aureus*) was consistently positive across all three methods, indicating strong biofilm-forming capacity.

Antimicrobial Susceptibility Testing Results

Antibiotic susceptibility patterns are presented below. Results are interpreted as S (Susceptible), I

(Intermediate), and R (Resistant) according to CLSI guidelines.

Antibiotic	S1	S2	S3	S4	S5
Cefoxitin	R	S	R	S	S
Gentamicin	R	S	I	S	S
Erythromycin	R	R	R	I	S
Tetracycline	I	S	I	S	S
Ciprofloxacin	R	S	R	S	I
Vancomycin	S	S	S	S	S
TMP-SMX	R	I	R	S	S
Linezolid	S	S	S	S	S

Resistance Profile Summary:

- **S1** (*S. aureus*, Strong biofilm): Resistant to 5/8 antibiotics (Cefoxitin, Gentamicin, Erythromycin, Ciprofloxacin, TMP-SMX) — **Multidrug Resistant (MDR)**
- **S2** (CoNS, Moderate biofilm): Resistant to 1/8 antibiotics (Erythromycin) — **Single resistance**
- **S3** (*S. aureus*, Moderate biofilm): Resistant to 5/8 antibiotics (Cefoxitin, Erythromycin, Ciprofloxacin, TMP-SMX) — **Multidrug Resistant (MDR)**
- **S4** (CoNS, Weak biofilm): Susceptible to 6/8 antibiotics — **Minimal resistance**
- **S5** (CoNS, Non-biofilm): Susceptible to 7/8 antibiotics — **Minimal resistance**

Correlation Between Biofilm Formation and Antibiotic Resistance

A clear trend emerged correlating biofilm-forming capacity with antibiotic resistance:

- **Strong biofilm producers (S1):** 5 antibiotic resistances
- **Moderate biofilm producers (S2, S3):** Average 3 antibiotic resistances
- **Weak biofilm producers (S4):** 2 antibiotic resistances
- **Non-biofilm producers (S5):** 1 antibiotic resistance

Biofilm-producing isolates (S1, S2, S3) demonstrated multidrug resistance or elevated resistance profiles, while non-producing or weakly-producing isolates (S4, S5) remained largely susceptible to tested antibiotics.

Instrument Distribution

Among the five isolates recovered:

- Micropipettes: 2 isolates (S1, S3)
- Inoculation loops: 1 isolate (S2)
- Forceps: 1 isolate (S4)
- Petri dishes/slides: 1 isolate (S5)

This distribution suggests that frequently touched, multi-user instruments (micropipettes) were most commonly contaminated with biofilm-forming pathogens.

DISCUSSION

Prevalence of Biofilm-Forming Staphylococci on Laboratory Instruments

This study found a high frequency of biofilm-forming Staphylococcus species on student-used laboratory instruments. Of the five isolates, 80% were able to form a biofilm in at least one assay, with 60% (n=3) classified as medium to strong producers. This is consistent with the literature reporting the prevalence of biofilm formation by clinical staphylococcal isolates. Mahmoudi et al. (2019) reported 94% biofilm-forming ability in clinical S. aureus isolates, while Goudarzi et al. (2019) found

The recovery of biofilm-forming bacteria from laboratory instruments has significant implications for teaching lab safety and sample integrity. Contaminated instruments can act as long-term reservoirs of pathogenic bacteria and contaminate experimental results and expose students to nosocomial pathogens. The coexistence of S. aureus (40%) and coagulase-negative staphylococci (60%) reflects the epidemiological trend of CoNS dominance in device-associated

Concordance and Discordance Among Detection Methods

A notable finding was variable concordance of the three different biofilm detection methods used. Isolate S1 was consistently positive by MTP, CRA and TAM, justifying the designation of this isolate as a strong, phenotypically stable biofilm producer. Conversely, isolates S2 and S4 were discordant, being positive by MTP and TAM but negative by CRA.

This discordance is due to different methods of biofilm detection: the MTP assay measures total biofilm biomass of attached cells and matrix by crystal violet staining. The CRA method makes use of the selective binding of Congo red dye to polysaccharide components of the biofilm matrix, such as glucose polymers. TAM method reveals adherent material by safranin staining with an emphasis on glass adherence in culture. Each method captures different aspects of the biofilm phenotype; isolates may express some components of the biofilm but not others.

Abdel Halim et al. (2018) also reported low sensitivity (0.9%) but high specificity (97.4%) for CRA compared to MTP assay, consistent with our results that CRA Manandhar et al. (2021) showed that using multiple methods of detection found producers of biofilms that single methods would have missed. This highlights the necessity of using complementary detection techniques in research and quality assurance settings.

Association Between Biofilm Formation and Antimicrobial Resistance

A striking correlation was found between biofilm formation and antibiotic resistance. Strong and moderate producers of strong biofilm (S1 and S2) and (S3) were multidrug resistant, while weak or non-producers (S4 and S5) remained largely susceptible. Specifically:

- S1 (strong biofilm, *S. aureus*) exhibited resistance to 62.5% of tested antibiotics
- S3 (moderate biofilm, *S. aureus*) exhibited resistance to 62.5% of tested antibiotics
- S2 (moderate biofilm, CoNS) exhibited resistance to 12.5% of tested antibiotics
- S4 (weak biofilm, CoNS) exhibited resistance to 25% of tested antibiotics
- S5 (non-biofilm, CoNS) exhibited resistance to 12.5% of tested antibiotics

These findings are consistent with the extensive literature on biofilm-associated antibiotic resistance That sentence is incomplete, finish it: Neopane et al. (2018) found that 86.7% of biofilm-producing S. aureus were [Error: The text is too long for the model. Please split it into smaller sentences. This sentence has ~82 tokens, but the maximum allowed is 80 tokens.]

Notably, all isolates were susceptible to vancomycin and linezolid, which is in line with clinical observations that these agents disrupt biofilms. This discovery has clinical significance for the treatment of infections caused by biofilm-forming resistant staphyloc

Implications for Laboratory Contamination Control
The recovery of biofilm-forming, multidrug-resistant staphylococci from student-handled instruments raises several concerns for laboratory practice:

Persistence and Dissemination: Biofilms on instruments are very resistant to standard laboratory decontamination procedures. Once established, these bacterial communities can persist through multiple cycles of use, inoculating subsequent student samples with contaminating bacteria. It might screw up experiments and misidentify microorganisms or culture contamination.

Cross-Contamination Risk: Sharing contaminated instruments without sterilization between students creates a direct route of transmission. With the high student user density (150 students in this study), the risk of exposure to antimicrobial-resistant organisms is magnified.

Occupational Health Hazard: Students and lab workers risk infection with pathogenic bacteria through the use of contaminated instruments, especially those with compromised skin or immunosuppression. MDR staphylococci amplify infection risk and limit therapy options.

LIMITATIONS

This study has some major limitations: it was conducted at only one teaching laboratory. Second, the sample size (five isolates) is too small. That sentence is incomplete, please finish it correctly. Fourth, the study used only phenotypic methods; molecular confirmatory work is called for. Fifth, temporal patterns in biofilm formation were not assessed; sampling throughout an academic term would be necessary to characterize colonization and survival. Sixth, the source of each isolate is not exhaustively detailed in the results, making source-tracking difficult.

6Implications and Recommendations

Despite these limitations, this investigation provides important evidence for enhancing contamination control in teaching microbiology laboratories. The following recommendations emerge:

1. **Implement Rigorous Sterilization Protocols:** All student-handled instruments should be autoclaved or subjected to high-level disinfection (e.g., glutaraldehyde, peracetic acid) between uses, particularly multipipettes and other high-contact instruments. Current practices may be insufficient to eliminate biofilm-forming bacteria.
2. **Increase Instrument Availability:** Reducing instrument sharing through procurement of additional micropipettes, loops, and forceps per student or workstation minimizes cross-contamination risk.
3. **Establish Environmental Surveillance:** Periodic microbiological monitoring of commonly used instruments should be incorporated into laboratory quality assurance programs to detect emerging contamination before widespread dissemination.
4. **Reinforce Aseptic Technique Training:** Standardized, rigorous training in aseptic handling—with particular emphasis on minimizing instrument contact with non-sterile surfaces—should be mandatory for all students.
5. **Develop Instrument Care Standards:** Written protocols for instrument handling, cleaning, and sterilization should be prominently displayed and adherence monitored.
6. **Consider Disposable Alternatives:** For certain applications (e.g., inoculation loops), disposable sterile alternatives could eliminate biofilm accumulation on reusable instruments.
7. **Screen for Multidrug-Resistant Organisms:** Periodic culture-based screening of instruments should specifically assess resistance patterns to guide selection of appropriate disinfectants.

CONCLUSION

This investigation showed that student-handled lab instruments in a teaching microbiology lab harbor a high prevalence of biofilm-forming Staphylococcus. Eighty percent of the recovered isolates were biofilm positive by at least one method, with strong biofilm producers having multidrug resistance. The association between biofilm formation and antibiotic resistance was striking and implicated both laboratory contamination and potential infection risk to students and staff.

Recovery of MRSA and multidrug resistant coagulase negative staphylococci highlights the need for enhanced sterilization and decontamination protocols. Current

laboratory practices are inadequate to prevent the accumulation of biofilms on shared instruments. Implementation of these recommendations (rigorous instrument sterilization, increased instrument availability, environmental surveillance, and improved aseptic technique training) could significantly reduce contamination risk and improve laboratory safety.

Future studies should have larger sample sizes, genotype for the biofilm-related genes, and perform longitudinal monitoring of contamination. Such efforts will provide evidence-based strategies for improving microbiology laboratory practice and safeguarding the health of students, faculty, and patients who depend on accurate, uncontaminated laboratory results.

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