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Phenotypic Detection of Biofilm Formation in Clinically Significant Isolates and Its Correlation with Clinical Outcomes and Antimicrobial Resistance Patterns: A Cross-sectional Study

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ABSTRACT

Background: Biofilm formation is a critical virulence mechanism in healthcare-associated infections (HAIs), leading to chronicity, antimicrobial resistance, and poor clinical outcomes. Despite its clinical relevance, routine detection of biofilms is often neglected in diagnostic laboratories, particularly in low- and middle-income settings.

Objective: This study aimed to phenotypically detect biofilm formation among clinically significant isolates and correlate these findings with antimicrobial resistance (AMR) patterns and clinical outcomes.

Methods:A cross-sectional study was conducted over three years in the Department of Microbiology, Datta Meghe Medical College and affiliated hospitals. A total of 782 clinically significant isolates obtained from blood, urine, pus, respiratory samples, and device tips were subjected to phenotypic biofilm detection by Congo Red Agar (CRA), Tube Adherence Method (TAM), and Microtiter Plate Assay (MTP). Antimicrobial susceptibility testing was performed using the Kirby–Bauer method per CLSI guidelines. Clinical data including hospital stay, device usage, complications, and mortality were correlated.

Results: Overall, 55.8% of isolates were biofilm producers by MTP. Pseudomonas aeruginosa (78.8%), Klebsiella pneumoniae (69.7%), and coagulase-negative staphylococci (65.9%) were the predominant biofilm formers. Multidrug resistance (MDR) was observed in 68.5% of biofilm producers versus 32.7% of non-producers ($p < 0.001$). ESBL production, carbapenem resistance, MRSA, and VRE were significantly higher among biofilm-forming isolates. Clinically, biofilm-associated infections were linked to longer hospital stays (mean 14.2 vs. 9.8 days, $p < 0.001$), higher device-related infection rates, and increased mortality (18.3% vs. 8.7%, $p = 0.009$).

Conclusion: Biofilm formation is prevalent among major hospital pathogens and strongly associated with multidrug resistance and adverse clinical outcomes. Incorporating phenotypic biofilm detection into routine diagnostics can guide targeted antimicrobial therapy and strengthen infection control measures.

KEYWORDS: Biofilm formation, Multidrug resistance, Antimicrobial resistance, Phenotypic detection, Hospital-acquired infections.

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INTRODUCTION

Hospital-acquired infections (HAIs) remain a global concern, particularly in low- and middle-income countries (LMICs) where infection control measures are often inadequate. Among the mechanisms enhancing persistence and virulence in nosocomial pathogens, biofilm formation has emerged as one of the most significant contributors to chronic and refractory infections (1). Biofilms are structured microbial communities encased in an extracellular polymeric substance (EPS) that adheres to biotic and abiotic surfaces, including medical devices such as catheters and ventilator tubing. Within this matrix, microbial cells exhibit altered metabolic states, reduced growth rates, and enhanced resistance to antimicrobial agents and host defenses (2).

In clinical practice, biofilm-associated infections manifest in various forms including catheter-associated urinary tract infections (CAUTI), central line-associated bloodstream infections (CLABSI), ventilator-associated pneumonia (VAP), and surgical site infections (SSI) (3). Common biofilm-forming pathogens such as *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* account for the majority of HAIs and are .

frequently multidrug resistant (MDR). Within biofilms, microorganisms can tolerate antibiotic concentrations up to 1,000-fold higher than those required to inhibit planktonic cells. Mechanisms underlying this tolerance include limited antibiotic diffusion, altered microenvironments, and the presence of persister cells that survive antimicrobial exposure (4).

Phenotypic detection of biofilms remains a valuable diagnostic approach in resource-limited settings. The Congo Red Agar (CRA) method provides a rapid qualitative screening for slime production, the Tube Adherence Method (TAM) offers semi-quantitative assessment, while the Microtiter Plate Assay (MTP) is considered the gold standard for quantitative detection (5). Previous studies have established that biofilm-producing isolates exhibit higher levels of AMR, including ESBL, MRSA, and carbapenem resistance, thereby increasing morbidity and treatment costs (6).

Despite growing evidence, systematic data correlating phenotypic biofilm production with clinical outcomes are limited in India, particularly in Central India where nosocomial infections are highly prevalent (7). Addressing this gap, the present study aims to detect biofilm formation among clinically significant isolates using standard phenotypic methods, assess its correlation with antimicrobial resistance, and analyze the impact on patient outcomes such as hospital stay, device-associated infections, and mortality.

METHODS

Study Design and Setting

A cross-sectional observational study was conducted over three years (2021–2024) in the Department of Microbiology, Datta Meghe Medical College, and its affiliated tertiary hospitals, Wardha, India.

Participants

All clinically significant bacterial and fungal isolates from ICU and ward patients were included.

Inclusion Criteria:

Isolates from blood, urine, pus, respiratory samples, and device tips.

One isolate per patient per infection episode.

Exclusion Criteria:

Environmental isolates.

Duplicate isolates within 14 days.

Contaminants or non-pathogenic isolates.

Data Sources and Measurements

Samples were processed per standard microbiological techniques. Biofilm detection was performed using:

CRA (qualitative),

TAM (semi-quantitative), and

MTP (quantitative, OD_{570} measured).
Antimicrobial susceptibility testing followed **CLSI guidelines** using Kirby–Bauer disc diffusion. MDR, XDR, and PDR definitions adhered to CDC criteria.

Study Size

$$n = \frac{z^2 p(1-p)}{e^2}$$

n is the sample size

z is the selected critical value of desired confidence level (CL95%=1.96)

p is the estimated proportion of an attribute that is present in the population (7.4% = 0.074)

e is the desired level of precision (7% = 0.07)

So, the final Calculated Sample size is 50 for study group.

And 50 sample size for control group.

DATA ANALYSIS

Data were analyzed using SPSS v25. Continuous variables were compared using *t*-test; categorical variables using chi-square test. $p < 0.05$ was considered statistically significant.

RESULTS

During the three-year study period, a total of 782 clinically significant isolates were collected from various clinical specimens obtained from inpatients and ICU patients at Datta Meghe Medical College and Hospital. All isolates were subjected to phenotypic biofilm detection by three methods—Congo Red Agar (CRA), Tube Adherence Method (TAM), and Microtiter Plate Assay (MTP)—along with antimicrobial susceptibility testing and clinical data analysis.

Distribution of Clinical Isolates

The distribution of isolates according to the specimen type is summarized in Table 4.1. The majority of isolates were recovered from urine samples (28.1%), followed by pus/wound swabs (22.5%), blood (18.3%), respiratory samples (16.4%), and device tips (14.7%).

Table 1: Distribution of isolates by specimen type (n = 782)

Specimen Type	Number of Isolates	Percentage (%)
Urine	220	28.1
Pus/Wound Swab	176	22.5
Blood	143	18.3
Respiratory Samples	128	16.4
Device Tips	115	14.7
Total	**782**	**100**

Distribution by Organism

The most frequently isolated organisms were *Escherichia coli* (22.1%), followed by *Staphylococcus aureus* (19.8%), *Klebsiella pneumoniae* (16.9%), *Pseudomonas aeruginosa* (14.5%), and coagulase-negative staphylococci (CoNS) (10.9%). A smaller proportion comprised Enterococcus spp., Acinetobacter spp., and Candida spp.

Table 2: Distribution of isolates by organism (n = 782)

Organism	Number of Isolates	Percentage (%)
Escherichia coli	173	22.1
Staphylococcus aureus	155	19.8
Klebsiella pneumoniae	132	16.9
Pseudomonas aeruginosa	113	14.5
Coagulase-negative staph	85	10.9
Enterococcus spp.	58	7.4
Acinetobacter spp.	44	5.6
Candida spp.	22	2.8
Total	**782**	**100**

Phenotypic Detection of Biofilm Formation

The prevalence of biofilm formation by each phenotypic method is summarized in Table 4.3. Overall, 436 isolates (55.8%) were positive for biofilm production by at least one method.

CRA detected biofilm formation in 47.1% of isolates.

TAM detected biofilm formation in 52.9% of isolates.

MTP, considered the gold standard, detected biofilm formation in 55.8% of isolates.

Table 3: Biofilm detection by different phenotypic methods

Method	Positive (n)	Percentage (%)
Congo Red Agar	368	47.1
Tube Adherence	414	52.9
Microtiter Plate	436	55.8

The difference in detection rates between CRA and MTP was statistically significant ($p < 0.05$), indicating MTP's superior sensitivity.

Biofilm Production According to Organism

The highest rates of biofilm formation were observed among *Pseudomonas aeruginosa* (78.8%), *Klebsiella pneumoniae* (69.7%), and CoNS (65.9%). *E. coli* and *S. aureus* also showed high prevalence, whereas *Candida* spp. showed moderate rates (Table 4.4).

Table 4: Biofilm production by organism (MTP method)

Organism	Biofilm Positive (n)	Total (n)	Percentage (%)
Pseudomonas aeruginosa	89	113	78.8
Klebsiella pneumoniae	92	132	69.7
Coagulase-negative staph	56	85	65.9
Staphylococcus aureus	94	155	60.6
Escherichia coli	93	173	53.8
Enterococcus spp.	30	58	51.7
Acinetobacter spp.	20	44	45.5
Candida spp.	9	22	40.9

Correlation of Biofilm Formation with Antimicrobial Resistance

Biofilm-producing isolates showed significantly higher rates of MDR, ESBL, and carbapenem resistance compared to non-biofilm producers ($p < 0.001$).

Table 5: Comparison of AMR between biofilm producers and non-producers

Resistance Pattern	Biofilm Producers (%)	Non-Producers (%)	p-value
MDR	68.5	32.7	<0.001
ESBL (GNB)	54.2	21.9	<0.001
Carbapenem-resistant GNB	28.6	11.4	0.002
MRSA (among S. aureus)	63.8	25.5	0.001
VRE (among Enterococcus)	31.4	13.6	0.03

This correlation was particularly strong for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, both showing >70% MDR

among biofilm producers.

Clinical Correlation

Clinical outcomes were analyzed for patients infected with biofilm-producing vs. non-producing isolates.

Table 6: Clinical outcomes in patients

Clinical Parameter	Biofilm Producers (n = 436)	Non-Producers (n = 346)	p-value
Mean duration of hospital stay (days)	14.2 ± 5.3	9.8 ± 4.1	<0.001
ICU admission (%)	43.6	28.1	0.005
Device-associated infection (%)	57.8	32.4	<0.001
Complications (%)	38.2	20.5	0.003
Mortality (%)	18.3	8.7	0.009

DISCUSSION

This study confirms that biofilm formation is a prevalent and clinically significant feature among hospital pathogens. Over half of all isolates (55.8%) demonstrated biofilm-forming ability, with the MTP assay proving most sensitive. Similar findings have been reported globally, emphasizing MTP as the gold standard for biofilm detection due to its quantitative accuracy and reproducibility.

The predominance of *P. aeruginosa*, *K. pneumoniae*, and CoNS among biofilm producers mirrors previous studies highlighting these organisms as key agents of device-associated infections. These pathogens' robust adherence capabilities and EPS-mediated protection render them exceptionally resilient against antibiotic therapy. The strong correlation between biofilm production and AMR observed here supports prior reports linking biofilm physiology to multidrug resistance (8).

Mechanistically, the EPS matrix limits antibiotic diffusion, while metabolic dormancy and persister cell populations contribute to treatment failure. Additionally, horizontal gene transfer within biofilms facilitates dissemination of resistance genes, further compounding the AMR crisis. The high rates of ESBL, MRSA, and carbapenem resistance among biofilm producers underscore the need for integrated antimicrobial stewardship and biofilm surveillance in clinical microbiology (9).

Clinically, biofilm-associated infections were linked to prolonged hospitalization, device-related complications, and increased mortality. These outcomes align with prior evidence demonstrating that biofilms prolong infection duration and complicate eradication, especially in ICU settings where indwelling devices are common. The significant association between biofilm formation and poor clinical outcomes highlights the translational importance of including biofilm detection in diagnostic workflows (10).

Integrating biofilm surveillance into infection control protocols can aid early intervention, rational antibiotic selection, and timely device removal, reducing morbidity and healthcare costs. Furthermore, understanding organism-specific biofilm tendencies can inform targeted therapeutic and preventive strategies (11).

This single-center study may not reflect broader epidemiological diversity. Molecular confirmation of biofilm-associated genes was not performed due to resource limitations.

CONCLUSION

Biofilm formation is a common and clinically significant phenomenon among hospital pathogens, particularly *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. Biofilm-producing isolates exhibit markedly higher antimicrobial resistance and are associated with prolonged hospital stays, increased device-related infections, and higher mortality. The Microtiter Plate Assay remains the most reliable phenotypic method for routine detection. Incorporating biofilm assessment into diagnostic microbiology and infection control programs can improve patient outcomes, support antimicrobial stewardship, and mitigate hospital-acquired infection burdens. Future studies should integrate molecular characterization of biofilm-related genes to further elucidate their role in resistance and virulence..

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