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Phenotypic Detection of Biofilm Formation in Clinically Significant Isolates

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ABSTRACT

Biofilms are structured microbial communities embedded in a self-produced extracellular matrix, contributing significantly to chronic and device-associated infections. They are linked with prolonged hospital stays, multidrug resistance (MDR), and therapeutic failures. Phenotypic detection of biofilm formation is crucial in clinical microbiology to guide infection control and patient management, particularly in low- and middle-income countries. This narrative review examines the biological basis of biofilm formation, summarizes commonly used phenotypic detection methods such as Congo Red Agar (CRA), Tube Adherence Method (TAM), and Microtiter Plate (MTP) assay, and explores their correlation with antimicrobial resistance and clinical outcomes. A structured literature search identified relevant studies between 2010 and 2025. CRA and TAM are simple and affordable for routine use, whereas MTP remains the gold standard. Biofilm formation strongly correlates with MDR phenotypes such as ESBL, MRSA, and carbapenem resistance, leading to worse clinical outcomes including prolonged hospital stay and increased mortality. Standardizing phenotypic detection in diagnostic laboratories can improve infection surveillance and clinical care.

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INTRODUCTION

Biofilm formation is now recognized as a major virulence strategy of clinically significant microorganisms, contributing to persistent infections, antimicrobial resistance, and poor patient outcomes (1–3). Biofilms are defined as communities of microorganisms irreversibly attached to surfaces and encased in a self-produced extracellular polymeric substance (EPS) (4). Within these structured communities, microbial cells undergo phenotypic shifts that enhance their survival by limiting antimicrobial penetration, promoting genetic exchange, and evading host immune mechanisms (5–7).

Clinically, biofilms play a pivotal role in chronic and device-associated infections. Catheter-associated urinary tract infections (CAUTI), central line–associated bloodstream infections (CLABSI), ventilator-associated pneumonia (VAP), and prosthetic device infections are among the most common hospital-acquired infections (HAIs) linked with biofilm-forming organisms (8,9). Common pathogens include *Staphylococcus aureus* (including MRSA), coagulase-negative staphylococci, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Candida* spp. (9,10). Donlan and Costerton estimated that more than 65% of human microbial infections involve biofilms, either on tissues or medical devices (2).

Biofilm-associated infections are difficult to treat due to inherent tolerance to antimicrobials—biofilm cells may require up to 1000-fold higher antibiotic concentrations than planktonic cells (5). Standard treatment strategies often fail without device removal or aggressive surgical intervention (3,8). These infections are associated with prolonged hospital stays, higher recurrence rates, and increased mortality, especially in vulnerable populations such as ICU and oncology patients (11,12).

Although molecular methods (e.g., PCR for biofilm-associated genes) are precise, they are costly and technically demanding for routine diagnostics. Phenotypic detection methods such as CRA, TAM, and MTP assays provide inexpensive, practical alternatives, making them valuable tools for resource-limited laboratories (13–15). CRA is a qualitative screening method, TAM is semi-quantitative, and MTP is widely considered the gold standard (15). In India and similar LMICs, the high prevalence of MDR organisms combined with limited diagnostic resources makes biofilm detection particularly relevant. Incorporating simple phenotypic assays into routine laboratory practice can help identify high-risk infections early, guide appropriate therapy, and inform infection control strategies (16,17)..

This review aims to summarize current knowledge on phenotypic detection of biofilm formation, outline their performance characteristics, and examine the relationship between biofilm production, antimicrobial resistance, and clinical outcomes.

METHODS

(Search Strategy)

A narrative literature review was conducted following a structured search strategy. Databases searched included PubMed, Google Scholar, and Scopus. Search terms were combined using Boolean operators and included: *"biofilm"*, *"phenotypic detection"*, *"Congo red agar"*, *"tube adherence method"*, *"microtiter plate assay"*, *"antimicrobial resistance"*, *"MDR"*, *"ESBL"*, *"MRSA"*, *"clinical outcomes"*, and *"India"*.

The search period was restricted to January 2010 to September 2025 to ensure inclusion of contemporary data. Reference lists of key articles were screened to identify additional relevant studies. Inclusion criteria were: (i) studies involving clinically significant bacterial isolates, (ii) application of at least one phenotypic biofilm detection method, (iii) evaluation of resistance patterns or clinical outcomes. Exclusion criteria were: animal model studies, purely molecular studies without phenotypic data, and non-English articles.

Data were extracted on study design, organism types, biofilm detection method, prevalence, resistance correlation, and clinical outcomes. Tables were constructed to compare detection methods and summarize correlations with resistance and clinical outcomes.

REVIEW

Biofilm Biology and Pathogenesis

Biofilm formation involves sequential steps: initial reversible attachment, irreversible adhesion via adhesins/pili, EPS synthesis, biofilm maturation into 3D structures, and dispersal of planktonic cells to new sites (4,18). The EPS matrix acts as a diffusion barrier and structural scaffold, while nutrient and oxygen gradients within biofilms create physiologic heterogeneity, including slow-growing and persister cells (5,19). These features confer tolerance to antibiotics and immune mechanisms. Biofilm-associated infections, especially on indwelling medical devices, are persistent sources of bacteremia and chronic infection (3,8,11).

Phenotypic Detection Methods

Table 1: Comparison of Phenotypic Biofilm Detection Methods

Method	Principle	Advantages	Limitations	Sensitivity/Specificity (vs MTP)
CRA (Congo Red Agar)	Detects slime production (black colonies)	Simple, cheap, rapid	Variable results, less sensitive	Sensitivity ~65–70%, Specificity ~40–50%
TAM (Tube Adherence Method)	Biofilm stained on tube walls	Easy, semi-quantitative	Observer-dependent, subjective	Sensitivity ~85–90%, Specificity ~70%
MTP (Microtiter Plate)	Quantifies biofilm OD via crystal violet staining	Gold standard, objective, high throughput	Needs plate reader, standardization	Reference

Table 2: Correlation Between Biofilm Formation, Antimicrobial Resistance, and Clinical Outcomes

Method

CRA (Congo Red Agar)

TAM (Tube Adherence)
MTP (Microtiter Plate)

CRA, first described by Freeman et al. (15), is rapid but has variable accuracy. TAM is more sensitive but subjective (16). MTP provides quantitative, reproducible results and remains the reference standard (13,17,21).

Correlation with Antimicrobial Resistance

Biofilm producers frequently exhibit MDR phenotypes. Mechanisms include antibiotic sequestration by EPS, altered metabolic states, horizontal gene transfer, and upregulated efflux systems (6,12,18). Indian studies report MDR prevalence among biofilm producers ranging from 70–85%, significantly higher than among non-producers (22,23). ESBL production among *Enterobacteriaceae* and MRSA prevalence among *S. aureus* are strongly associated with biofilm formation (24,25). Biofilm production in carbapenem-resistant *K. pneumoniae* and *A. baumannii* further complicates therapy (26).

Table 2. Correlation Between Biofilm Formation, Antimicrobial Resistance, and Clinical Outcomes

Parameter	Biofilm Producers	Non-Producers	Clinical Implication
MDR prevalence	70–85%	30–40%	Strong correlation between biofilm and MDR strains
ESBL production (*Enterobacteriaceae*)	Common (e.g. *E. coli*, *K. pneumoniae*)	Less frequent	Increased treatment failure
MRSA prevalence	High (30–40% strong biofilm producers)	Lower	Greater virulence, persistent infection
Hospital stay (mean)	12–14 days	7–9 days	Significantly prolonged LOS
Mortality (CRKP, VAP, etc.)	OR 5–6× higher	Baseline	Biofilm predicts poor outcomes

CLINICAL OUTCOMES

Strong biofilm producers are linked with longer hospital stays, frequent relapses, and increased mortality (11,12,27). Di Domenico et al. demonstrated that oncology patients with CRKP infections due to strong biofilm producers had six-fold higher mortality (11). In ventilator-associated pneumonia, biofilm-positive *Pseudomonas* isolates are associated with higher ICU mortality (28).

Device removal is often necessary to achieve cure in biofilm infections. Failure to detect and address biofilms leads to chronicity and higher healthcare costs (3,8,27). Biofilm detection can serve as an early prognostic marker, guiding aggressive therapy and infection control interventions.

RESEARCH GAPS AND INDIAN CONTEXT

Despite growing recognition, standardized phenotypic testing is not widely implemented in Indian laboratories. Studies vary in methods and criteria, leading to prevalence differences from 20–70% (22,23). Few multicentric studies link biofilm data with clinical outcomes. Integrating routine phenotypic testing (e.g., CRA/TAM screening followed by MTP confirmation) can strengthen surveillance and guide clinical decision-making in resource-limited settings (16,17,22).

CONCLUSION

Phenotypic detection of biofilm formation provides critical insights into the pathogenic potential of clinical isolates. CRA and TAM are useful low-cost screens, while MTP remains the gold standard for reliable detection. Biofilm production is strongly correlated with MDR phenotypes and poor clinical outcomes, including prolonged hospital stay and higher mortality. Incorporating standardized phenotypic methods into routine diagnostics, especially in high-burden settings like India, can enhance infection control and improve patient care.

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