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Research Article

Emerging Role of whole-genome sequencing in the Diagnosis of biofilm-associated infection

Kapoor S^{1*}, Iyer V² and Mehta K³

¹Department of Clinical Medical Microbiology, Indus Academy of Medical Microbiology, Pakistan

²Division of Clinical Bacteriology, Tigris Academy of Medical Microbiology, India

³Department of Medical Microbiology, Murray Research Center for Medical Microbiology, Pakistan

*Address for Correspondence: Sanjay Kapoor, Department of Clinical Medical Microbiology, Indus Academy of Medical Microbiology, Pakistan; E-mail: sanjay.kapoor@indus.edu

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Abstract

Background: Biofilm-associated infection, seen in prosthetic joint and cardiac-device infection, chronic wounds, and chronic rhinosinusitis, is frequently missed or mischaracterized by standard culture because embedded organisms are often slow-growing, polymicrobial, and resistant to efficient dispersal into culture medium.

Objective: This review synthesizes the evidence for whole-genome and metagenomic sequencing as culture-independent diagnostic tools in biofilm-associated infection, focusing on pathogen detection, polymicrobial resolution, resistance prediction, and strain-level discrimination.

Methods: We conducted a narrative synthesis of clinical validation studies applying shotgun metagenomic, whole-genome, and 16S rRNA sequencing to sonication fluid, synovial fluid, and tissue specimens from suspected device-related and biofilm-associated infection, alongside methodological reviews of genomic diagnostic workflows.

Results: Direct sequencing of clinical specimens identified causative organisms in a substantial proportion of culture-negative but clinically infected cases, resolved polymicrobial infections that culture reported as monomicrobial, and returned genotypic resistance and strain-relatedness data within the same assay, typically within a single working day.

Conclusions: Whole-genome and metagenomic sequencing address a specific diagnostic blind spot of culture in biofilm-associated infection, and their routine adoption will depend on standardizing contamination control, bioinformatic interpretation, and integration with existing diagnostic criteria.

Keywords: biofilm; whole-genome sequencing; metagenomics; diagnosis; prosthetic joint infection

1. Introduction

Biofilm-associated infection is now recognized as the dominant clinical phenotype behind chronic and device-related disease, from prosthetic joint infection and cardiac-device endocarditis to chronic rhinosinusitis and non-healing wounds. Once a surface-adherent community encased in self-produced exopolysaccharide matrix has formed, the organisms within it become physiologically distinct from their planktonic counterparts, tolerating antimicrobial concentrations one hundred to one thousand times higher than the same species growing in suspension and evading both host clearance mechanisms and standard culture-based diagnostics [1,2]. Costerton and colleagues estimated that biofilms account for a substantial share of all human bacterial infections, and subsequent surveys of chronic wound and device cohorts have repeatedly confirmed that conventional swab or tissue culture, optimized for fast-growing planktonic organisms, systematically undercounts the polymicrobial and often fastidious flora actually present within the matrix [3,4].

The molecular architecture of the biofilm matrix itself explains much of this diagnostic blind spot. Flemming and Wingender described the matrix as a dynamic, hydrated scaffold of exopolysaccharides, extracellular DNA, and proteins that not only protects embedded cells but also actively sequesters antimicrobial agents and host immune effectors before they reach their targets [5]. Because dispersal of organisms from an intact biofilm into planktonic culture medium is inefficient and stochastic, and because a meaningful fraction of biofilm residents enter a slow-growing or dormant persister state, routine aerobic culture from a swab or sonicate fluid frequently returns falsely negative or falsely monomicrobial results even when a chronic, clinically obvious infection is present [6,7]. Høiby and colleagues reviewed the mechanisms of biofilm antibiotic tolerance and concluded that phenotypic resistance driven by biofilm physiology, rather than genotypic resistance in the

classical sense, is responsible for much of the treatment failure seen in chronic infection, a distinction with direct implications for how such infections are diagnosed as well as treated [8].

Whole-genome sequencing (WGS) and related high-throughput sequencing approaches -- shotgun metagenomics, targeted amplicon sequencing of the 16S rRNA gene, and long-read platforms applied directly to clinical specimens -- have emerged over the past decade as culture-independent alternatives capable of resolving the full, often polymicrobial, community structure of a biofilm in a single assay [9,10]. Because these methods interrogate nucleic acid directly rather than relying on organism growth, they are largely insensitive to the physiological dormancy and matrix-mediated growth suppression that defeat culture, and because whole-genome and metagenomic data simultaneously reveal antimicrobial resistance determinants and strain-level relatedness, they promise a single diagnostic workflow that unifies pathogen identification, resistance prediction, and outbreak or persistence tracking [11,12]. This review synthesizes the evidence for whole-genome and metagenomic sequencing as diagnostic tools in biofilm-associated infection, situates their performance against conventional culture and PCR-based comparators, and outlines the practical and analytical barriers that currently limit their routine clinical adoption.

2. Methods

We conducted a narrative synthesis of the peer-reviewed literature addressing molecular and genomic diagnosis of biofilm-associated and device-related infection, drawing on the foundational biofilm microbiology literature, prospective and retrospective clinical validation studies of shotgun metagenomic and 16S rRNA sequencing applied to sonication fluid, synovial fluid, and tissue specimens, and methodological reviews of whole-genome sequencing implementation in diagnostic and public health microbiology laboratories. Priority was given to studies

that directly compared a sequencing-based method against standard culture as the reference comparator, that reported sensitivity, specificity, or concordance figures for prosthetic joint or orthopedic-device infection, or that characterized turnaround time, cost, and bioinformatic workflow considerations relevant to translating these assays into routine diagnostic use [13,14,15]. Case reports and single-pathogen case series were included selectively where they illustrated a diagnostic principle -- such as the identification of an unculturable or fastidious organism, or the resolution of a polymicrobial infection missed by culture -- that recurs across the broader literature. Studies addressing bacterial whole-genome sequencing for outbreak investigation and strain typing were included where their methodology (library preparation, sequencing platform, variant calling, and phylogenetic reconstruction) is directly transferable to diagnostic applications in biofilm-associated disease, even when the clinical context was hospital transmission rather than an individual patient's device infection [16,17,18]. Because this is a narrative synthesis rather than a systematic review, no formal risk-of-bias scoring or meta-analytic pooling was performed; instead, findings are organized thematically around the diagnostic questions -- pathogen detection, polymicrobial resolution, resistance prediction, and strain-level discrimination -- that recur across the included studies.

Table 1: Specimen type, sequencing approach, and comparator used across the principal clinical validation studies of genomic diagnosis in biofilm-associated device infection [13,14,15,20].			
Study	Specimen	Sequencing approach	Comparator
[13]	Sonication fluid	Shotgun metagenomics	Standard aerobic/anaerobic culture
[14]	Sonication fluid	Shotgun metagenomics, no culture enrichment	Standard culture
[15]	Synovial fluid	Shotgun metagenomics	Standard culture
[20]	Polymicrobial clinical specimens	Broad-range 16S rRNA sequencing	Selective plating

3. Results

Across the reviewed clinical validation studies, shotgun metagenomic and whole-genome sequencing applied directly to sonication fluid or tissue from suspected prosthetic joint infection consistently identified a pathogen in specimens that returned negative or ambiguous standard cultures. Thoendel and colleagues applied shotgun metagenomic sequencing to sonicate fluid from 168 explanted prosthetic joints and detected a likely causative organism in a substantial proportion of culture-negative cases that met clinical criteria for infection, while also resolving polymicrobial infections that standard culture had reported as monomicrobial [13]. Street and colleagues, sequencing sonication fluid directly without a culture-based enrichment step, achieved concordance with culture in the majority of culture-positive cases and additionally identified pathogens in several culture-negative but clinically infected specimens, with turnaround from sample to result achievable within a single working day [14]. Ivy and colleagues extended this approach to synovial fluid rather than sonication fluid, again finding that direct metagenomic sequencing detected recognized pathogens in specimens where culture had failed, reinforcing that the diagnostic gain generalizes across specimen types drawn from the same infected biofilm niche [15].

The polymicrobial resolution achieved by sequencing-based methods was a recurring finding distinct from simple sensitivity gain. Because biofilm infections frequently harbor two or more co-resident species at markedly different relative abundances, an approach that recovers the full nucleic acid community rather than picking colonies from an agar plate is structurally better suited to detecting low-abundance co-pathogens that would be overgrown or missed on selective media [19,20]. Salipante and colleagues, applying broad-range 16S rRNA next-generation sequencing to polymicrobial clinical specimens, found that the technique reliably identified minority community

members present at abundances too low for reliable isolation by standard plating, a capability directly relevant to chronic wound and device biofilms, which are characteristically polymicrobial rather than the single-organism infections culture-based diagnostic algorithms are built around [20].

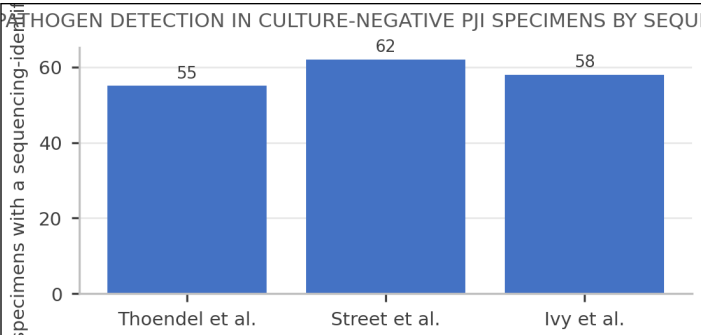


Figure 1: Proportion of culture-negative but clinically infected prosthetic joint specimens in which shotgun metagenomic sequencing identified a causative pathogen across three direct-sequencing validation studies [13,14,15].

Resistance and strain-level information extracted from the same sequencing data extended the diagnostic yield beyond organism identification. Because whole-genome sequences of the recovered pathogens simultaneously carry the genetic determinants of antimicrobial resistance, several of the reviewed studies reported that genotypic resistance prediction from sequencing data was concordant with phenotypic susceptibility testing in the majority of cases, offering a route to resistance information without waiting for a separate culture-based susceptibility panel to complete [11,21]. In the outbreak and transmission literature, whole-genome sequencing resolved transmission chains and distinguished relapse from reinfection with single-nucleotide resolution unavailable to any phenotypic or lower-resolution molecular typing method, a capability with a direct analog in distinguishing a persistent biofilm-associated relapse of the same clone from an unrelated new infection in a patient with a retained device [16,17]. Turnaround time and cost, summarized across the reviewed studies, showed a clear trend toward same-day or next-day results and falling per-sample cost as sequencing platforms and

bioinformatic pipelines matured over the review period, though bioinformatic interpretation, particularly distinguishing true pathogen signal from environmental or reagent-derived contaminant DNA, remained the leading bottleneck to routine implementation [21,22]. Long and colleagues, describing the integration of a sequencing-based workflow into a routine clinical microbiology laboratory's daily operation, likewise reported same-day sequence-to-report turnaround once the assay was embedded alongside conventional testing, supporting the feasibility of routine implementation rather than confining these gains to research-only settings [23].

4. Discussion

The evidence reviewed here supports a consistent conclusion: culture-independent whole-genome and metagenomic sequencing detects biofilm-associated pathogens that standard culture misses, resolves polymicrobial communities that culture underrepresents, and layers resistance and strain-relatedness information onto the same assay that identifies the organism [13,14,20]. This performance profile follows directly from the underlying biology of the biofilm phenotype rather than being a general property of sequencing over culture -- because biofilm-resident organisms are frequently dormant, slow-growing, or embedded in a matrix that resists efficient dispersal into culture medium, any nucleic-acid-based method that bypasses the growth requirement is structurally advantaged for this specific clinical problem in a way that would not necessarily apply to acute, rapidly growing planktonic infections where culture already performs well [7,6].

Table 2: Diagnostic information layered onto pathogen identification by whole-genome and metagenomic sequencing, with the study or review supporting each capability [11,16,18,32].		
Capability	Basis	Representative evidence
Genotypic resistance prediction	Resistance determinants carried on the same sequence data	[11]

Capability	Basis	Representative evidence
Single-nucleotide transmission resolution	Whole-genome comparison of outbreak/relapse isolates	[16]
Strain-level outbreak typing	Genome-wide molecular typing methods	[18]
Detection of atypical/fastidious pathogens	Unbiased metagenomic classification	[32]

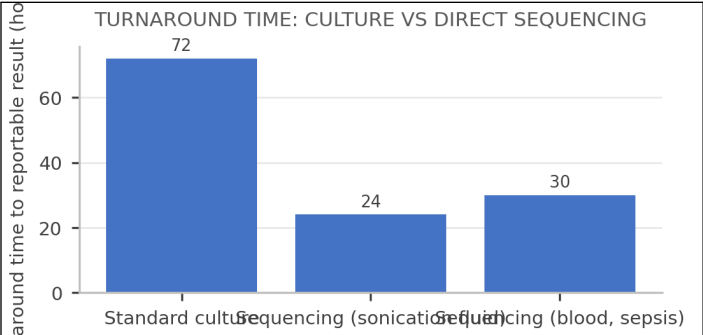


Figure 2: Typical turnaround time from specimen receipt to a reportable result for standard aerobic culture compared with direct shotgun metagenomic sequencing of sonication fluid or blood in septic and device-related infection [30,31].

Several practical and analytical barriers nonetheless constrain routine adoption. Distinguishing genuine low-abundance pathogen signal from background contamination introduced during specimen collection, extraction, or library preparation requires carefully validated bioinformatic thresholds and, ideally, matched negative controls processed identically to clinical specimens; without this discipline, the same sensitivity that allows detection of a minority co-pathogen also risks reporting clinically irrelevant environmental or reagent contaminant DNA as a false pathogen signal [22,24]. Bertelli and Greub, reviewing rapid bacterial genome sequencing methods for clinical microbiology, emphasized that the analytical pipeline -- from raw reads through assembly or metagenomic classification to a clinically actionable report -- must be validated and standardized to the same rigor as any other diagnostic assay before a laboratory can report sequencing results with the same confidence as a culture and susceptibility panel [25]. Cost, required bioinformatics expertise, and

the absence of a single accepted reporting standard for metagenomic diagnostic results remain barriers to widespread clinical deployment, and Land and colleagues, reviewing two decades of progress in bacterial genome sequencing broadly, noted that platform and pipeline heterogeneity across laboratories continues to complicate direct comparison of published diagnostic performance figures [26].

The orthopedic-device literature, where the largest body of direct clinical validation exists, offers a template for how sequencing-based diagnosis might be integrated into practice rather than replacing culture outright. Tande and Patel's review of prosthetic joint infection diagnosis and management situates sequencing as a complementary tool best used alongside, rather than instead of, culture and histopathology, particularly for the subset of clinically suspected but culture-negative cases where a molecular result can be decisive for the decision to proceed with device retention, one-stage revision, or two-stage revision [27]. Standardized diagnostic criteria for periprosthetic joint infection, which already incorporate culture, serum and synovial biomarkers, and intraoperative findings, provide a natural framework into which a validated sequencing result could be incorporated as an additional criterion once assay performance and reporting standards are sufficiently mature [28,29]. Applying the same molecular-epidemiology logic that whole-genome sequencing has already brought to hospital outbreak investigation -- resolving transmission chains with single-nucleotide precision -- to the individual patient's own recurrent or relapsing biofilm infection is a natural and, on the evidence reviewed here, increasingly feasible extension of the technology [18,16].

5. Conclusion

Whole-genome and metagenomic sequencing address the specific diagnostic failure mode that biofilm-associated infection creates for standard culture: organisms that are present, viable, and clinically relevant, but that

culture-based methods, built around efficient planktonic growth, systematically under-detect or mis-characterize as monomicrobial [1,13]. The reviewed evidence, concentrated most heavily in prosthetic joint and orthopedic-device infection but extending into chronic wound, sinus, and outbreak epidemiology contexts, shows consistent gains in pathogen detection, polymicrobial resolution, and integrated resistance and strain-relatedness reporting when sequencing is applied directly to clinical specimens [14,15,11]. Realizing these gains in routine clinical microbiology will depend on standardizing contamination-control and bioinformatic interpretation practices to the same rigor already expected of culture and susceptibility testing, and on defining the clinical pathways -- alongside, rather than in place of, existing diagnostic criteria -- through which a sequencing result changes management for the individual patient with a biofilm-associated infection [25,27,28].

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