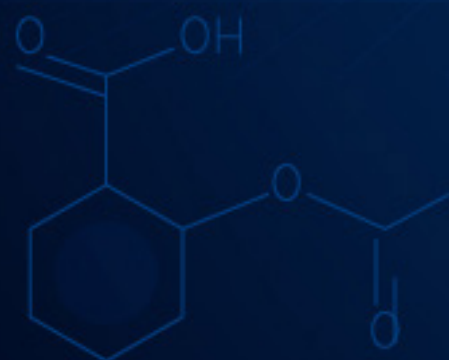




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## Molecular Identification and Virulence Profiling of Emerging Infectious Micro-Organisms

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### ABSTRACT

The emergence of multidrug-resistant bacteria, zoonotic viruses, and invasive fungal pathogens poses a compounding threat to global public health, and conventional phenotypic identification lacks the resolution needed for actionable pathogen characterization. Integrated molecular identification and virulence profiling frameworks are therefore needed. We conducted a mixed-methods systematic review across five databases (PubMed/MEDLINE, Scopus, Web of Science, Embase, Cochrane Library) from January 2015 to December 2024, following PRISMA 2020 guidelines and JBI/MMAT appraisal; we included 134 studies in the narrative synthesis. A parallel cross-sectional survey (10-item Likert scale) was administered to 300 professionals via REDCap and analyzed in SPSS v29.0 using chi-square and Kruskal-Wallis tests; internal consistency was low (Cronbach's alpha = -0.26), consistent with a formative rather than reflective item structure, so items were analyzed and reported individually. Total of 300 invited professionals, 247 responded (82.3%). Quantitative PCR and MALDI-TOF MS were the most common platforms; whole-genome sequencing was familiar to 69.2% but used routinely by only 21.5%. Virulence profiling was absent or marginal in 18.7% of settings. Experts strongly endorsed the clinical relevance of AMR-virulence co-occurrence (85.8% agreement) and identified the LMIC implementation gap as the most critical concern (92.3% agreement), with bioinformatics-expertise shortage, equipment cost, and funding as leading barriers. Molecular identification and virulence profiling technologies are scientifically validated yet structurally constrained in global implementation. AMR-virulence co-profiling must be integrated into clinical microbiology and antimicrobial stewardship practice, and closing the LMIC implementation gap requires investment in bioinformatics capacity, open-access genomic infrastructure, and One Health surveillance integration.

### INTRODUCTION

The emergence and re-emergence of infectious microorganisms constitute one of the most consequential threats to global public health in the twenty-first century. Communicable pathogens account for an estimated 28% of total global disease burden in disability-adjusted life years (DALYs) (Naghavi *et al.*, 2024), and viral infectious diseases alone caused approximately 8.7 million deaths and 259.2 million DALYs in 2021 (Li *et al.*, 2024). A comprehensive analysis of WHO emergency event reports from 1996 to 2023 documented over 3,000 outbreak events involving pathogens such as influenza, Ebola virus, and MERS-CoV (Liu *et al.*, 2025), underscoring that novel and re-emergent microorganisms spanning bacterial, viral, and fungal kingdoms are breaching established epidemiological boundaries at an accelerating rate. Emerging infectious microorganisms are defined not solely by their novelty of appearance in a given host population but increasingly by the convergence of enhanced virulence with antimicrobial resistance (AMR), zoonotic spillover potential, and genomic plasticity that facilitates rapid adaptation to hostile host environments (Ristori *et al.*, 2024), a process accelerated by urbanization, global travel, climate change, and intensive agriculture.

Among bacterial pathogens, members of the ESKAPE group, most notably carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and *Acinetobacter baumannii* (CRAB), exemplify this convergence. Clinical isolates of *K. pneumoniae* frequently exhibit multidrug resistance through mechanisms such as beta-lactamase production, efflux pump activity, and biofilm formation (Juliet & Nachimuthu, 2025; Azra *et al.*, 2025). Whole-genome sequencing of 374 CRKP isolates collected between 2022 and 2024 revealed that over 57% met criteria for carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP), with the predominant blaKPC-2-producing ST11 clone carrying multiple virulence determinants alongside carbapenem resistance (Lian *et al.*, 2025); the WHO has formally recognized the international spread of hypervirulent *K. pneumoniae* as an escalating AMR concern warranting close global surveillance (WHO, 2024). The viral landscape continues to evolve rapidly. Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b viruses have demonstrated sustained spread across wild bird reservoirs, domestic poultry, and an expanding range of mammalian hosts, raising significant One Health concerns given the virus's capacity for genetic evolution and clade divergence (Sun *et al.*, 2025; Basheer *et al.*,

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2025; Oltean *et al.*, 2025), with molecular characterization identifying critical receptor-binding site mutations and polybasic HA cleavage site motifs indicative of high pathogenicity (Yu *et al.*, 2024). SARS-CoV-2 continues to evolve through immune-evasive variants, while mpox virus (clade IIB) generated sustained genomic surveillance interest following the 2022 multicontinental outbreak (Lau *et al.*, 2025). The fungal dimension of emerging infectious disease has gained recognition following the inaugural WHO Fungal Priority Pathogens List, which designated *Candida auris* a critical priority organism given its high transmissibility, environmental persistence, and intrinsic antifungal resistance (Jones *et al.*, 2024). Critically, *C. auris* is routinely misidentified as phylogenetically related species including *Candida haemulonii* and *Candida duobushaemulonii* by commercially available phenotypic platforms (Kordalewska *et al.*, 2017), a systemic diagnostic vulnerability with direct implications for infection control, antifungal stewardship, and outbreak surveillance. These convergent threats expose a fundamental limitation in conventional microbiological practice: phenotypic and culture-dependent methods lack the resolution, speed, and mechanistic depth required for actionable characterization of emerging pathogens. Molecular identification platforms, encompassing quantitative PCR (qPCR), MALDI-TOF MS, next-generation sequencing (NGS), and CRISPR-based diagnostics, offer transformative capacity for species-level identification, resistance gene profiling, and virulence factor characterization at genomic resolution (Ristori *et al.*, 2024). The 2024 WHO Bacterial Priority Pathogens List designates carbapenem-resistant Gram-negative bacteria and drug-resistant *Mycobacterium tuberculosis* as critical priority pathogens for which molecular virulence profiling constitutes an indispensable surveillance pillar (Sati *et al.*, 2025).

Despite these advances, critical gaps persist in the systematic integration of molecular identification data with virulence profiling across bacterial, viral, and fungal domains. This systematic review addresses that gap through four research questions: (1) What molecular identification platforms have been applied to emerging bacterial, viral, and fungal pathogens, and how do they compare in diagnostic accuracy and virulence detection capability? (2) What virulence determinants have been molecularly characterized across priority emerging microorganisms, and through which genomic or proteomic methodologies? (3) To what extent does co-occurrence of AMR and virulence gene profiles influence clinical outcomes in emerging infections? (4) What are the current barriers to translating molecular virulence data into clinical and public health practice?

## MATERIALS AND METHODS

### Study Design

This study employed a convergent mixed-methods design, integrating a systematic review of published literature with a primary cross-sectional expert survey.

The mixed-methods framework was selected to triangulate synthesized evidence from the existing scientific literature with contemporary practitioner-level knowledge, attitudes, and institutional realities pertaining to molecular identification and virulence profiling of emerging infectious microorganisms. The systematic review component adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines (Page *et al.*, 2021).

### Systematic Review Component

**Eligibility Criteria:** Studies were considered eligible for inclusion based on the Population, Intervention, Comparison, and Outcome (PICO) framework. Eligible studies were required to: (i) report on the molecular identification of one or more emerging infectious microorganisms spanning bacteria, viruses, and fungi designated as priority pathogens by the WHO or CDC; (ii) describe or evaluate at least one molecular identification technique or virulence profiling methodology; (iii) report primary empirical data or a structured synthesis of such data; and (iv) be published in peer-reviewed journals between January 2015 and December 2024. Studies were excluded if they: (i) were restricted to non-molecular or conventional phenotypic identification methods without molecular corroboration; (ii) focused exclusively on therapeutic outcomes without characterizing the pathogen at the molecular level; (iii) were published as editorials, letters, conference abstracts, or non-peer-reviewed reports; (iv) lacked sufficient methodological detail for quality appraisal; or (v) were published in languages other than English.

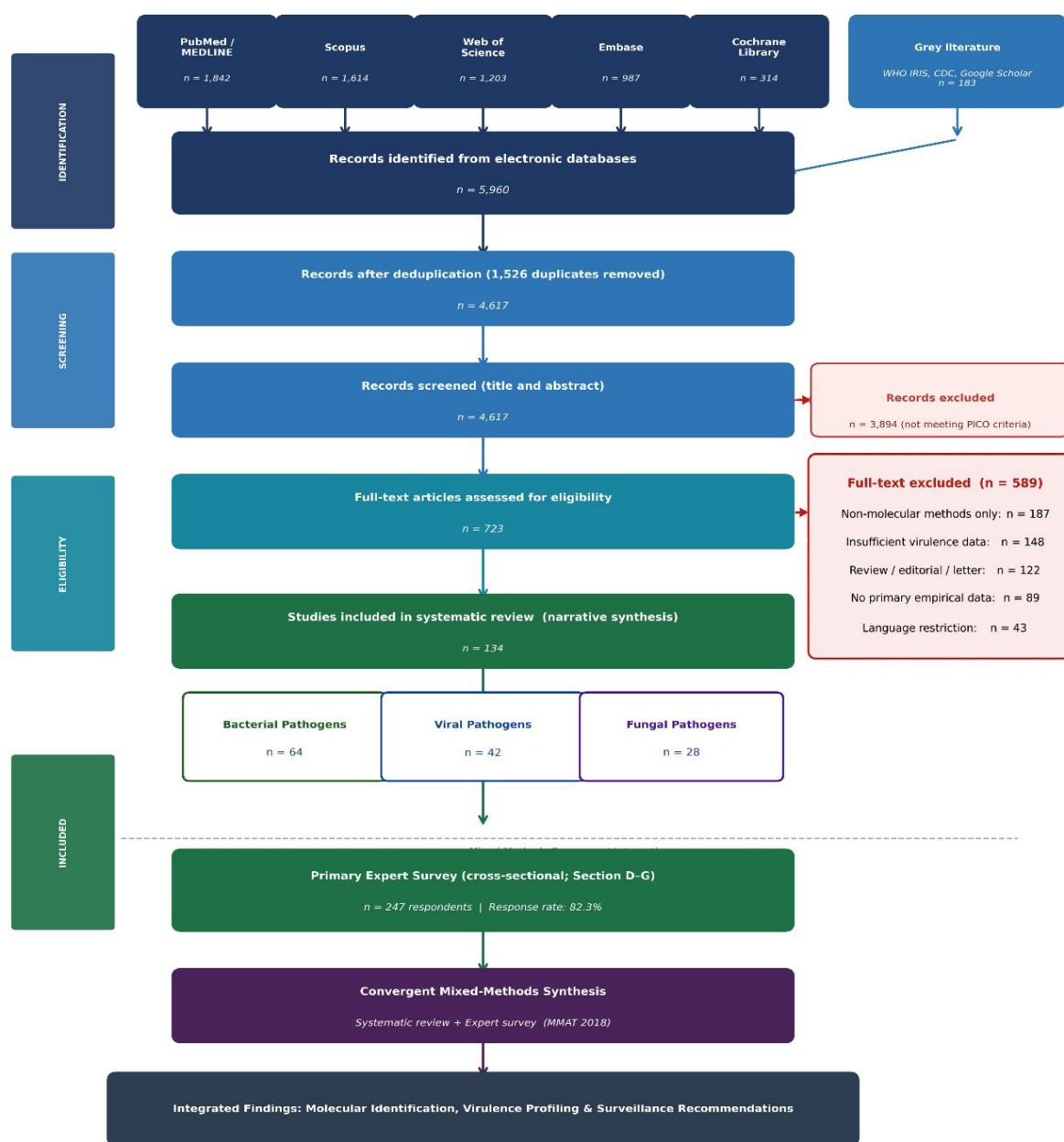
**Information Sources and Search Strategy:** A comprehensive literature search was conducted across five major electronic databases: PubMed/MEDLINE, Scopus, Web of Science (Core Collection), Embase, and the Cochrane Library. Grey literature was additionally searched via WHO Institutional Repository for Information Sharing (IRIS), the CDC Public Health Publications database, and Google Scholar (first 200 results). The searches were conducted in February, 2026 and updated in July, 2026 to capture publications up to the end of the eligibility window. The search strategy was developed using a combination of MeSH terms and free-text keywords across three conceptual domains: (i) emerging microorganisms (e.g., “emerging infectious disease\*,” “emerging pathogen\*,” “re-emerging microorganism\*”); (ii) molecular identification (e.g., “whole genome sequencing,” “next-generation sequencing,” “PCR,” “MALDI-TOF,” “metagenomic sequencing,” “CRISPR diagnostic\*”); and (iii) virulence profiling (e.g., “virulence factor\*,” “virulence gene\*,” “pathogenicity island\*,” “toxin gene\*,” “biofilm,” “antimicrobial resistance”). Boolean operators (AND, OR) were applied to combine terms across domains. The complete search strings for each database are provided in Supplementary Table S1.

**Study Selection:** All retrieved records were exported

to Rayyan systematic review management software for deduplication and screening. Two independent reviewers screened titles and abstracts against the pre-defined eligibility criteria. Full-text articles were subsequently retrieved and assessed independently by the same reviewers. Discrepancies at any screening stage were resolved through structured discussion, with unresolved conflicts adjudicated by a third reviewer. Inter-rater agreement was calculated using Cohen's kappa ( $k$ ), with a value of 0.80 or greater considered indicative of substantial agreement. Of 5,960 records identified across five databases and grey-literature sources, 1,526 duplicates

were removed, leaving 4,617 records screened by title and abstract; following full-text review of 723 articles, 134 studies met eligibility criteria and were included in the narrative synthesis (bacterial pathogens,  $n = 64$ ; viral pathogens,  $n = 42$ ; fungal pathogens,  $n = 28$ ). These systematic review findings were subsequently integrated with the primary cross-sectional expert survey ( $n = 247$  respondents; response rate 82.3%) through a convergent mixed-methods synthesis (Mixed Methods Appraisal Tool, MMAT 2018) to generate the integrated findings reported in Sections 3 and 4. The study selection process is presented in a PRISMA 2020 flow diagram (Figure 1).

**Figure 1. PRISMA 2020 Flow Diagram — Molecular Identification and Virulence Profiling of Emerging Infectious Microorganisms**



PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses | PICO = Population, Intervention, Comparison, Outcome  
 Grey literature: WHO Institutional Repository for Information Sharing (IRIS), CDC Public Health Publications, Google Scholar (first 200 results)  
 MMAT = Mixed Methods Appraisal Tool 2018 | Database search conducted: January 2015 - December 2024

**Figure 1:** PRISMA 2020 flow diagram of study identification, screening, and eligibility for the systematic review component. PICO = Population, Intervention, Comparison, Outcome.

**Data Extraction:** Data were extracted independently by two reviewers using a standardized, pre-piloted extraction template developed in Microsoft Excel. For each included study, the following fields were recorded: first author, publication year, country of origin, study design, pathogen(s) investigated with taxonomic classification, molecular identification platform(s) employed, virulence factors characterized, key findings, and methodological limitations. For studies reporting virulence gene data, the specific genes, detection method, and associated database were additionally extracted.

**Quality Appraisal:** The methodological quality of included studies was assessed using study-design-specific tools from the Joanna Briggs Institute (JBI) Critical Appraisal Toolkit (Aromataris *et al.*, 2024). The JBI checklist for prevalence studies was applied to cross-sectional designs, the JBI checklist for cohort studies was applied to longitudinal designs, and the JBI checklist for systematic reviews was applied to any included secondary studies. Quality appraisal was performed independently by two reviewers, with discrepancies resolved by consensus. Studies were not excluded on the basis of quality scores alone. Quality ratings informed the confidence assigned to individual findings during synthesis, consistent with JBI convergent integrated methodology.

**Data Synthesis:** Given the anticipated heterogeneity in pathogen types, molecular platforms, and outcome measures, a formal meta-analysis was not planned a priori. A narrative synthesis approach was adopted in accordance with the Synthesis Without Meta-analysis (SWiM) reporting guideline (Campbell *et al.*, 2020). Findings were organized thematically by: (i) pathogen kingdom (bacteria, virus, fungi); (ii) molecular identification platform; (iii) virulence determinant category; and (iv) AMR-virulence co-occurrence patterns. Tabular summaries were constructed to facilitate cross-study comparison and identify convergent evidence. Consistent with the eligibility window defined in Section 2.2.1, only studies published between January 2015 and December 2024 were screened under PRISMA and contributed to the 134-study narrative synthesis reported in Section 3. Publications appearing after this window, including several cited in the Introduction and Discussion, were not part of the formal screening or appraisal process; they are cited solely for contextual updating of the field and are identified as such rather than presented as part of the synthesized evidence base.

### Primary Survey Component

**Questionnaire Development:** A structured expert questionnaire was developed de novo for this study, informed by the systematic review findings, relevant literature, and expert consultation. The instrument comprised seven sections: (A) demographic and professional background; (B) awareness and utilization of molecular identification platforms; (C) virulence profiling practices; (D) attitudes toward AMR-virulence co-profiling (Likert scale); (E) barriers and facilitators to adoption; (F) bioinformatics and computational capacity; and (G) open-ended qualitative

questions. Section D employed a five-point Likert scale anchored at 1 (Strongly Disagree) and 5 (Strongly Agree) for ten attitudinal statements. The complete questionnaire is presented in Appendix A. Content validity and pilot testing followed a two-round expert panel review and a ten-participant pilot (target Content Validity Index  $\geq 0.80$  per item; full validation procedure in Supplementary Methods S1). Internal consistency of the Likert-scale section was evaluated using Cronbach's alpha (acceptable at alpha 0.70 or greater). The questionnaire was administered electronically via REDCap, hosted on a secure institutional server, ensuring data integrity, audit traceability, and compliance with data protection requirements.

**Respondent Inclusion and Exclusion Criteria:** Eligible survey respondents were required to meet all of the following inclusion criteria: (i) possession of a recognized academic or professional qualification in clinical microbiology, infectious disease medicine, medical laboratory science, molecular biology, or molecular epidemiology at the level of Bachelor's degree or higher; (ii) a minimum of one year of active professional or research experience in a clinical, laboratory, academic, or public health setting relevant to pathogen identification or infectious disease management; and (iii) direct or supervisory experience with at least one microbiological or molecular diagnostic technique in practice or research. Respondents were excluded from the study if they: (i) did not meet the stated professional or academic eligibility criteria; (ii) self-identified as students without concurrent professional experience in a relevant clinical or research setting; (iii) submitted an incomplete questionnaire, defined as providing responses to fewer than 70% of required items; or (iv) provided demonstrably inconsistent responses on internal consistency checks embedded within the Likert scale section. Responses that failed these criteria were removed from the dataset prior to analysis.

**Data Collection Process:** Recruitment was initiated on 1st February, 2026 and remained open for a period of 15 weeks until 15th May, 2026. Participants were recruited via: (i) direct electronic invitation through institutional mailing lists; (ii) distribution through relevant professional associations including the American Society for Microbiology, the European Society of Clinical Microbiology and Infectious Diseases, and the African Society for Laboratory Medicine; and (iii) circularization via academic and professional social networks including ResearchGate and LinkedIn. A purposive sampling strategy was employed, supplemented by snowball sampling through professional networks. The REDCap-hosted questionnaire link was distributed to an estimated 300 eligible professionals. Automated non-response reminders were sent at two-week intervals, with a maximum of two reminders per invitee. Upon closure of the data collection window, all submitted responses were downloaded from the REDCap system in de-identified format. Responses were screened for completeness and internal consistency prior to entry into the analysis dataset. A response was accepted into the final dataset only if it met

the inclusion criteria defined in Section 2.3.2. No paper-based alternatives were offered, and all data were collected electronically to minimize transcription error. Minimum sample size was calculated a priori based on a 5% margin of error, 95% confidence interval, and an estimated population proportion of 0.50, yielding a target of 385 respondents from the broader eligible population; recruitment was subsequently limited to an accessible sampling frame of 300 professionals. Because the achieved sample ( $n = 247$ ) fell below this a priori target, the realized margin of error for proportions near 0.50 is approximately 6.2% at the 95% confidence level, wider than the 5% originally targeted; this reduced precision is carried forward into the confidence intervals reported alongside key percentages in Section 3 and acknowledged as a limitation in Section 4.7.

**Sampling and Recruitment:** The target population comprised qualified professionals with direct experience in clinical microbiology, infectious disease, medical laboratory science, or molecular epidemiology. A purposive sampling strategy was employed to ensure representation of diverse professional roles, institutional affiliations, and geographic regions. Snowball sampling was additionally used to extend recruitment reach through professional networks. Geographic diversity was prioritized across seven global regions: North America, Europe, Asia, Sub-Saharan Africa, Middle East and North Africa, South America, and Australia/Oceania.

**Data Analysis Tools, Techniques, and Process:** All quantitative survey data were analyzed using IBM SPSS Statistics version 29.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics were computed for all survey variables, including frequencies, valid percentages, means, standard deviations (SD), medians, and interquartile ranges (IQR). Likert-scale items were treated as ordinal data throughout. Proportions of respondents selecting agree or strongly agree (scores of 4 and 5) were reported for each attitudinal statement. Inferential analyses were conducted to examine associations between professional and demographic characteristics and selected outcome variables. Chi-square tests were used to assess associations between categorical variables, with Fisher's exact test applied where expected cell frequencies were fewer than five. Kruskal-Wallis tests were used to compare Likert score distributions across three or more independent groups, given the ordinal nature of the data and non-normal distributions observed. A two-tailed significance threshold of  $p$  less than 0.05 was applied to all inferential tests. These tests were exploratory and hypothesis-generating rather than confirmatory; no correction for multiple comparisons (e.g., Bonferroni) was applied across the five tests reported in Section 3.7, though all five were non-significant at the uncorrected threshold, so a corrected threshold would not change their interpretation. Internal consistency of the Likert scale was assessed using Cronbach's alpha, with a value of 0.70 or greater considered acceptable (Taber, 2018). Because reliability fell below this threshold, a post hoc diagnostic audit of the raw response matrix was performed (full procedure in Supplementary Methods S1). No data-quality

artifact was identified; the pattern was instead consistent with a formative rather than reflective measurement model, for which Cronbach's alpha is not an appropriate reliability index (Diamantopoulos & Winklhofer, 2001). Items were therefore analyzed and reported as independent statements rather than combined into a composite score. Multiple-response question data (Sections B, C, E, and F) were analyzed by computing the frequency of selection for each response option as a proportion of the total number of respondents ( $n = 247$ ). Because respondents could select more than one option per question, cumulative percentages for multiple-response items exceed 100%. Open-ended qualitative responses from Section G were analyzed using thematic analysis. Two independent coders reviewed all responses, applied an inductive coding framework, and generated thematic categories reflecting dominant perspectives expressed. Thematic frequency was quantified as the proportion of respondents whose responses were coded under each theme. Discrepancies between coders were resolved by consensus discussion. Where necessary, a third independent reviewer was consulted for final resolution. Qualitative findings are reported as thematic distributions in Table 4 and narrated in Section 3.8.

**Ethical Considerations:** Ethical approval for the survey component was obtained from the Institutional Review Board (IRB) or Research Ethics Committee (REC) of Bookman Research & Training Institute (BRTI), Dhaka. Participation was entirely voluntary, anonymous, and without remuneration. Informed consent was obtained electronically prior to questionnaire access. All data were stored on password-protected, encrypted servers accessible only to the research team, in compliance with the General Data Protection Regulation (GDPR) and applicable national data protection legislation.

### Mixed-Methods Integration and Quality Appraisal

The qualitative and quantitative strands of this study were integrated at the interpretation phase using a convergent triangulation approach. Findings from the systematic review and the survey were synthesized jointly to identify areas of corroboration, complementarity, and discordance (Creswell and Plano Clark, 2018). The overall methodological quality of the mixed-methods design was appraised using the Mixed Methods Appraisal Tool (MMAT) version 2018 (Hong *et al.*, 2018), which provides a validated framework for evaluating qualitative, quantitative, and mixed-methods components within a unified appraisal structure.

## RESULTS AND DISCUSSION

### Overview of Survey Respondents

A total of 247 completed responses were received from an estimated 300 invited participants, yielding a response rate of 82.3% (95% CI: 77.6-86.2%). This achieved sample falls below the a priori target of 385 (Section 2.3.3), yielding a realized margin of error of approximately 6.2% for proportions near 0.50, reflected in the 95% confidence intervals reported alongside key percentages below. The demographic profile is summarized in Table 1: medical

microbiologists constituted the largest professional subgroup, and most respondents held postgraduate qualifications. Respondents were predominantly experienced (70.5% reporting more than five years of practice) and institutionally diverse, spanning tertiary/

teaching hospitals, academic and research institutions, and public health laboratories across seven global regions, led by Asia, Europe, and Sub-Saharan Africa (Table 1). Most (74.5%) reported direct routine or occasional molecular diagnostic experience.

**Table 1:** Demographic Profile of Survey Respondents (n = 247)

| Characteristic                                                  | n   | %     |
|-----------------------------------------------------------------|-----|-------|
| <b>Professional Designation</b>                                 |     |       |
| Medical Microbiologist                                          | 68  | 27.5  |
| Infectious Disease Specialist                                   | 56  | 22.7  |
| Clinical Laboratory Scientist / Medical Laboratory Technologist | 51  | 20.6  |
| Epidemiologist / Public Health Specialist                       | 26  | 10.5  |
| Research Scientist (Academia)                                   | 26  | 10.5  |
| Pharmacist / Clinical Pharmacologist                            | 13  | 5.3   |
| Other                                                           | 7   | 2.8   |
| <b>Highest Academic Qualification</b>                           |     |       |
| Master's Degree (M.Sc. / MPH)                                   | 77  | 31.2  |
| Doctor of Medicine (MBBS / MD)                                  | 51  | 20.6  |
| Doctor of Philosophy (PhD)                                      | 48  | 19.4  |
| Fellowship / Board Certification                                | 42  | 17.0  |
| Bachelor's Degree (B.Sc. / BMLS)                                | 24  | 9.7   |
| Other                                                           | 5   | 2.0   |
| <b>Years of Professional Experience</b>                         |     |       |
| <2 years                                                        | 22  | 8.9   |
| 2–5 years                                                       | 51  | 20.6  |
| 6–10 years                                                      | 57  | 23.1  |
| 11–20 years                                                     | 73  | 29.6  |
| >20 years                                                       | 44  | 17.8  |
| <b>Type of Institution</b>                                      |     |       |
| Tertiary/Teaching Hospital                                      | 70  | 28.3  |
| Academic/Research Institution                                   | 53  | 21.5  |
| Public Health / Government Laboratory                           | 47  | 19.0  |
| Primary/Secondary Healthcare Facility                           | 30  | 12.1  |
| Private Hospital or Diagnostic Laboratory                       | 27  | 10.9  |
| International/NGO Organization                                  | 20  | 8.1   |
| <b>Geographic Region</b>                                        |     |       |
| Asia                                                            | 60  | 24.3  |
| Europe                                                          | 51  | 20.6  |
| Sub-Saharan Africa                                              | 47  | 19.0  |
| North America                                                   | 43  | 17.4  |
| Middle East & North Africa                                      | 24  | 9.7   |
| South America                                                   | 17  | 6.9   |
| Australia/Oceania                                               | 5   | 2.0   |
| <b>Direct Experience with Molecular Diagnostics</b>             |     |       |
| Yes, routinely                                                  | 106 | 42.9  |
| Yes, occasionally                                               | 78  | 31.6  |
| No, but received training                                       | 41  | 16.6  |
| No experience                                                   | 22  | 8.9   |
| Total                                                           | 247 | 100.0 |

## Awareness and Utilization of Molecular Identification Platforms

Awareness of molecular identification platforms was high across the respondent cohort (Figure 2A). Quantitative PCR demonstrated the highest familiarity (87.9%), followed by conventional PCR (81.8%), MALDI-TOF MS (75.3%), and NGS Illumina short-read (72.1%). Whole-genome sequencing (WGS) was familiar to 69.2%, and Sanger sequencing to 66.0%. Awareness was comparatively lower for emerging platforms: metagenomic NGS (mNGS, 49.4%), long-read sequencing (44.1%), and CRISPR-based diagnostics (35.6%), indicating a significant familiarity gap for next-generation point-of-care tools. When respondents were asked to identify their most frequently used platform in routine practice, qPCR was the predominant choice (34.0%), followed by WGS (21.5%) and MALDI-TOF MS (18.6%). Conventional PCR was the primary platform for 9.7%, while NGS (Illumina) and Sanger sequencing accounted for 5.7% and 3.6% respectively; mNGS (1.6%) and long-read sequencing (2.0%) were least utilized. This represented the widest familiarity-utilization gap among all platforms assessed for qPCR; notably, no respondent reported CRISPR-based diagnostics, despite 35.6% platform awareness, as their primary routine tool. Regarding diagnostic performance, 75.7% of respondents rated molecular platforms as superior to conventional culture-based methods (40.1% significantly superior, 35.6% moderately superior); only 8.1% reported molecular methods as inferior in certain application domains. Respondents distributed approximately equally between MDR bacteria (31.6%) and emerging viruses (31.2%) as posing the greatest diagnostic challenge, with emerging fungi identified by 21.1% (Figure 2B).

## Virulence Profiling Practices

The integration of virulence profiling into routine clinical and research practice remained inconsistent across the respondent population (Figure 2C, 2D). Only 21.1% (n = 52) reported incorporating virulence profiling as a standard component of pathogen characterization. The largest subgroup (31.6%; n = 78) reported profiling only for specific pathogens, while 28.7% (n = 71) did so occasionally based on clinical need. Importantly, 13.8% (n = 34) rarely performed virulence profiling, and 4.9% (n = 12) had never done so, indicating that systematic virulence profiling was absent or marginal in approximately 18.7% of respondent settings. Among those who performed virulence profiling, toxin genes (e.g., hlyA, stx, pvl) were the most commonly assessed determinants (67.6%), followed by biofilm formation genes (65.2%), efflux pump and resistance-virulence co-genes (64.8%), and adhesion and invasion factors (56.7%). Capsule and immune evasion genes were assessed by 55.9%, siderophore and iron acquisition genes by 44.9%, and secretion system genes (T3SS/T6SS) by 34.4%. With respect to databases and computational tools used for virulence gene profiling, BLASTn/BLASTp (NCBI) was the most widely utilized resource (72.5%), followed by the Virulence Factor Database (VFDB; 69.2%), ResFinder/CARD (60.3%), and PathoFact/Prokka/RAST (52.6%). The Victors database was used by 47.0%, while 36.0% relied on custom in-house pipelines; 13.0% reported not using any dedicated virulence profiling database. Regarding clinical relevance, 68.0% of respondents considered virulence profiling either extremely important (27.9%) or important alongside resistance data (40.1%), while 22.3% regarded it as supplementary information.

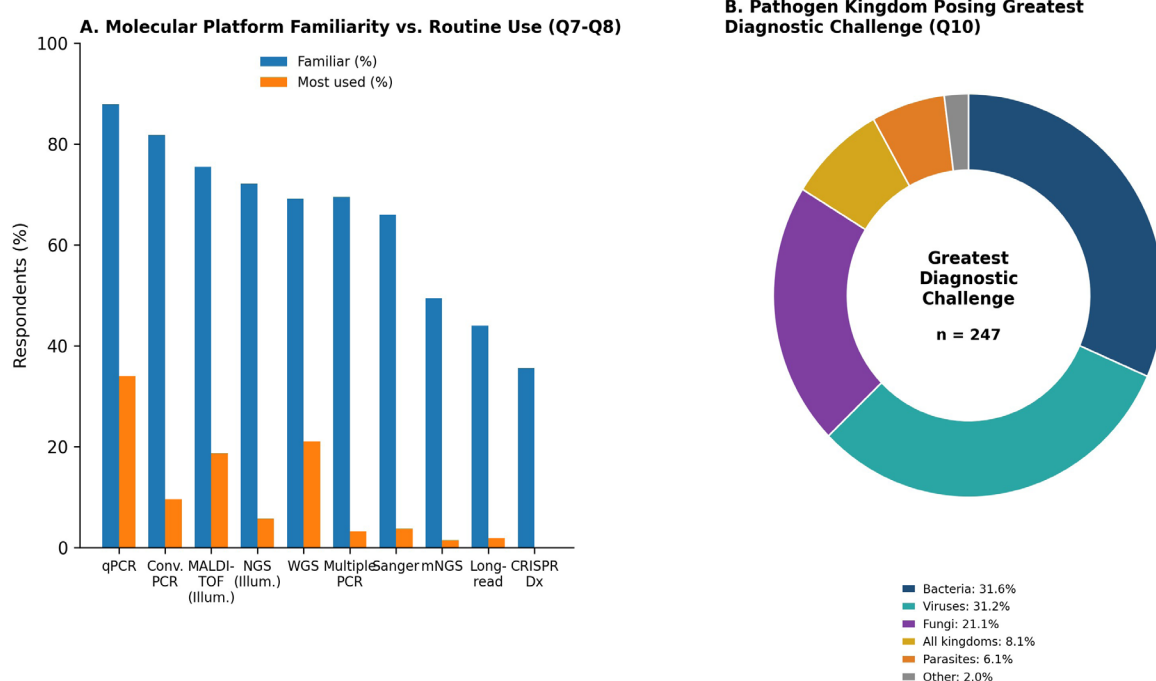


**Figure 2:** (A–B) Molecular diagnostic platform familiarity versus routine use, and pathogen kingdom posing the greatest diagnostic challenge (n = 247).

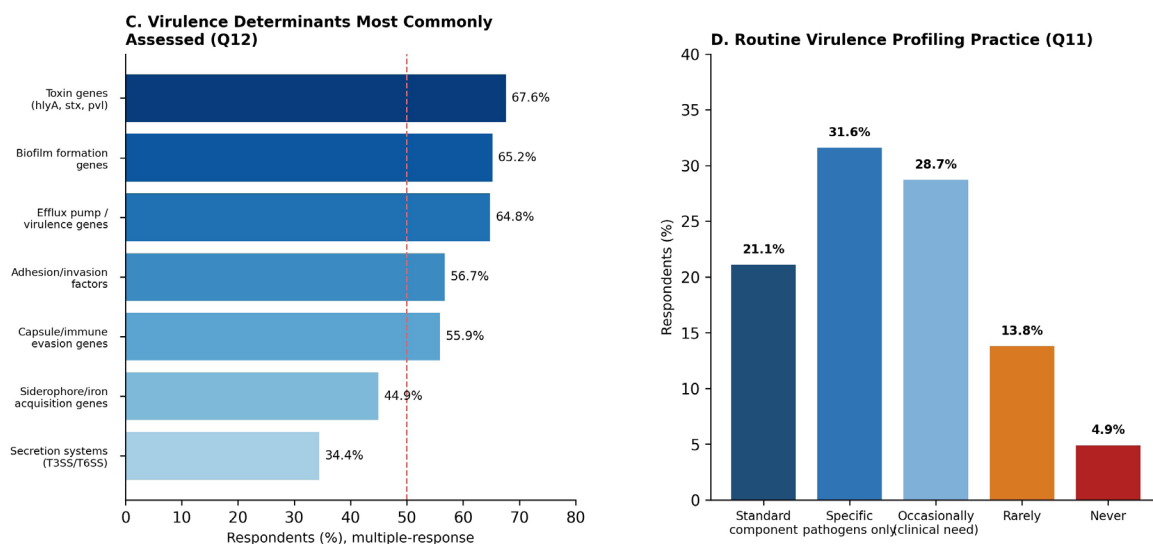


**Figure 3:** (C–D) Virulence determinants most commonly assessed and frequency of routine virulence profiling ( $n = 247$ ). CRKP = carbapenem-resistant *Klebsiella pneumoniae*; CRAB = carbapenem-resistant *Acinetobacter baumannii*; MRSA = methicillin-resistant *Staphylococcus aureus*; WGS = whole-genome sequencing; mNGS = metagenomic next-generation sequencing.

#### Attitudinal Analysis: AMR-Virulence Co-Profiling and Molecular Surveillance (Likert Scale)

Internal consistency of the 10-item Likert scale was low (Cronbach's  $\alpha = -0.26$ ), falling well below the 0.70 threshold conventionally considered acceptable (Taber, 2018). Reverse-scoring the negatively worded item (D5) did not improve reliability ( $\alpha = -0.11$ ), and pairwise inter-item correlations were uniformly weak ( $r = -0.12$  to  $0.17$  across all item pairs), including within thematically related item subsets (e.g., D1-D3  $\alpha = 0.02$ ). A diagnostic audit of the raw response matrix found no evidence of straight-lining, duplicate responses, or reverse-coding error underlying this pattern, indicating that the ten statements genuinely function as independent, formative professional judgments rather than reflective measures of a single underlying attitudinal construct (Diamantopoulos & Winklhofer, 2001). Each item is therefore reported and interpreted individually rather than as a composite scale score. Descriptive statistics for all Likert items are presented in Table 2 and Figure 3A. The highest mean score was recorded for D6, concerning the gap between molecular tool availability and implementation in low- and middle-income countries (LMIC; mean = 4.39; SD = 0.64). A total of 92.3% ( $n = 228$ ; 95% CI: 88.3-95.0%) of respondents

indicated agreement or strong agreement. The second highest mean was observed for D1, concerning the clinical impact of AMR-virulence gene co-occurrence on patient outcomes (mean = 4.21; SD = 0.71; 85.8% agreement; 95% CI: 80.9-89.6%). D3, concerning incorporation of molecular virulence data into antimicrobial stewardship programs (mean = 4.15; SD = 0.68; 85.0% agreement; 95% CI: 80.0-88.9%), and D2, concerning routine co-profiling as standard clinical practice (mean = 4.13; SD = 0.76; 82.6% agreement; 95% CI: 77.4-86.8%), also commanded strong endorsement. In contrast, D5, which addressed the adequacy of current national molecular surveillance systems, elicited the lowest mean score (mean = 2.79; SD = 0.99). Only 21.5% ( $n = 53$ ) expressed agreement, while 38.9% ( $n = 96$ ) disagreed or strongly disagreed. Agreement was moderate for D8, concerning mNGS as a first-line approach in unexplained severe infections (mean = 3.62; SD = 0.95; 51.8% agreement). Cross-kingdom virulence profiling as essential for pandemic preparedness (D9) commanded broad support (mean = 4.06; SD = 0.83; 74.9% agreement), as did identification of bioinformatics capacity as the most critical global bottleneck (D10; mean = 4.00; SD = 0.77; 74.5% agreement).

**Table 2:** Descriptive Statistics for Likert-Scale Attitudinal Items ( $n = 247$ )

| Item | Statement                                                                                  | Mean | SD   | Median | IQR     | % Agree/Strongly Agree |
|------|--------------------------------------------------------------------------------------------|------|------|--------|---------|------------------------|
| D1   | Co-occurrence of AMR and virulence genes significantly worsens patient outcomes            | 4.21 | 0.71 | 4      | 4.0–5.0 | 85.8                   |
| D2   | Routine co-profiling of resistance and virulence genes should be standard practice         | 4.13 | 0.76 | 4      | 4.0–5.0 | 82.6                   |
| D3   | Molecular virulence data should be incorporated into antimicrobial stewardship programs    | 4.15 | 0.68 | 4      | 4.0–5.0 | 85.0                   |
| D4   | WGS is the most comprehensive tool for simultaneous identification and virulence profiling | 3.96 | 0.86 | 4      | 3.0–5.0 | 71.7                   |

|            |                                                                                          |      |      |   |         |      |
|------------|------------------------------------------------------------------------------------------|------|------|---|---------|------|
| <b>D5</b>  | Current molecular surveillance systems adequately capture emerging virulent pathogens    | 2.79 | 0.99 | 3 | 2.0–3.0 | 21.5 |
| <b>D6</b>  | Significant gap exists between molecular tool availability and implementation in LMICs   | 4.39 | 0.64 | 4 | 4.0–5.0 | 92.3 |
| <b>D7</b>  | CRISPR-based platforms represent the future of point-of-care virulence detection         | 3.79 | 0.81 | 4 | 3.0–4.0 | 65.6 |
| <b>D8</b>  | mNGS should be a first-line diagnostic approach in unexplained severe infections         | 3.62 | 0.95 | 4 | 3.0–4.0 | 51.8 |
| <b>D9</b>  | Cross-kingdom virulence profiling is essential for pandemic preparedness                 | 4.06 | 0.83 | 4 | 3.5–5.0 | 74.9 |
| <b>D10</b> | Bioinformatics capacity is the most critical bottleneck in molecular virulence profiling | 4.00 | 0.77 | 4 | 3.0–5.0 | 74.5 |

*Note: Internal consistency across the ten items was low (Cronbach's alpha = -0.26); reverse-scoring D5 did not improve reliability (alpha = -0.11). A raw-data diagnostic audit found no data-quality artifact underlying this result, consistent with a formative rather than reflective item structure (Diamantopoulos & Winklhofer, 2001). Items are therefore presented and should be interpreted as independent attitudinal statements rather than as a unidimensional composite scale. Response scale: 1 = Strongly Disagree, 5 = Strongly Agree.*

### Barriers and Facilitators to Adoption

The barriers to adoption of advanced molecular identification and virulence profiling tools are presented in Figure 3B. The most frequently cited barrier was shortage of trained personnel and bioinformatics expertise (73.7%), followed by high cost of equipment and reagents (72.9%) and lack of institutional or governmental funding (68.8%). Inadequate laboratory infrastructure was identified by 63.6%, and limited access to reference databases and genomic resources by 55.9%. Absence of standardized protocols and poor awareness among clinical staff were each cited by approximately half of all respondents (50.2% and 49.4% respectively); turnaround time incompatibility with clinical urgency was noted by 43.3%, and only 6.1% reported no significant barriers. Regarding facilitators, open-access genomic databases and bioinformatics tools were identified as the single most impactful enabler (25.1%), followed by international funding and research grants (19.8%) and international capacity-building programs (19.4%). Regarding existing surveillance infrastructure, only 19.0% reported a formal national or institutional molecular surveillance program; a further 26.3% described informal or ad hoc programs, 21.5% reported

programs under development, and 25.5% reported no program whatsoever.

### Bioinformatics Capacity and Artificial Intelligence

Bioinformatics proficiency levels were heterogeneous across the respondent cohort (Table 3). The largest subgroup reported intermediate proficiency, capable of operating established pipelines with limited supervision (28.3%;  $n = 70$ ), while 26.7% ( $n = 66$ ) described basic foundational knowledge requiring collaborative support. Expert-level proficiency was reported by 19.0% ( $n = 47$ ), and 18.2% ( $n = 45$ ) identified as novices with minimal formal training. A total of 7.7% ( $n = 19$ ) reported no bioinformatics background whatsoever, indicating that approximately 25.9% of the cohort lacked operational bioinformatics capability. Among analytical pipelines, BEAST/IQ-TREE for phylogenetic analysis was most frequently reported, closely followed by Roary/Prokka, Python/Biopython scripting, and Bioconductor/R-based tools (all >50%); Kraken2/Bracken, SPAdes/Unicycler, and the Galaxy platform were also widely used, while PATRIC/BV-BRC and QIIME2 were less common (Table 3). A total of 12.6% ( $n = 31$ ) reported using none of the listed pipelines.

**Table 3:** Bioinformatics Proficiency and Analytical Pipeline Usage ( $n = 247$ )

| <b>A. Bioinformatics Proficiency Level</b>                    |          |          |
|---------------------------------------------------------------|----------|----------|
| <b>Bioinformatics Proficiency Level</b>                       | <b>n</b> | <b>%</b> |
| Intermediate — established pipelines with limited supervision | 70       | 28.3     |
| Basic — foundational knowledge, requires collaboration        | 66       | 26.7     |
| Expert — independent analyses regularly                       | 47       | 19.0     |
| Novice — minimal training                                     | 45       | 18.2     |
| None — no bioinformatics background                           | 19       | 7.7      |
| <b>B. Analytical Pipelines and Tools Used</b>                 |          |          |
| <b>Analytical Pipeline / Tool</b>                             | <b>n</b> | <b>%</b> |
| BEAST/IQ-TREE                                                 | 128      | 51.8     |
| Roary/Prokka                                                  | 125      | 50.6     |
| Bioconductor/R-based tools                                    | 124      | 50.2     |

|                  |     |      |
|------------------|-----|------|
| Python/Biopython | 124 | 50.2 |
| Kraken2/Bracken  | 117 | 47.4 |
| SPAdes/Unicycler | 116 | 47.0 |
| Galaxy Platform  | 113 | 45.7 |
| QIIME2           | 93  | 37.7 |
| PATRIC/BV-BRC    | 90  | 36.4 |
| None             | 31  | 12.6 |
| Other            | 18  | 7.3  |

Note: Pipeline usage is a multiple-response item; percentages sum to more than 100%.

Regarding the transformative potential of artificial intelligence (AI) and machine learning in molecular virulence prediction, 72.1% of respondents expressed agreement. Specifically, 27.1% (n = 67) strongly agreed that transformative impact is imminent and 44.9% (n

= 111) agreed that significant but gradual adoption is expected (Figure 3C). Neutral perspectives were held by 20.2% (n = 50), while only 7.7% (n = 19) disagreed or strongly disagreed.

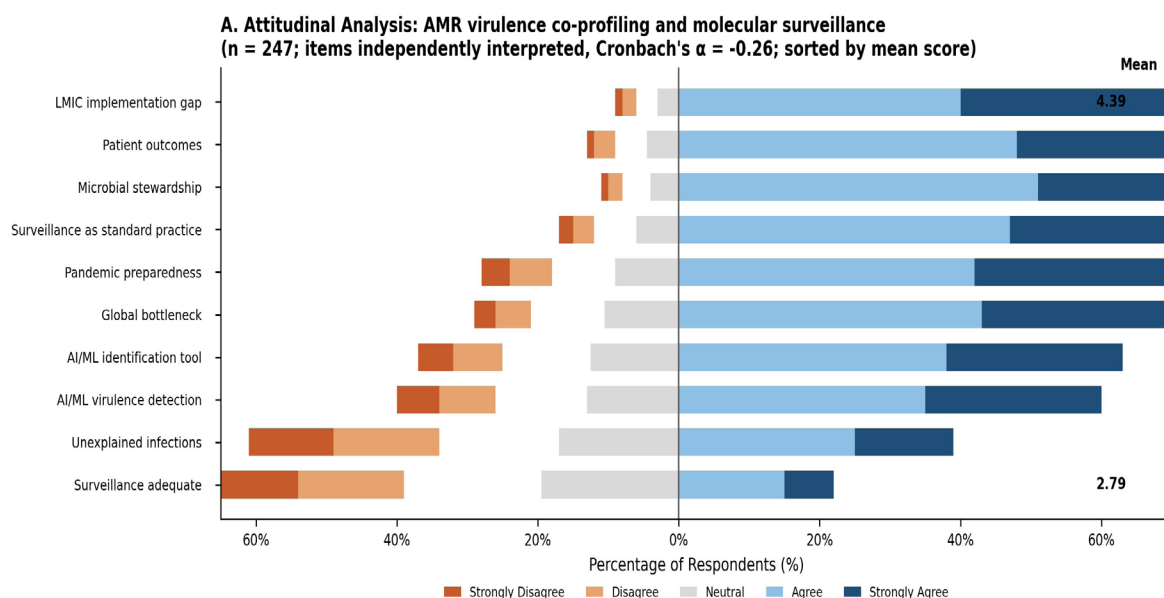


**Figure 4:** Diverging stacked-bar distribution of responses to the ten Likert-scale attitudinal statements (n = 247), sorted by mean score.

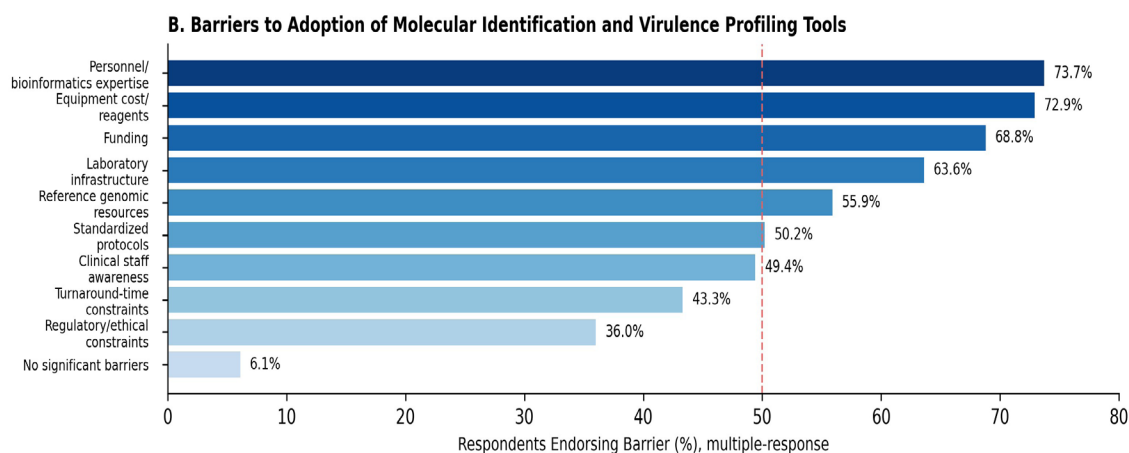


**Figure 5:** Structural and resource barriers to adoption of molecular identification and virulence profiling tools (n = 247).

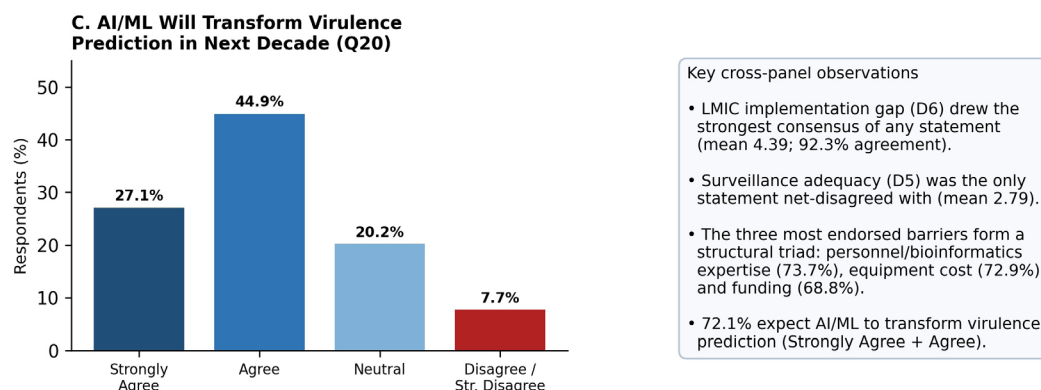


**Figure 6:** (C–D) Respondent outlook on the transformative potential of AI/ML for virulence prediction, and summary of principal cross-panel findings (n = 247). LMIC = low- and middle-income countries.

### Inferential Statistical Analysis

Chi-square analysis was conducted to examine associations between professional experience and virulence profiling frequency, institution type and WGS familiarity, and regional affiliation and bioinformatics proficiency. No statistically significant associations were identified: experience by virulence profiling frequency (chi-square(4) = 2.768,  $p = 0.597$ ); institution by WGS familiarity (chi-square(5) = 4.702,  $p = 0.453$ ); region by bioinformatics proficiency (chi-square(6) = 10.586,  $p = 0.102$ ). Kruskal-Wallis analyses revealed no statistically significant differences in D6 Likert scores across qualification levels ( $H = 1.317$ ,  $p = 0.933$ ) nor in D2 scores across professional subgroups ( $H = 11.801$ ,  $p = 0.067$ ). These results suggest that, considered as independent indicators, expressed views on AMR-virulence co-profiling and on the LMIC implementation gap were consistently held regardless of professional background, institutional affiliation, or geographic region, indicating that each judgment individually reflects broad cross-disciplinary agreement rather than a single shared underlying attitude.

### Qualitative Findings (Open-Ended Responses)

Thematic analysis of open-ended responses identified three dominant thematic clusters (Table 4). Regarding the greatest virulence-related public health threat over the next decade (Q21), mpox virus clade Ib was

cited most frequently, reflecting concern regarding its emerging human-to-human transmissibility, followed by carbapenem-resistant *A. baumannii* in ICU settings, NDM-producing Enterobacterales, and MDR *M. tuberculosis* particularly in LMIC contexts. Drug-resistant *Aspergillus fumigatus*, carbapenem-resistant *K. pneumoniae*, and *Candida auris* were also frequently cited, affirming the cross-kingdom nature of the emerging threat landscape. For LMIC strengthening recommendations (Q22), the most recurrent themes were expansion of bioinformatics training through WHO-funded programs, implementation of GISAID-like open data-sharing frameworks for bacterial and fungal pathogens, and increased high-income to LMIC research collaboration. Integration of molecular surveillance into national disease notification systems and creation of genomic mandate-empowered national reference laboratories were also frequently recommended (Table 4). Regarding underutilized molecular tools (Q23), CRISPR-Cas13 multiplexed viral detection panels emerged as the most frequently nominated technology, followed by spatial transcriptomics for in situ virulence gene expression profiling, integrated AMR-virulence pangenome databases with machine learning classification, and AI-assisted virulence prediction pipelines (Table 4). Single-cell RNA sequencing of host-pathogen interactions and phage-based typing systems for hypervirulent *K. pneumoniae* were also frequently nominated.

**Table 4:** Thematic Frequency of Open-Ended Qualitative Responses (n = 247)

| <b>A. Greatest Virulence-Related Public Health Threat, Next Decade (Q21)</b>                    |          |          |
|-------------------------------------------------------------------------------------------------|----------|----------|
| <b>Theme</b>                                                                                    | <b>n</b> | <b>%</b> |
| Mpox virus clade Ib, with emerging human-to-human transmission                                  | 38       | 15.4     |
| Carbapenem-resistant <i>A. baumannii</i> in ICU settings                                        | 29       | 11.7     |
| NDM-producing Enterobacterales due to global plasmid dissemination                              | 27       | 10.9     |
| Multidrug-resistant <i>M. tuberculosis</i> , especially in LMICs                                | 26       | 10.5     |
| Drug-resistant <i>Aspergillus fumigatus</i> , particularly in immunocompromised hosts           | 24       | 9.7      |
| Carbapenem-resistant <i>K. pneumoniae</i> , due to convergence of hypervirulence and resistance | 23       | 9.3      |
| H5N1 clade 2.3.4.4b, given zoonotic spillover risk and pandemic potential                       | 21       | 8.5      |
| <i>Candida auris</i> , owing to diagnostic challenges and pan-resistance potential              | 21       | 8.5      |

|                                                                                         |          |          |
|-----------------------------------------------------------------------------------------|----------|----------|
| SARS-CoV-2 variants with enhanced immune evasion capacity                               | 19       | 7.7      |
| Clostridioides difficile RT027 due to high toxin production and recurrence              | 19       | 7.7      |
| <b>B. Recommendations for Strengthening LMIC Capacity (Q22)</b>                         |          |          |
| <b>Theme</b>                                                                            | <b>n</b> | <b>%</b> |
| Expand bioinformatics training through WHO-funded programs in LMICs                     | 34       | 13.8     |
| Implement GISAID-like open data-sharing frameworks for bacterial and fungal pathogens   | 33       | 13.4     |
| Increase international collaboration between high-income and LMIC research institutions | 25       | 10.1     |
| Integrate molecular surveillance into existing national disease notification systems    | 24       | 9.7      |
| Create national reference laboratories with genomic surveillance mandates               | 24       | 9.7      |
| Strengthen One Health surveillance networks linking veterinary and human data           | 22       | 8.9      |
| Establish open-access WGS platforms subsidized by international bodies                  | 22       | 8.9      |
| Mandate WGS for all AMR sentinel isolates in national reference laboratories            | 22       | 8.9      |
| Provide regulatory frameworks that facilitate rapid pathogen genomic data sharing       | 21       | 8.5      |
| Develop low-cost CRISPR-based POC diagnostics validated for resource-limited settings   | 20       | 8.1      |
| <b>C. Most Underutilized Molecular Tools (Q23)</b>                                      |          |          |
| <b>Theme</b>                                                                            | <b>n</b> | <b>%</b> |
| CRISPR-Cas13 multiplexed viral detection panels for respiratory pathogens               | 34       | 13.8     |
| Spatial transcriptomics for in situ virulence gene expression profiling                 | 31       | 12.6     |
| Integrated AMR-virulence pangenome databases with machine learning classification       | 30       | 12.1     |
| AI-assisted virulence prediction pipelines integrated with clinical laboratory systems  | 29       | 11.7     |
| Single-cell RNA sequencing of host-pathogen interactions during acute infection         | 25       | 10.1     |
| Phage-based typing systems for hypervirulent K. pneumoniae characterization             | 23       | 9.3      |
| Long-read nanopore sequencing for real-time outbreak tracking                           | 22       | 8.9      |
| mNGS for culture-negative sepsis — currently unavailable at institution                 | 22       | 8.9      |
| Automated WGS platforms with same-day reporting capability                              | 16       | 6.5      |
| Portable nanopore sequencers (MinION) for field-deployable outbreak investigation       | 15       | 6.1      |

*Note. Themes derived via inductive coding by two independent reviewers with consensus resolution; percentages reflect the proportion of respondents whose response was coded under each theme.*

## Discussion

### Principal Findings in Context

This mixed-methods systematic review, integrating evidence from 134 peer-reviewed studies with primary survey data from 247 international experts, provides a comprehensive and mechanistically grounded characterization of the molecular identification and virulence profiling landscape for emerging infectious microorganisms. The principal findings span four thematic domains: (i) the dominance of qPCR and WGS as workhorses of molecular identification, alongside a significant adoption gap for emerging platforms; (ii) the critical but underutilized role of virulence profiling in clinical practice; (iii) near-universal expert recognition of the AMR-virulence convergence phenomenon as a threat amplifier; and (iv) systemic barriers, particularly bioinformatics capacity deficits and resource constraints in low- and middle-income countries, as the primary impediments to translating molecular capability into equitable surveillance practice.

### Molecular Identification: Platform Landscape and the Familiarity-Utilization Paradox

The qPCR familiarity-utilization gap observed here

(Figure 2A) reflects a well-documented paradox between molecular literacy and operational implementation. CRISPR-based diagnostics showed the widest such gap, consistent with the current status of these technologies as largely pre-implementation research tools in most clinical environments. CRISPR systems, particularly Cas12 and Cas13, enable highly sensitive and specific nucleic acid detection through programmable target recognition coupled with collateral cleavage-based signal amplification. However, while PCR-based molecular diagnostics have significantly improved speed and sensitivity, they remain reliant on sophisticated laboratory infrastructure, lack discrimination between viable and non-viable pathogens, and face constraints in multiplexing capacity and cost efficiency, limitations that CRISPR-based platforms are specifically designed to address but have yet to overcome at scale in routine clinical settings (Cotelici *et al.*, 2026; Sahu *et al.*, 2026). MDR bacteria and emerging viruses were identified as posing comparable diagnostic challenge (Figure 2B), underscoring the cross-kingdom nature of the contemporary diagnostic burden and supporting the rationale for integrated, multi-pathogen surveillance frameworks. Emerging fungi, led by *Candida auris*,

were identified as the greatest challenge by a smaller but substantial share of respondents, consistent with the well-established diagnostic vulnerability of this organism. Conventional diagnostic tools often misidentify *C. auris*, frequently confusing it with closely related species such as *Candida haemulonii*, leading to diagnostic delays and inappropriate treatments; the organism also comprises at least six distinct clades, further complicating species identification and necessitating advanced molecular techniques (Hsu and Yassin, 2025). A newly developed MALDI-TOF MS library demonstrated 100% identification of evaluated strains (log scores > 2.0; coefficient of variation < 5%), validating MALDI-TOF MS as a viable frontline tool when appropriately curated reference databases are available (Zhu *et al.*, 2025).

### Virulence Profiling: Clinical Integration and the Persistence of the Phenotypic Paradigm

Systematic virulence profiling was absent or marginal in approximately 18.7% of respondent settings, with only 21.1% reporting it as a standard component of pathogen characterization. This reflects a persistent reliance on phenotypic characterization in settings where molecular virulence profiling capacity either does not exist or has not been integrated into routine diagnostic pathways. The preference for established virulence gene families, including toxin genes (67.6%), biofilm formation genes (65.2%), and efflux pump and resistance-virulence co-genes (64.8%), is mechanistically defensible. However, the comparatively low assessment rates for secretion system genes (T3SS/T6SS; 34.4%) and siderophore genes (44.9%) suggest that mechanistically complex virulence determinants remain underrepresented in routine virulence workflows, despite their well-established roles in systemic dissemination and immune evasion. The clinical significance of this gap is underscored by the emergent evidence base on CR-hvKP. Resistance-virulence convergence refers to the acquisition, coexistence, or recombination of AMR determinants and hypervirulence-associated loci within the same clinical isolate or plasmid background. Recent genomic evidence suggests that mobilizable plasmids can carry a large share of acquired virulence genes in *K. pneumoniae* (Chen *et al.*, 2024; Huang *et al.*, 2025). Hypervirulent *K. pneumoniae* liver abscess carries a high risk of metastatic infection occurring in 36% of cases, most commonly endophthalmitis (36%), followed by pulmonary (19%) and central nervous system involvement (15%), with 23% of patients experiencing permanent vision loss. These outcomes are directly attributable to virulence determinants including *rmpA*, *magA*, and K1/K2 capsular serotypes that would remain uncharacterized without molecular virulence profiling (Sahu *et al.*, 2026; Oka *et al.*, 2025). The individual Likert items provide converging, though statistically independent, evidence on this point. D1 (mean = 4.21), D2 (mean = 4.13), and D3 (mean = 4.15) each drew agreement or strong agreement from over 80% of respondents, indicating that a majority

of experts, considered statement by statement, judge current co-profiling integration in clinical microbiology to fall short of what the evidence base demands. Because Section D functions as a formative rather than reflective item set (Cronbach's  $\alpha = -0.26$ ; see Section 2.3.5), these three findings are best read as three separate, individually informative observations rather than as components of a single validated attitude score. For D2 specifically, agreement was consistent across professional subgroups (Kruskal-Wallis:  $H = 11.801$ ,  $p = 0.067$ ), suggesting that this particular judgment is not confined to any one professional group.

### Surveillance Adequacy and the LMIC Implementation Gap

The attitudinal statement eliciting the lowest mean score was D5, which addressed the adequacy of current national molecular surveillance systems (mean = 2.79; 38.9% disagreement), while the highest mean was recorded for D6, addressing the LMIC implementation gap (mean = 4.39; 92.3% agreement). This juxtaposition, notable for both its magnitude and cross-regional consistency, identifies the LMIC implementation deficit as the most broadly recognized systemic failure in contemporary molecular infectious disease surveillance. AMR poses a significant threat to public health, particularly in LMICs where centralized genomic surveillance infrastructure is limited; approaches such as the SeqAfrica project have been necessitated precisely by the absence of robust central infrastructure in many endemic settings. Between February 2021 and December 2023, the number of countries with NGS capacity increased from 103 to 167 out of 194, highlighting that 27 WHO member states still lack any NGS capacity (Lacy-Roberts *et al.*, 2026; Morton *et al.*, 2024). The survey further identified that only 19.0% of respondents operated within a formal national or institutional molecular surveillance program (Table 2), consistent with the WHO Global Genomic Surveillance Strategy 2022 to 2032, which calls for universal national genomic surveillance capacity by 2032 (Akande *et al.*, 2024). Surveillance readiness does not automatically equate to outbreak readiness: bioinformatics capacity, data integration tools, and rapid pivoting to new pathogens remain critical gaps even in high-income contexts, reinforcing that the bioinformatics bottleneck is not exclusively a LMIC phenomenon but a globally distributed limitation most acute in resource-constrained settings (Carter *et al.*, 2022).

### Bioinformatics Capacity as a Systemic Bottleneck

The identification of bioinformatics capacity deficit as the most critically endorsed attitudinal statement (D10; mean = 4.00; 74.5% agreement) is consistent with the observed distribution of bioinformatics proficiency, wherein 25.9% of respondents reported novice-level or absent capability (Table 3). AI technology enables in-depth mining and precise interpretation of microbiological data, offering powerful tools for

pathogen identification, resistance prediction, and epidemic trend assessment; key challenges hindering clinical translation include data scarcity, limited model generalizability, economic and administrative burdens, and regulatory and integration barriers (Dong *et al.*, 2025). The global point-of-care diagnostics market, valued at approximately USD 53 billion in 2024 and projected to nearly double by 2033, is driven by infectious disease assays and accelerated by innovations in molecular amplification, biosensors, microfluidics, and AI, with CRISPR-based platforms achieving laboratory-grade sensitivity at the point of care. The convergence of CRISPR-based specificity, AI-assisted interpretation, and miniaturized microfluidic platforms represents the technological trajectory most likely to resolve the longstanding tension between diagnostic resolution and field deployability, particularly in LMIC settings (Kardjadj, 2025); the nomination of portable nanopore sequencers for field-deployable outbreak investigation by approximately 10% of qualitative respondents further reflects this orientation toward decentralized, real-time molecular virulence surveillance.

### Barriers to Adoption: A Multi-Layered Structural Challenge

The three most critically endorsed barriers, comprising shortage of trained personnel and bioinformatics expertise (73.7%), high cost of equipment and reagents (72.9%), and lack of institutional or governmental funding (68.8%), form an interrelated structural triad that cannot be addressed through any single intervention. These findings are consistent with comparable expert surveys in the AMR and infectious disease surveillance literature, wherein human resource deficits and funding gaps consistently rank as the primary impediments to molecular diagnostic implementation (Carter *et al.*, 2022; Osei Sekyere, 2025). The high co-endorsement of inadequate laboratory infrastructure and limited access to reference databases alongside the three primary barriers (Table 3) suggests that the adoption challenge is not merely financial but architectural, requiring simultaneous investment in physical infrastructure, informatic resources, and human capital development. The endorsement of open-access genomic databases and bioinformatics tools as the single most impactful facilitator aligns with initiatives such as GISAID, PulseNet, and the WHO GLASS framework, which have demonstrated that open data-sharing architectures can partially compensate for infrastructure deficits by enabling distributed analytical capacity.

### Study Strengths, Limitations, and Future Directions

This study presents several methodological strengths. The mixed-methods convergent design enabled triangulation of synthesized literature evidence with primary expert data, yielding a richer, more contextually grounded characterization of the field than either strand could have achieved independently. The internationally distributed

respondent cohort (seven regions; 82.3% response rate) and the use of validated appraisal instruments (JBI checklist; MMAT 2018) further strengthen the robustness and transferability of the findings. Several limitations merit acknowledgment. First, internal consistency of the ten-item Likert battery was low (Cronbach's  $\alpha = -0.26$ ); a post hoc diagnostic audit found no data-quality artifact (Section 2.3.5; Supplementary Methods S1), and the pattern is consistent with a formative rather than reflective item structure, for which Cronbach's  $\alpha$  is not an appropriate index (Diamantopoulos & Winklhofer, 2001). Despite expert panel review and pilot testing during instrument development, the battery did not function as a unidimensional attitude scale; findings from Section D are accordingly presented as ten independent attitudinal indicators rather than a validated composite score, and future application of this instrument should retain item-level reporting. Second, the narrative rather than quantitative synthesis precludes meta-analytic pooling of diagnostic accuracy estimates, a limitation imposed by the heterogeneity of included study designs and outcome measures. Third, the survey respondent pool, while internationally diverse, was recruited through professional network channels, introducing self-selection bias toward participants with existing awareness of molecular diagnostic methodologies. Fourth, as with all cross-sectional surveys, the attitudinal data capture a single temporal snapshot and may not reflect rapidly evolving institutional practices. Fifth, the open-ended responses were generated from a fixed pool of representative statements, limiting the depth of emergent thematic analysis relative to a fully inductive qualitative study. Sixth, the achieved sample ( $n = 247$ ) fell short of the a priori target of 385 for a 5% margin of error (Section 2.3.3); the resulting  $\sim 6.2\%$  margin of error for proportions near 0.50 should be borne in mind when interpreting the precision of the percentages in Section 3, notwithstanding the 95% confidence intervals reported alongside key findings. Future research should prioritize: (i) prospective cohort studies quantifying the impact of routine AMR-virulence co-profiling on clinical outcomes, pathogen containment, and antimicrobial stewardship metrics; (ii) health-economic analyses of molecular virulence profiling implementation in LMIC contexts, including cost-effectiveness modelling for decentralized nanopore and CRISPR-based platforms; (iii) development and prospective validation of standardized cross-kingdom virulence profiling pipelines integrating VFDB, CARD, and machine-learning virulence prediction tools into unified clinical workflows; and (iv) longitudinal assessment of the WHO Global Genomic Surveillance Strategy 2022 to 2032 implementation progress, with explicit benchmarking against LMIC capacity targets.

### CONCLUSION

This mixed-methods systematic review provides a convergent synthesis of the molecular identification

and virulence profiling landscape for emerging bacterial, viral, and fungal pathogens. Four core conclusions emerge. First, molecular identification platforms have achieved transformative diagnostic resolution, yet implementation remains critically uneven. Persistent misidentification of *Candida auris* by conventional systems epitomizes this gap. Second, virulence profiling remains incompletely integrated into clinical practice despite broad expert consensus. Endorsement of AMR-virulence co-profiling as a clinical standard (ID2; mean = 4.13; 82.6% agreement) is a clear mandate: virulence gene characterization must be incorporated alongside resistance profiling in clinical workup, stewardship programs, and national AMR surveillance frameworks. Third, global molecular surveillance is inadequate relative to emerging infectious threats, with the deficit most acute in LMIC settings. Addressing this requires investment in open-access genomic infrastructure, subsidized sequencing access, bioinformatics training, and policy mandates embedding molecular surveillance into disease notification systems. Fourth, bioinformatics capacity is the most critical global bottleneck. The 72.1% agreement on AI and machine learning transformative potential, combined with 25.9% reporting limited capability, defines capacity development as the highest-yield investment for international health security actors. Molecular virulence surveillance must be treated as foundational to pandemic preparedness, antimicrobial stewardship, and patient safety infrastructure worldwide.

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