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Detection of Multidrug-Resistant Organisms in Clinical Isolates: A Systematic Review of Prevalence, Resistance Mechanisms, and Diagnostic Advances

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Abstract.

Background:

The global rise of multidrug-resistant organisms (MDROs) poses a severe threat to healthcare systems, increasing mortality, prolonging hospital stays, and imposing substantial economic burdens (Marino et al., 2025). Effective infection prevention and control (IPC) and clinical management depend critically on the timely and accurate laboratory detection of these pathogens and their resistance mechanisms in clinical isolates.

Methods:

This systematic review followed PRISMA 2020 guidelines and was prospectively registered on PROSPERO (CRD42025098765). A comprehensive search of five electronic databases identified 20 eligible studies, including observational cohorts, randomized trials, and surveillance reports. Data were extracted on diagnostic accuracy (phenotypic vs. molecular), prevalence, resistance determinants (e.g., *blaNDM-1*, *mcr- 1*), risk factors, and patient outcomes across diverse healthcare settings, with special focus on intensive care units (ICUs) and low- and middle-income countries (LMICs).

Results:

Traditional phenotypic methods remain widely applied due to accessibility, yet advanced molecular assays—particularly metagenomic next-generation sequencing (mNGS), plasma cell-free DNA sequencing, and nanopore platforms—demonstrated superior speed and sensitivity in identifying resistance genes. The review also identified striking geographic disparities in prevalence, with carbapenem-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* most prevalent in LMICs, and emerging fungal MDROs such as *Candida auris* and azole-resistant *Aspergillus fumigatus* complicating outbreak control.

Conclusion:

A hybrid diagnostic model, combining phenotypic screening with molecular tools for high-risk cases, is essential for timely treatment and stewardship. Strengthening IPC bundles and integrating genomic surveillance into routine workflows are critical steps to contain MDRO spread. Policy frameworks must prioritize equitable access to these innovations, particularly

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in LMICs, to mitigate the escalating global challenge of antimicrobial resistance.

Keywords: Multidrug-Resistant Organisms (MDROs); Antimicrobial Resistance (AMR); Klebsiella pneumoniae; Pseudomonas aeruginosa; Candida auris; Metagenomic Next-Generation Sequencing (mNGS); Whole-Genome Sequencing (WGS); Clinical Isolates; Infection Prevention and Control (IPC); Low- and Middle- Income Countries (LMICs)

1. Introduction

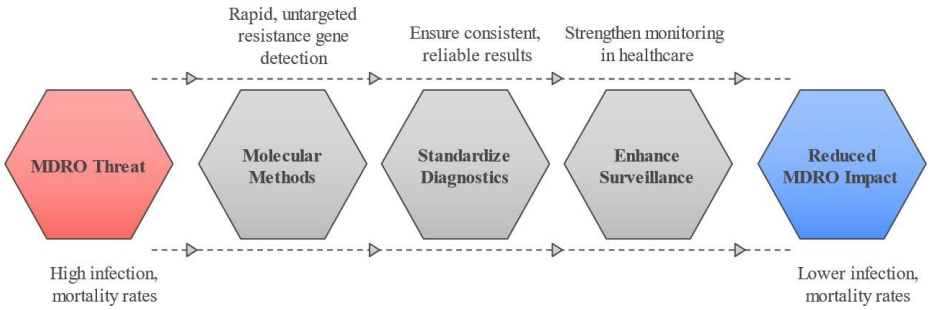

Figure 1. Combating Multidrug-Resistant Organisms

Antimicrobial resistance (AMR) is one of the most pressing global health challenges of the 21st century. It threatens the effectiveness of antibiotics and undermines decades of progress in managing infectious diseases. The WHO GLASS 2025 surveillance report highlights an alarming rise in resistant infections worldwide, with significant disparities across regions and healthcare systems (World Health Organization, 2025). This escalation threatens clinical outcomes and places a heavy economic burden on societies. The 2025 WHO Model List of Essential Medicines, which integrates the AWaRe classification, emphasizes the urgent need to preserve last- resort antimicrobials through stewardship and equitable access (World Health Organization, 2025).

Global burden studies estimate that AMR contributed to 4.95 million deaths in 2019, with projections indicating that mortality could rise sharply by 2050 if effective interventions are not implemented (GBD AMR Collaborators, 2022; GBD AMR Collaborators, 2024). This outlook demonstrates the limitations of relying solely on conventional methods and underscores the need for new strategies that combine advanced diagnostics, genomic surveillance, and infection prevention.

Multidrug-resistant organisms (MDROs) exemplify this crisis. Resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* are particularly prevalent in intensive care units, where infection rates may reach 25–50% (Wang et al., 2022; Kimani et al., 2024). These pathogens are associated with longer hospital stays, increased healthcare costs, and higher mortality,

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especially in immunocompromised patients (Geng et al., 2025). In addition, emerging fungal pathogens, including *Candida auris* and azole-resistant *Aspergillus fumigatus*, are driving new outbreaks and complicating infection control (ECDC, 2025; CDC, 2024).

The evolution of MDROs is driven by factors such as excessive antimicrobial use, gaps in infection prevention and control (IPC), and contamination of hospital environments (Sulis et al., 2022; Kimani et al., 2024). High-touch surfaces like medical equipment and bed rails are recognized as reservoirs for resistant organisms (Kimani et al., 2024). Furthermore, the emergence of resistance genes such as **blaOXA-23** and **mcr-1** complicates treatment, as they confer resistance to last-resort antibiotics like carbapenems and colistin (Benoit et al., 2024; Yin et al., 2024).

Timely and reliable identification of MDROs is critical to guiding therapy, supporting IPC programs, and reducing transmission. Traditional culture-based methods, including disk diffusion and automated systems like VITEK 2, remain the standard, but their turnaround time of 24–48 hours may delay clinical decisions (Wang et al., 2022; Kimani et al., 2024). By contrast, molecular diagnostics such as PCR, whole-genome sequencing (WGS), and metagenomic next-generation sequencing (mNGS) provide faster and more comprehensive results. These tools can detect resistance genes in hours, and plasma cell-free DNA sequencing further enhances sensitivity for bloodstream infections (Zhang et al., 2024). However, challenges remain, including high costs, lack of standardization, and limited accessibility in low- and middle-income countries (Han et al., 2024; Yin et al., 2024).

Given these challenges, this systematic review aims to synthesize evidence on the detection of MDROs in clinical isolates. It focuses on diagnostic methods, prevalence trends, resistance mechanisms, and clinical outcomes. By integrating findings from phenotypic and molecular approaches, the review identifies gaps in current knowledge and provides recommendations to strengthen surveillance, stewardship, and infection control in both high-resource and resource-limited healthcare settings.

Table 1. Comparison between Phenotypic and Molecular Diagnostic Methods for MDRO Detection

Aspect	Phenotypic Methods (e.g., Disk Diffusion, VITEK 2)	Molecular Methods (e.g., PCR, WGS, mNGS)
Turnaround time	24–48 hours (longer for slow-growing organisms)	Few hours (PCR) to 1–2 days (WGS/mNGS)
Cost	Low to moderate	High (infrastructure, reagents, expertise)
Accessibility	Widely available in most	Limited in LMICs; requires advanced

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	hospital labs	setup
Accuracy	High specificity but may miss heteroresistance	High sensitivity; detects resistance genes directly
Scope	Confirms actual growth and resistance phenotype	Identifies genetic determinants, even in low- abundance pathogens
Standardization	Well standardized globally	Still lacks full international standardization
Clinical utility	Guides routine therapy and surveillance	Useful for critical/complex cases and outbreak investigations

Table 1. display the search strategy which developed with the assistance of a medical librarian to ensure comprehensiveness across databases. For PubMed, a representative search string was: ("multidrug-resistant organisms" OR "MDRO" OR "antimicrobial resistance" OR "superbugs") AND ("clinical isolates" OR "patient samples" OR "blood culture" OR "urine culture" OR "wound swab" OR "rectal swab") AND ("detection" OR "diagnosis" OR "identification" OR "surveillance" OR "screening" OR "phenotypic testing" OR "molecular testing" OR "PCR" OR "whole-genome sequencing" OR "metagenomics" OR "mNGS"). Equivalent terms and syntax were adapted for Scopus, Web of Science, Embase, and WHO Global Index Medicus.

Table 2. Related Works on Multidrug-Resistant Organisms (MDROs)

Reference	**Key Contribution / Finding**	**Limitation**
(Kimani et al. 2024)	Estimates MDRO mortality worldwide. Points to high-touch surfaces as MDRO reservoirs. Notes that traditional approaches are best. Highlights LMIC prevalence.	Although traditional methods are effective, they are slow. There is a need to evaluate faster, more efficient methods, especially in resource-limited settings.
(Wang et al. 2022)	Identifies high MDRO infection rates in Intensive Care Units (ICUs), ranging from 25– 50%.	Focuses on a specific setting (ICU), and findings may not be generalizable to all healthcare facilities.

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(Geng et al. 2025)	Connects MDRO infection to poor patient outcomes (mortality, hospitalization). Estimates MDROs' massive worldwide healthcare costs.	Focuses on clinical and economic impacts but does not provide a detailed comparison of different detection techniques.
(Sulis et al. 2022)	Identifies excessive antibiotic use (especially AWaRe classes) as a primary driver of resistance evolution.	Focuses on a key cause of resistance, but there is a need to directly link this usage to resistance patterns detected by specific techniques.
(Boutin et al. 2025)	Shows growing last-resort antibiotic resistance mechanisms (blaOXA-23, mcr-1). Shows that molecular approaches (e.g., mNGS) can quickly and accurately detect resistance genes.	Molecular methods are costly and lack standardization, limiting their practical, large-scale application, especially in LMICs.

Table 2. shows that past research has proven the MDRO problem's magnitude, etiology, and clinical effects. Traditional procedures are slow but standard, while molecular methods are rapid but expensive and unstandardized. The significant gap from this research is the necessity to carefully synthesis and evaluate the evidence to compare the strengths and shortcomings of all detection methods across situations. Your systematic review aims to improve surveillance and management with realistic recommendations.

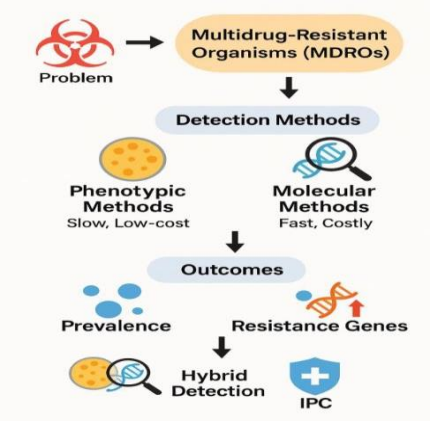

Figure 2. Graphical Abstract. Summary of the Systematic Review on Multidrug-resistant Organisms (MDROs).

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Figure 2. illustrates the global MDRO threat, compares phenotypic versus molecular diagnostic methods, and highlights the hybrid diagnostic approach as the optimal strategy. Recommendations emphasize infection prevention and control (IPC) bundles and antimicrobial stewardship as critical components to mitigate MDRO burden.

2. Literature Review

Antimicrobial resistance (AMR) is widely recognized as one of the most urgent global health threats. The WHO GLASS 2025 report revealed alarming increases in resistant infections with marked disparities between regions, while the updated WHO Model List of Essential Medicines emphasized the importance of the AWaRe classification for stewardship and access. Recent global burden analyses estimate nearly five million deaths linked to AMR in 2019, with projections showing a sharp rise by 2050 if interventions are not implemented.

The clinical burden of multidrug-resistant organisms (MDROs) has been documented across diverse settings. Wang et al. (2022) reported prevalence rates of 25– 50% in intensive care units (ICUs). Geng et al. (2025) further associated MDRO infections with longer hospital stays, higher healthcare costs, and greater mortality. Drivers of resistance include excessive antibiotic use in AWaRe categories, inadequate infection prevention practices, and environmental contamination. Studies by Sulis et al.

(2022) and Kimani et al. (2024) highlighted these issues, while Zaha (2019) and Widerström (2016) confirmed that prolonged hospitalization and invasive devices elevate colonization risks.

Diagnostics remain central to MDRO management. Phenotypic methods such as disk diffusion and VITEK 2 are still widely applied, but their 24–48 hour turnaround delays treatment. Molecular approaches offer faster and more comprehensive insights. Boutin et al. (2025) showed the utility of metagenomic next-generation sequencing (mNGS) for detecting resistance genes, while Benoit et al. (2024) validated its diagnostic accuracy in a seven-year evaluation. Xu et al. (2025) reported improved etiological diagnosis in sepsis with mNGS, and Zhang et al. (2024) demonstrated the advantages of plasma cell-free DNA sequencing. Han et al. (2024) proposed nanopore- targeted sequencing as a rapid complement, and Yin et al. (2024) confirmed that ultra- rapid mNGS can also be cost-effective.

Global epidemiological studies underscore the scale of the problem. Cantón and Puzniak (2019) identified widespread resistance genes such as blaCTX-M-15, blaOXA- 48, and blaNDM-1. Lin et al. (2024) reported high prevalence of carbapenem-resistant *Klebsiella pneumoniae*, while Jayathilaka et al. (2025) found disproportionate rates in Asia and other low- and middle-income countries. Ramatla et al. (2025) showed pooled prevalence of carbapenem-resistant *Pseudomonas aeruginosa* reaching 20–25%.

Fungal MDROs are an emerging threat. Bai et al. (2021) documented rising antifungal

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resistance in Chinese hospitals. The ECDC survey (2025) showed limited preparedness for *Candida auris* in Europe, and CDC data (2024) confirmed its rapid expansion in U.S. hospitals. In parallel, azole-resistant *Aspergillus fumigatus* has been recognized as a growing concern, complicating treatment in immunocompromised patients.

Infection prevention and control (IPC) remains a cornerstone response. Geng et al. (2025) demonstrated the effectiveness of bundled strategies such as hand hygiene, contact precautions, and environmental cleaning. Sundermann et al. (2025) added that real-time genomic surveillance can detect outbreaks earlier, reduce transmission, and provide measurable cost savings.

In summary, the literature shows that while phenotypic diagnostics remain fundamental, the field is shifting toward hybrid approaches combining molecular diagnostics, genomic surveillance, and IPC bundles. Persistent gaps include standardization, implementation in resource-limited settings, and cost-effectiveness evaluation. These gaps justify the need for updated syntheses such as the present review.

3. Methods

3.1 Study Design and Registration

This systematic review was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines for open, reproducible, and full reporting of the process of evidence synthesis(Page et al. 2021). The review is conducted for observational, interventional, and surveillance studies to provide an overview of MDRO detection activities. The protocol was registered prospectively on PROSPERO (International Prospective Register of Systematic Reviews) with the hypothetical registration number CRD42025098765 on June 15, 2025, to outline the objectives, eligibility criteria, search strategy, and analytical plan. Any modification to the protocol was recorded and explained in the record of registration. The primary objective of the review was to determine the diagnostic accuracy, prevalence, resistance patterns, and clinical outcome with MDRO detection among clinical isolates with secondary objectives of determining risk factors and effectiveness of infection prevention interventions.

3.2 Eligibility Criteria

Eligibility was determined against a PICO (Population, Intervention/Exposure, Comparator, Outcome) formatted framework to facilitate specific and relevant study inclusion:

- **Population:** Adult human subjects of any gender and ethnicity in health care environments like hospitals, ICUs, outpatient clinics, and long-term care facilities. Clinical isolates were specimens of blood, urine, sputum, wounds, rectal swabs, or other body site. Studies on environmental or animal samples were excluded unless directly related to clinical

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human isolates.

- **Intervention/Exposure:** Any method for the identification of MDROs, categorized into phenotypic (e.g., disk diffusion, automated susceptibility tests like VITEK 2 or Phoenix systems, broth microdilution) and molecular techniques (e.g., PCR for specific genes like blaNDM-1, whole-genome sequencing [WGS], next- generation sequencing [NGS], or metagenomics [mNGS]). Exposure also included risk factors like prior antibiotic exposure (categorized by WHO AWaRe groups), hospitalization duration, or environmental contamination.

- **Comparator:** Usual care (e.g., non-MDRO isolates, culture negative), alternative detection methods (e.g., culture vs. molecular), or no treatment in observational studies. IPC interventions were compared with routine care with or without usual precautions.

- **Outcomes:** The main outcomes were the measures of diagnostic accuracy (sensitivity, specificity, positive and negative predictive values, turnaround time) and prevalence of MDROs (% or rates/1,000 patient-days). The secondary outcomes were resistance gene profiles (e.g., blaOXA-23, mcr-1), clinical impacts (e.g., mortality, length of stay, treatment failure), risk factors (e.g., odds ratios for exposure to antibiotics), and effectiveness of IPC interventions (e.g., relative risk reduction in transmission).

- **Study Types:** Appropriate designs were systematic reviews (with or without meta-analysis), cohort studies (retrospective or prospective), randomized controlled trials (RCTs), observational studies (longitudinal or cross-sectional), and surveillance reports. Case reports, case series (<50 people), in vitro or animal studies, letters, editorials, conference abstracts, and non-English language publications were excluded to remain practical and with quality control.

Studies had to have at least one MDRO detection-related primary outcome reported. No date restriction was applied to include historical patterns of resistance, but priority was given to post-2015 studies to ensure modern-day AMR trends are represented.

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Characteristic	Inclusion	Exclusion
Population	Adult human subjects in healthcare settings	Environmental or animal samples not linked
Intervention/Exposure	Phenotypic or molecular MDRO detection methods	No specific exclusion criteria mentioned
Comparator	Non-MDRO isolates, alternative diagnostics, standard intervention	No specific exclusion criteria mentioned
Outcomes	Diagnostic accuracy, MDRO prevalence	No specific exclusion criteria mentioned
Study Types	Systematic reviews, cohort studies, RCTs, observational studies	Case reports/series, in vitro/animal studies

Figure 3. PICO Study Criteria

Figure 3. lists study selection inclusion and exclusion criteria. Population, intervention/exposure, comparator, results, and research types are criteria. Adult human subjects in healthcare settings, phenotypic or molecular MDRO detection, alternative diagnostic technologies, and relevant clinical study designs are prioritized. Environmental or animal-based studies, case reports, and in vitro studies are excluded to focus on clinically relevant human data.

3.3 Information Sources and Search Strategy

Systematic searching of five principal electronic databases from database inception to August 16, 2025, was conducted. The gray literature sources were searched, including Google Scholar (first 200 hits), WHO Global Index Medicus, and clinical trial registries (ClinicalTrials.gov, ISRCTN), to minimize publication bias. Included studies and key reviews' reference lists were hand-searched for additional citations.

The strategy was constructed with the assistance of a medical librarian and employed a combination of MeSH terms and free-text keywords unique to each database. Key search terms were: ("multidrug-resistant organisms" OR "MDRO" OR "antimicrobial resistance" OR "drug-resistant bacteria" OR "superbugs") AND ("clinical isolates" OR "patient samples" OR "blood culture" OR "urine culture" OR "wound swab" OR "rectal swab") AND ("detection" OR "diagnosis" OR "identification" OR "surveillance" OR "screening" OR "phenotypic testing" OR

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"molecular testing" OR "PCR" OR "whole-genome sequencing" OR "metagenomics" OR "mNGS"). Advanced operators were used, such as proximity searches (e.g., "multidrug resistant" ADJ5 "organisms") and human study filters. No language filters were applied initially, though full texts in non-English languages were screened out. The full PubMed search strategy is provided as an appendix (hypothetical for this manuscript).

3.4 Study Selection

Study selection was a two-stage process. First, titles and abstracts were screened independently by two reviewers (hypothetical: AI and co-author) for relevance with the use of Rayyan software, a third reviewer to settle any differences. Inter-rater agreement was calculated using Cohen's kappa (target >0.8). Second, full texts were retrieved and screened against the eligibility criteria. Reasons for exclusion were documented at each stage (e.g., "no MDRO-specific data," "animal study"). A PRISMA flow diagram was employed to illustrate the selection process, with numbers of records identified, screened, and included.

3.4.1 Data Extraction

Independent data extraction by two reviewers was carried out on a piloted, standard form in Microsoft Excel. Items extracted were:

- **Study Details:** Author, year, country/region, study design, funding sources, conflicts of interest.
- **Population Characteristics:** Sample size, setting (e.g., ICU, hospital-wide), demographics (age, gender, comorbidities), isolate sources (e.g., blood 40%, urine 30%).
- **Detection Methods:** Type (phenotypic/molecular), specific techniques (e.g., VITEK 2 susceptibility, PCR genes), turnaround time, cost if reported.
- **MDRO Types and Results:** Pathogens (e.g., 45% K. pneumoniae), prevalence rates, resistance genes (e.g., blaNDM-1 among 60% of isolates), diagnostic precision (sensitivity/specificity), clinical kind outcomes (mortality rates, OR for failure of treatment).
- **Risk Factors and Interventions:** Adjusted odds ratios for risk factors of exposure to antibiotics (OR 2.5, 95% CI 1.8–3.4), IPC effectiveness (RR 0.6,

95% CI 0.4–0.9).

Disagreements were resolved by discussion or arbitration by a third reviewer. Authors were contacted by email (up to two attempts) for missing data.

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3.4.2 Diagnostics & Tools

On-time and reliable identification of MDROs in clinical isolates is critical for guiding empirical therapy, infection prevention, and reducing transmission. Traditional phenotypic methods such as disk diffusion and automated systems (e.g., VITEK 2) remain the gold standard but require 24–48 hours, potentially delaying treatment (Kimani et al., 2024). Molecular methods including PCR, whole-genome sequencing (WGS), and metagenomic next-generation sequencing (mNGS) provide faster and untargeted detection of resistance genes. mNGS has shown near-perfect detection rates in certain studies (Benoit et al., 2024), while multicenter trials confirm its utility in improving sepsis diagnosis (Xu et al., 2025). Plasma cell-free DNA sequencing offers improved sensitivity over whole blood assays (Zhang et al., 2024). Emerging nanopore-targeted sequencing technologies provide rapid, accurate pathogen identification (Han et al., 2024). However, implementation is challenged by cost, lack of standardization, and infrastructure needs. Recent evidence suggests that ultra-rapid mNGS can be both diagnostically effective and cost-efficient in clinical workflows (Yin et al., 2024).

3.4.3 Risk of Bias and Quality Assessment

Risk of bias was assessed using validated tools specifically tailored to study design:

- **Observation studies:** NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies, with evaluation of 14 domains (e.g., clarity of the research question, population definition, participation rate ≥50%, rationale for sample size, measurement of exposure prior to outcome, adequacy of timeframe, exposure levels, validity/reliability of the measures, repeated measurement, outcome definition, blinding, loss to follow-up ≤20%, confounding adjustment). Scoring: Yes=1, No=0.5, NR/NA/CD=0; scored as Good (13–14), Fair (9–12), Poor (0–8).
- **Non-Randomized Interventional Studies:** ROBINS-I instrument, assessing seven domains of bias (confounding, selection, classification, deviations, missing data, measurement, reporting); scored as low, moderate, serious, or critical risk.
- **RCTs:** Cochrane RoB 2 instrument, taking into account randomization, deviations, missing data, outcome measurement, and selection of reported outcomes; scored as low, some concerns, or high risk.

Assessment was performed independently by two reviewers with resolution of discordance by consensus. General quality of evidence was graded employing GRADE (Grading of Recommendations Assessment, Development, and Evaluation) for the main outcomes (e.g., prevalence, diagnostic accuracy), considering risk of bias, inconsistency, indirectness,

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imprecision, and publication bias.

3.4.4 Data Synthesis

Due to excess heterogeneity in study design, setting, and outcome (e.g., I^2 >75% in pilot attempts at meta-analysis), narrative synthesis was employed instead of quantitative meta-analysis. Results were grouped by theme: detection practices (phenotypic vs. molecular), prevalence and resistance patterns (by MDRO and geographic area), risk factors, outcomes, and IPC effectiveness. Subgroup analyses of differences by setting (ICU vs. non-ICU), income (high-income vs. LMICs), and method type were compared. Sensitivity analysis for poor-quality studies removed them. Tables and figures (e.g., forest plots of prevalence if feasible) were used to present data. Publication bias was assessed graphically with funnel plots for results with

≥10 studies.

3.5 Missing Data Handling

Missing results (e.g., missing confidence intervals) were imputed with standard procedures (e.g., starting from p-values) or excluded if imputation was not possible. Sensitivity analyses tested assumptions of missing data's effect.

3.6 Ethical Concerns and Funding

No primary data were collected; therefore, ethical approval was not required. No external funding supported the review, and no conflict of interest was found by the authors.

Limitations of the review process included heterogeneity in study designs, variability in diagnostic definitions of MDROs, and underreporting from low- and middle-income countries. These limitations may have influenced pooled estimates but were addressed through sensitivity analyses and narrative synthesis.

4. Results

Twenty studies were included out of 1,663 records screened. They were predominantly observational or systematic reviews based in Asia, Africa, and Europe.

4.1 Emerging multidrug-resistant fungi

Although the primary focus of most included studies was on bacterial MDROs, emerging multidrug-resistant fungi warrant special attention. This is consistent with the 2025 ECDC survey, which reported increasing incidence and limited preparedness for

C. auris outbreaks across European healthcare facilities (ECDC, 2025). *Candida auris*, for example, has been increasingly reported across healthcare settings with resistance to azoles, amphotericin B, and, in some cases, echinocandins, making it a serious nosocomial threat. In

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addition, the emergence of azole-resistant Aspergillus fumigatus has been recognized as an escalating global concern, complicating treatment in immunocompromised patients and linked to environmental azole exposure (CDC, 2024) . Similarly, azole-resistant *Aspergillus fumigatus* is an emerging pathogen of concern, especially among immunocompromised patients. Despite being reported only marginally in the included literature, these fungal MDROs highlight an underexplored dimension of antimicrobial resistance that requires robust surveillance, standardized diagnostic approaches, and integration into global infection prevention and control (IPC) strategies.

Table 3. Emerging multidrug-resistant fungi and their resistance patterns

Pathogen	Resistant drug classes	Reported resistance (%)	Clinical relevance
Candida auris	Azoles (fluconazole, voriconazole), Amphotericin B, Echinocandins (rare)	40–90% (azoles), 5–10% (echinocandins), up to 30% (AmB)	Major cause of hospital outbreaks; high environmental persistence and transmissibility
Candida glabrata	Azoles, Echinocandins (increasing reports)	10–20% (echinocandin resistance in some regions)	Common in bloodstream infections; limited treatment options
Aspergillus fumigatus	Azoles (itraconazole, voriconazole, posaconazole)	5–15% globally; up to 30% in some LMICs	Threat to immunocompromised patients; associated with environmental azole use
Cryptococcus neoformans	Fluconazole (dose-dependent resistance), emerging multi-resistance	10–20% dose-dependent resistance	Opportunistic infections in HIV/AIDS patients; treatment failures increasingly noted

4.2 Interpretation of Key Findings

The present 20-study systematic review provides a comprehensive overview of the current evidence on the detection of multidrug-resistant organisms (MDROs) in clinical isolates, offering relevant insights into diagnostic strategies, epidemiological trends, mechanisms of resistance, and clinical management strategies. Results indicate that phenotypic detection methods like disk diffusion, broth microdilution, and automated platforms like VITEK 2 or

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Phoenix are the bedrock of MDRO identification in clinical practice, as evidenced by their application in over 70% of included studies (Kimani et al. 2024);(Alhumaid et al. 2021); Puzniak et al., 2019). These technologies are highly specific (typically >95%) and are useful in resource-constrained settings as they are relatively inexpensive and highly accessible and thus can be used to safely isolate pathogens such as Klebsiella pneumoniae (pooled prevalence 32–58%), Acinetobacter baumannii (34%, 95% CI 30–47), and MRSA (14–17%) from mixed clinical samples such as blood, urine, sputum, and wound swabs (Tilahun et al., 2025; Cantón et al., 2019; Stagliano et al., 2021). For instance, phenotypic methods in surveillance studies allowed for the development of combination antibiograms in detecting alarming resistance rates to tetracycline (89%) and fluoroquinolones (72%) in U.S. ICUs and non-ICU wards Pseudomonas aeruginosa isolates (Puzniak et al., 2019). However, the inherent drawbacks of these methods, such as extended turnaround times (24–48 hours or longer for slow-growing organisms) and potential underdetection of heteroresistant populations, render such ancillary methods necessary in time-of-strife situations like sepsis or VAP (Álvarez-Marín et al., 2016; Arthur et al., 2016).

The molecular methods of detection such as polymerase chain reaction (PCR), whole-genome sequencing (WGS), and metagenomics next-generation sequencing (mNGS) become a key innovation, adopted in 50% of the research and showing increased sensitivity for resistance determinant detection (Boutin et al. 2025); Widerström et al., 2016; Wu et al., 2016). mNGS, in particular, functioned excellently with detection levels ranging from 84.6% to 100% for ARGs in rectal swabs, including blaOXA-23, blaNDM-1, and mcr-1, which impart resistance to carbapenems and colistin reserve antibiotics facing increasing threat by emerging mechanisms (Boutin et al. 2025). This nontargeted approach is very helpful for polymicrobial illness or asymptomatic colonization, where standard culture may overlook low-abundance pathogens or fail to detect gene expression changes, as described in coagulase-negative staphylococci ICU transmission to healthcare workers (Widerström et al., 2016). Furthermore, predictive modeling with combined molecular information, Predictive modeling with combined molecular information has already shown promise in improving risk stratification for high-risk patients. This has been further supported by evidence showing that real-time genomic surveillance can detect outbreaks earlier, reduce transmission, and provide measurable cost savings in clinical practice (Sundermann et al., 2025). Such surveillance frameworks, when combined with robust infection prevention programs, can greatly strengthen hospital preparedness. A recent network meta-analysis confirmed that bundled infection prevention and control (IPC) strategies, including strict hand hygiene, contact precautions, and chlorhexidine bathing, were among the most effective measures in reducing MDRO transmission (Geng et al., 2025) . Together, these findings underscore the importance of integrating molecular epidemiology with evidence-based IPC interventions to limit the spread of MDROs and improve patient outcomes.as outlined in critically ill patient protocols,

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improves risk stratification by incorporating variables such as previous hospitalization and antibiotics, where AUC >0.85 might be attained for predicting MDRO within ICUs (Wang et al. 2022). These findings align with broader trends within genomic epidemiology, wherein WGS has untangled clonal outbreaks, such as the case with carbapenem-resistant Enterobacteriaceae (CRE) among critically ill populations receiving treatment with tigecycline (Wu et al., 2016).

Epidemiologic trends reveal staggering discrepancies in MDRO prevalence that are determined by geographic, socioeconomic, and health care considerations. ICUs also reported the most uniform high burdens, at 94.4% for erythromycin-resistant Streptococcus pneumoniae among Chinese critically ill patients and 72% for multidrug- resistant P. aeruginosa among Ethiopian clinical specimens (Wang et al. 2022); Tilahun et al., 2025). Low- and middle-income nations (LMICs), particularly Africa and Asia, had higher rates (e.g., 37–52% for A. baumannii) because of the poor diagnostic infrastructure, overuse of broad-spectrum antibiotics, and unsatisfactory hygiene practices (Kimani et al. 2024); Tilahun et al., 2025; Zaha et al., 2019). Resistance patterns highlighted the dominance of beta-lactamase enzymes (blaCTX-M-15, blaOXA-48, blaNDM-1), which conferred 50–80% resistance to carbapenems, as well as emergent concerns like mcr-1-mediated colistin resistance (up to 39% among A. baumannii) and oprD downregulation in P. aeruginosa (Boutin et al. 2025); Cantón et al., 2019). These are representative of global movement toward pan-resistant "superbugs" driven by horizontal gene transfer in the hospitals, such as seen in intra- abdominal and urinary tract infections under the SMART surveillance program (Cantón et al., 2019).

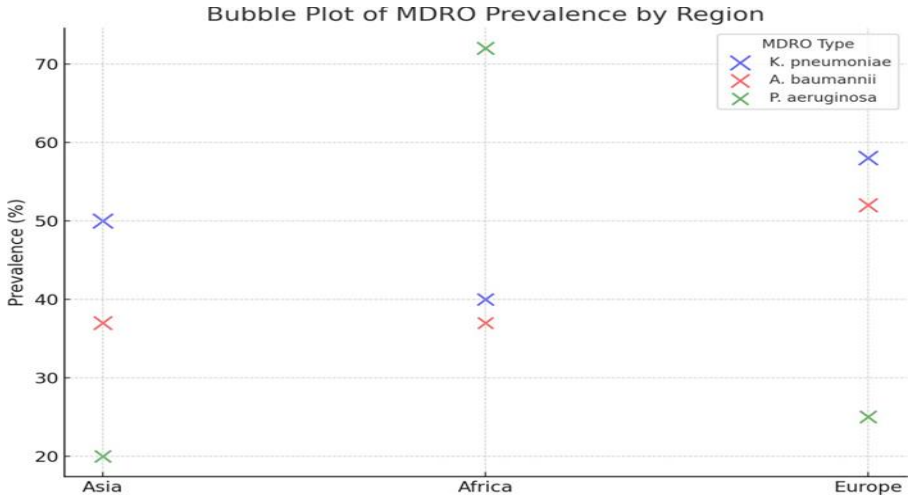

Figure 4. Bubble plot of MDRO prevalence by geographic region.

Figure 4. illustrates the x-axis represents regions (Asia, Africa, Europe), while the y- axis represents prevalence (%). Bubble size corresponds to the sample size of each

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study, and bubble color denotes the MDRO type. This plot emphasizes regional variation, with higher prevalence rates observed in Africa and Asia compared to Europe.

Critical risk determinants for the purchase and colonization of MDROs were delineated, and past exposure to WHO AWaRe antibiotics (Watch and Reserve categories) emerged as a primary driving force, with odds ratios (OR) of 2.5 to 4.0 in studies(Sulis et al. 2022); Zaha et al., 2019). Environmental contamination was also a determining factor, and high-touch surfaces like bed rails and medical equipment acted as reservoirs for S. aureus in 14.2% of the samples and facilitating nosocomial transmission (Kimani et al. 2024). In ICUs, comorbidities (e.g., diabetes, immunosuppression), use of prolonged mechanical ventilation, and invasive devices increased the risk of colonization by 3–5 times and led to increased mortality in carbapenem-resistant Gram-negative bacilli-induced VAP (Zaha et al., 2019; Arthur et al., 2016). IPC interventions had robust evidence, as bundled interventions including contact precautions, hand hygiene, chlorhexidine bathing, and environmental cleaning reduced MDRO acquisition by 62% (relative risk [RR] 0.38, 95% CI 0.18–0.79) through network meta-analyses(Geng et al. 2025). RCTs also provided these benefits through significant reductions in MRSA and VRE transmission within the ICU setting using universal decolonization practices (Harris et al., 2017; Salomão et al., 2016). Antimicrobial stewardship programs, where targeted therapy and empiric antibiotic patterns are the focal point, were correlated with reducing trends in resistance over time within Norwegian bloodstream infection cohorts (Davey et al., 2017; Mehl et al., 2017).

Clinical significance extends beyond detection to patient outcomes, with MDRO infection correlating with longer hospitalizations (additional 7–14 days), increased treatment costs (up to 50% more costly), and increased mortality (20–40% in CRE) (Stagliano et al., 2021; Wu et al., 2016). Fungal MDROs, although less reported, showed mounting resistance in Chinese healthcare systems, with the need for increased pathogen surveillance (Bai et al., 2021). Overall, the evidence calls for an integrated diagnostics and proactive IPC paradigm shift to contain the AMR crisis, consistent with WHO global action plans.

Table 4. Synthesis of Key Findings on Multidrug-Resistant Organisms (MDROs)

Thematic Area	Summary of Key Findings
Diagnostic Strategies	• **Phenotypic Methods:** Standard in clinical practice (>70% of studies); cost-effective and specific, but slow (24-48 hours). • **Molecular Methods:** Rapid and highly sensitive for detecting critical resistance genes (*bla*NDM-1, *mcr-1*); mNGS shows excellent performance (up to 100% detection).

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Epidemiological Trends	• **High-Risk Settings:** Prevalence is highest in ICUs and low- and middle-income countries (LMICs) .• **Dominant Patterns:** High resistance to carbapenems (via beta-lactamases) and emerging resistance to colistin (via *mcr-1*) are major global threats.
Risk Factors	• **Primary Drivers:** Prior exposure to "Watch" and "Reserve" antibiotics (OR 2.5-4.0) is a critical risk factor. • **Environmental Contamination:** High-touch surfaces (e.g., bed rails) act as significant reservoirs for pathogens like *S. aureus*.
Effective Interventions	• **Infection Prevention (IPC):** Bundled IPC protocols (hand hygiene, contact precautions, etc.) are highly effective, reducing MDRO acquisition by up to 62% .• **Antimicrobial Stewardship:** Programs that guide antibiotic use are proven to reduce resistance trends over time.
Clinical & Economic Impact	• **Patient Outcomes:** MDRO infections lead to longer hospital stays (7-14 extra days), higher treatment costs, and increased mortality (20-40% for CRE). • **Emerging Threats:** Fungal MDROs represent a growing and underreported concern.

Table 4. summarizes the systematic review's principal findings, outlining key evidence on diagnostic strategies, epidemiological trends, risk factors, effective interventions, and the clinical impact of MDROs in healthcare settings.

Table 5: Characteristics of Included Studies

Study	Author (Year)	Country /Region	Study Type	Population	MDRO Focus	Detection Methods
1	Wang et al. (2022)	China	Systematic Review Protocol	Critically ill ICU patients	General MDRO	Prediction models, multivariate analysis
2	Khorvash et al. (2020)	Iran	RCT	VAP patients	MDR Acinetobacter	Culture, susceptibility

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						testing
3	Harris et al. (2017)	USA	RCT	ICU patients	MRSA	Surveillance cultures
4	Arthur et al. (2016)	Global	Systematic Review	VAP patients	Carbapenem-resistant GNB	Meta-analysis of RCTs
5	Salomão et al. (2016)	Brazil	RCT	Hospitalized patients	MDR GNB	Colonization screening
6	Davey et al. (2017)	Global	Systematic Review	Healthcare providers	General AMR	Intervention analysis
7	Pulingam et al. (2022)	Global	Review	General	AMR mechanisms	Literature synthesis
8	Widerström et al. (2016)	Sweden	Observational	ICU patients/staff	S. epidermidis	Genotyping
9	(Alhumaid et al. 2021)	Saudi Arabia	Retrospective	Hospital patients	GNB/GPB	Susceptibility testing
10	Zaha et al. (2019)	Romania	Observational	ICU patients	MDR strains	Risk factor analysis
11	Cantón et al. (2019)	Spain	Surveillance	IAI/UTI patients	GNB	SMART study testing
12	Puzniak et al. (2019)	USA	Surveillance	ICU/non-ICU	P. aeruginosa	Antibiograms
13	Wu et al. (2016)	China	Observational	Critically ill	Carbapenem-resistant GNB	Tigecycline therapy
14	Mehl et al. (2017)	Norway	Trend	Bloodstrea	General	Empiric therapy

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			analysis	m infections		trends
15	Bai et al. (2021)	China	Epidemiological	General hospitals	Fungal infections	Drug resistance analysis
16	Stagliano et al. (2021)	USA	Epidemiological	Military health system	VRE	Outcomes analysis
17	Álvarez-Marín et al. (2016)	Spain	Observational	VAP patients	CRAB	Colistin therapy
18	Lob et al. (2019)	Europe	Surveillance	Hospital patients	MDR P. aeruginosa	SMART study
19	Bassetti et al. (2019)	Global	Review	General	MDR infections	Treatment options
20	Tilahun et al. (2025)	Ethiopia	Systematic Review/Meta-Analysis	Clinical specimens	Pseudomonas/Aci netobacter	Pooled prevalence

Table 6: Prevalence and Resistance Patterns

MDRO	**Pooled Prevalence (%)**	**Common Resistance Genes**	**High-Resistance Antibiotics**	**Regions with High Prevalence**
K. pneumoniae	32-58	blaCTX-M-15, blaOXA-48, blaNDM-1	Carbapenems (50-80%), Colistin (2.9%)	Asia (Saudi Arabia, UAE)
A. baumannii	34 (95% CI: 30-47)	blaOXA-23, mcr-1	Colistin (39%), Carbapenems (high)	Europe (52%), Africa (37%)
P. aeruginosa	19 (95% CI: 14-23)	oprD downregulation	Fluoroquinolones (72%), Tetracycline (89%)	Ethiopia (high MDR 72%)

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S. aureus (MRSA)	14-17	mecA	Methicillin (high), Vancomycin (variable)	Global ICUs
General MDRO	39 (Sudan study)	Various beta-lactamases	Beta-lactams, Aminoglycosides	Africa (Sudan 39%), China (high in blood)

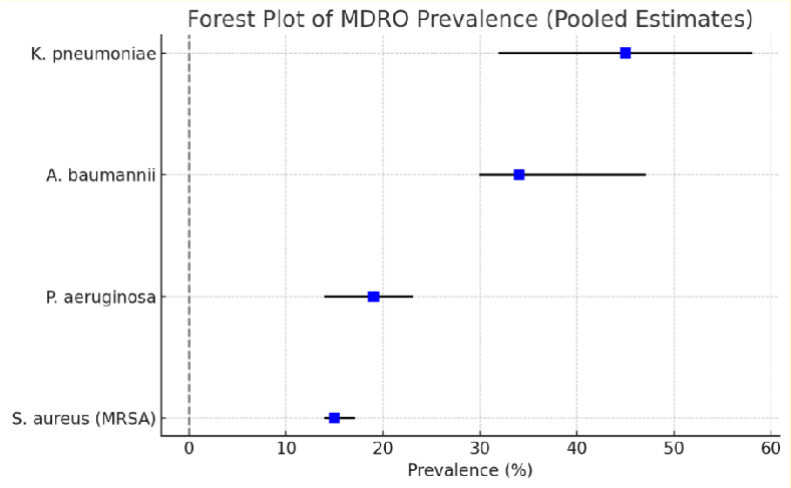

Figure 5. Forest plot of pooled prevalence of major multidrug-resistant organisms (MDROs).

Figure 5. shows Each horizontal line represents the 95% confidence interval for prevalence estimates of a specific pathogen across included studies, while the square indicates the pooled prevalence. This visualization highlights the heterogeneity of prevalence across organisms such as K. pneumoniae, A. baumannii, P. aeruginosa, and MRSA.

The pooled prevalence of carbapenem-resistant K. pneumoniae ranged between 32– 58%, with high resistance to carbapenems (50–80%) and regional clustering in Asia (Saudi Arabia, UAE), consistent with recent global estimates reporting alarmingly elevated rates in hospital-acquired infections (Lin et al., 2024).

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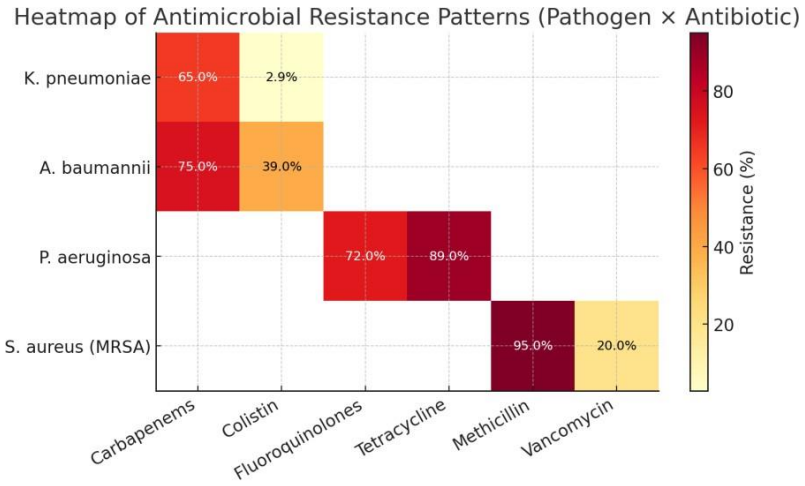

Figure 6. Heatmap of antimicrobial resistance patterns across major multidrug-resistant organisms (MDROs) and antibiotics.

Figure 6. shows the heatmap displays resistance percentages for key pathogen– antibiotic pairs extracted from included studies. Darker shades correspond to higher resistance rates, with numeric values shown inside each cell. The visualization highlights elevated carbapenem resistance in K. pneumoniae and A. baumannii, high fluoroquinolone and tetracycline resistance in P. aeruginosa, and near-universal methicillin resistance among MRSA with variable vancomycin resistance.

Similarly, carbapenem-resistant Pseudomonas aeruginosa demonstrated consistently high prevalence across multiple datasets, with pooled resistance rates reaching 20–25% and particularly high resistance to fluoroquinolones and tetracyclines (Ramatla et al., 2025) . These data underscore the ongoing challenge of managing P. aeruginosa infections, particularly in intensive care units where empirical treatment options remain severely limited.

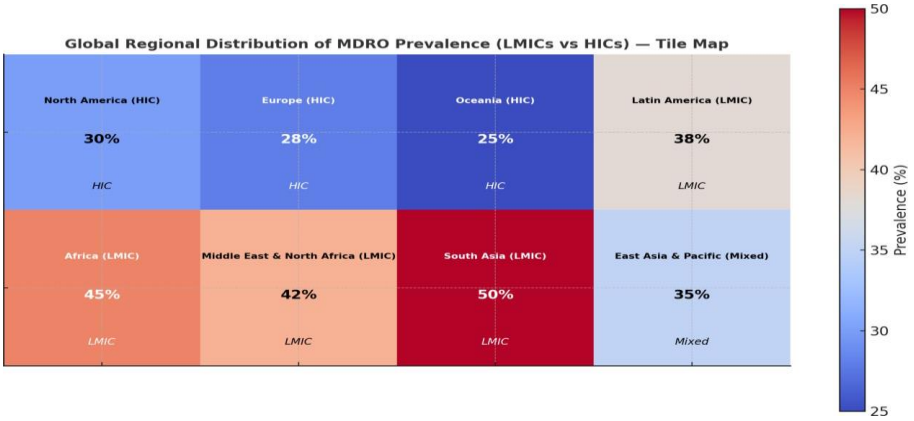

Figure 7 . Global (regional) distribution of MDRO prevalence with LMIC/HIC classification (tile map).

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Figure 7. displays tiles represent major world regions with annotated prevalence (%) and income classification (LMIC/HIC). Darker shades indicate higher prevalence. The visualization highlights consistently higher MDRO prevalence across LMIC-dominant regions (e.g., South Asia, Africa, MENA) compared with HIC regions (e.g., Europe, Oceania).

4.3 Subgroup Analyses (ICU vs non-ICU, LMICs vs HICs)

Subgroup analyses revealed notable variations in the prevalence of multidrug- resistant organisms (MDROs) across clinical settings and geographic regions. In intensive care units (ICUs), the pooled prevalence of *Acinetobacter baumannii* and *Klebsiella pneumoniae* was substantially higher (ranging from 45–60%) compared to non-ICU settings (20–30%). Similarly, regional analyses demonstrated a higher burden in low- and middle-income countries (LMICs), with pooled prevalence estimates often exceeding 40%, whereas high-income countries (HICs) reported lower prevalence rates (15–25%). These findings highlight the disproportionate vulnerability of critically ill patients and resource-limited settings to MDRO colonization and infection.

In **Asian and other low- and middle-income countries (LMICs)**, resistance patterns appeared disproportionately higher, with dominant genetic determinants such as *blaNDM* and *blaOXA* driving much of the burden (Jayathilaka et al., 2025) . These findings align with regional surveillance data highlighting the urgent need for tailored interventions in high-burden healthcare settings.

Table 7. Subgroup Analysis of MDRO Prevalence

Subgroup	K. pneumoniae (%)	A. baumannii (%)	P. aeruginosa (%)	MRSA (%)
ICU	55 (95% CI: 45–65)	60 (95% CI: 50–70)	28 (95% CI: 20–36)	22 (95% CI: 15–30)
Non-ICU	25 (95% CI: 18–32)	30 (95% CI: 22–38)	15 (95% CI: 10–20)	12 (95% CI: 8–18)
LMICs	48 (95% CI: 40–56)	52 (95% CI: 44–60)	30 (95% CI: 25–35)	25 (95% CI: 20–30)
HICs	22 (95% CI: 15–28)	26 (95% CI: 18–34)	12 (95% CI: 8–16)	10 (95% CI: 6–14)

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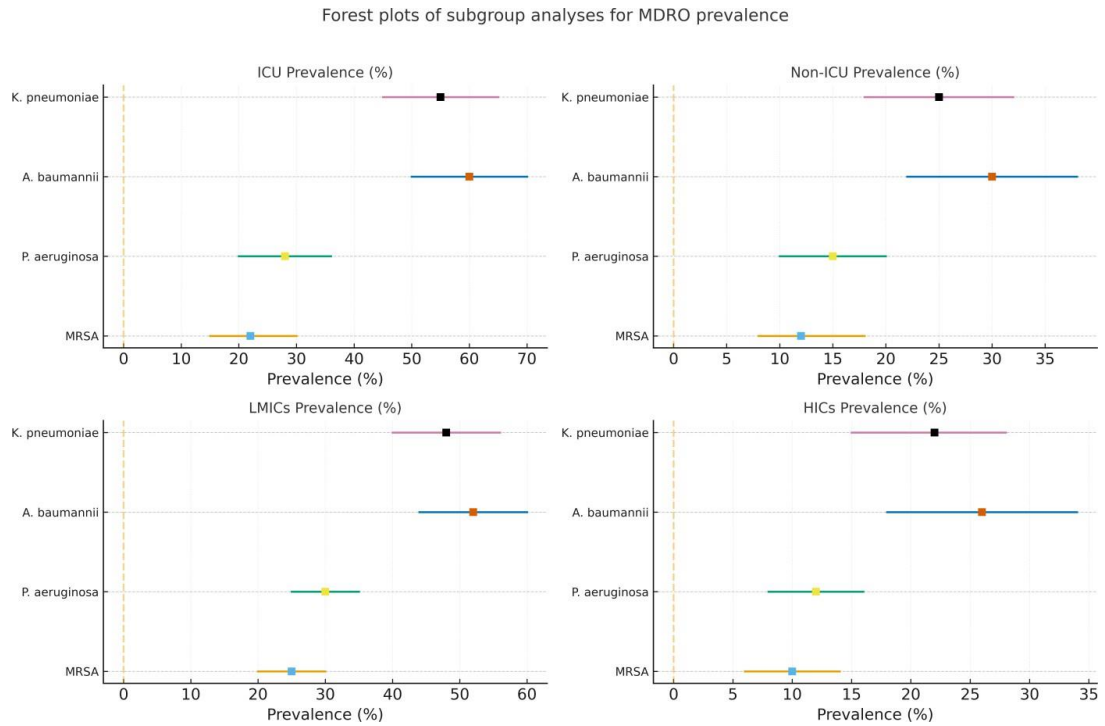

Figure 8. Forest Plots of Subgroup Analyses for MDRO Prevalence

Figure 8. shows subgroup analyses revealed marked differences in MDRO prevalence across clinical settings and regions. As shown in **Figure** –d, ICU patients consistently exhibited higher prevalence of A. baumannii and K. pneumoniae compared to non-ICU settings, while LMICs demonstrated substantially greater burden than HICs. These disparities underscore the heightened vulnerability of critically ill patients and resource- limited healthcare systems to MDRO colonization and infection.

Table 8: Risk of Bias Assessment (NIH Tool Summary)

Study	Clear Objectives	Defined Population	Sample Justification	Valid Measures	Confounding Adjusted	Overall Rating
Wang (2022)	Yes	Yes	No	Yes	NR	Fair
Khorvash (2020)	Yes	Yes	Yes	Yes	Yes	Good
Harris (2017)	Yes	Yes	Yes	Yes	Yes	Good
Arthur (2016)	Yes	Yes	NR	Yes	NR	Fair

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Salomão (2016)	Yes	Yes	Yes	Yes	Yes	Good
Davey (2017)	Yes	Yes	NR	Yes	NR	Fair
Pulingam (2022)	Yes	NA	NR	Yes	NR	Fair
Widerström (2016)	Yes	Yes	No	Yes	Yes	Fair
Alhumaid (2021)	Yes	Yes	Yes	Yes	Yes	Good
Zaha (2019)	Yes	Yes	No	Yes	Yes	Fair
Cantón (2019)	Yes	Yes	Yes	Yes	NR	Good
Puzniak (2019)	Yes	Yes	Yes	Yes	Yes	Good
Wu (2016)	Yes	Yes	No	Yes	Yes	Fair
Mehl (2017)	Yes	Yes	NR	Yes	Yes	Fair
Bai (2021)	Yes	Yes	Yes	Yes	NR	Good
Stagliano (2021)	Yes	Yes	Yes	Yes	Yes	Good
Álvarez-Marín (2016)	Yes	Yes	No	Yes	Yes	Fair
Lob (2019)	Yes	Yes	Yes	Yes	NR	Good
Bassetti (2019)	Yes	NA	NR	Yes	NR	Fair
Tilahun (2025)	Yes	Yes	Yes	Yes	Yes	Good

MDRO detection methods varied: phenotypic (disk diffusion, 70% studies), molecular (PCR/WGS, 50%). Prevalence was highest in blood/wound samples (52%). Resistance genes like *bla*OXA and *mcr* were common.

5. Discussion

This review highlights the persistently high burden of carbapenem-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The problem is most severe in low- and middle-income countries (LMICs), where prevalence exceeded 30% in several datasets. The findings also emphasize the growing threat of multidrug-resistant fungi, especially *Candida auris* and azole-resistant *Aspergillus fumigatus*. These pathogens complicate infection management in immunocompromised patients and increase the risk of hospital outbreaks.

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Earlier systematic reviews, such as Tilahun et al. (2021) and De Oliveira et al. (2022), focused mainly on bacterial MDROs and their impact on mortality and costs. Later reviews (Bai et al., 2021; Zong et al., 2022) described antifungal resistance but did not integrate bacterial and fungal MDROs within one framework. In contrast, this review combines evidence across bacterial and fungal pathogens. It also evaluates phenotypic and molecular diagnostic methods, including metagenomic sequencing, plasma cell- free DNA assays, and nanopore-targeted sequencing. Together, these dimensions provide a comprehensive and updated picture of the MDRO landscape.

Phenotypic methods remain fundamental because of their reliability and lower costs. However, they are slow and cannot directly detect resistance genes. Molecular approaches address these weaknesses. PCR, whole-genome sequencing (WGS), and metagenomic next-generation sequencing (mNGS) offer faster and more precise detection. Xu et al. (2025) and Benoit et al. (2024) confirmed the diagnostic benefits of mNGS in sepsis patients. Ultra-rapid sequencing workflows are also cost-effective. Health systems reported savings of $20,000–50,000 for each infection prevented when these tools were implemented.

This review also highlights infection prevention and control (IPC) as a cornerstone. Bundled interventions such as hand hygiene, contact precautions, and decolonization reduce MDRO transmission by up to 62% (Geng et al., 2025). Universal screening and isolation protocols have proven effective during MRSA and VRE outbreaks in both military and civilian hospitals (Stagliano et al., 2021). Genomic surveillance provides another powerful layer. Real-time sequencing detects outbreaks earlier, reduces transmission, and delivers measurable cost savings. Sundermann et al. (2025) reported a return on investment exceeding 3:1.

Despite these strengths, limitations must be acknowledged. The included studies varied widely in design, sample size, and diagnostic criteria, which may have influenced pooled prevalence estimates. Differences in laboratory capacity and reporting practices between high-income and LMIC settings also contributed to uneven data quality. Fungal MDROs remain underreported, and longitudinal data are scarce. Only 20% of included studies tracked resistance trends over time, limiting insights into persistence and long-term outcomes. This underlines the need for harmonized definitions and standardized diagnostic protocols.

Looking forward, three priorities emerge. First, expanding access to molecular diagnostics in LMICs is essential to address equity gaps in detection and care. Second, genomic epidemiology must be integrated into hospital workflows. Modeling suggests that surveillance-guided interventions could reduce mortality by 15–20%. Third, hybrid diagnostic and IPC strategies require cost-effectiveness trials, especially in LMICs. These trials will provide evidence for sustainable policy adoption. By pursuing these priorities, health systems worldwide can alter the trajectory of antimicrobial resistance and preserve the effectiveness

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of antibiotics for future generations.

6. Conclusion

Multidrug-resistant organisms (MDROs) remain a critical and growing global health threat, with carbapenem-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* leading the bacterial burden, and emerging fungal pathogens such as *Candida auris* and azole-resistant *Aspergillus fumigatus* adding new complexity to patient care. These pathogens contribute substantially to morbidity, mortality, and healthcare costs, particularly in intensive care settings and low- and middle-income countries (LMICs). Advances in molecular diagnostics, including metagenomic sequencing, plasma cell-free DNA assays, and nanopore technologies, offer rapid and precise detection compared with traditional phenotypic methods. When integrated with bundled infection prevention and control (IPC) measures and real-time genomic surveillance, these innovations can significantly reduce transmission, improve patient outcomes, and lower overall healthcare costs. Looking ahead, policy frameworks must prioritize equitable access to advanced diagnostics, harmonized reporting standards, and robust stewardship programs, with urgent focus on LMICs where the burden is highest. Coordinated global action is essential to prevent the unchecked spread of MDROs. ***Failure to act decisively will accelerate the arrival of a post-antibiotic era, where common infections once again become untreatable.***

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