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Review

Airborne Antibiotic-Resistant Bacteria—Challenge for Healthcare Environments

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Abstract

Antimicrobial resistance (AMR) is a growing global public health challenge. Its development is strongly associated with the inappropriate and excessive use of antimicrobial agents, leading to reduced treatment effectiveness, limited availability of therapeutic options, constraints on medical procedures, and an increasing economic burden. This narrative review synthesizes current knowledge on antibiotic-resistant bacteria detected in airborne samples from healthcare environments and examines their reported resistance profiles. The review focused on the bacterial species identified, methods used for antimicrobial susceptibility assessment, types of healthcare facilities investigated, and environmental and behavioral factors influencing the occurrence and dissemination of airborne antibiotic-resistant bacteria. The clinical relevance of the reported pathogens was discussed in the context of the WHO Bacterial Priority Pathogens List (BPPL), while the WHO AWaRe classification and TrACSS framework were used as complementary interpretative tools to contextualize resistance patterns and their implications for antimicrobial stewardship and AMR surveillance. The reviewed studies showed that airborne bacterial communities in healthcare settings were dominated by Gram-positive bacteria, particularly *Staphylococcus* spp. and *Bacillus* spp., while clinically relevant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were also frequently detected. Resistance to β-lactam antibiotics was the most frequently reported resistance pattern. Considerable heterogeneity in sampling strategies, antimicrobial susceptibility testing methods, and interpretive criteria limited direct comparison among studies. The findings highlight the need for standardized monitoring methods, long-term surveillance, and integrated environmental and clinical research to support infection prevention strategies and mitigate antimicrobial resistance.

Keywords: antibiotic resistance; bacteria; healthcare facilities

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1. Introduction

Air is one of the least studied environments with respect to the presence of antibiotic-resistant microorganisms [1]. In particular, greater attention should be paid to air quality in healthcare facilities, which remains insufficiently investigated [2]. The primary source of microorganisms in these environments is human activity; within one hour, individuals can emit between 1 and 10 million bacteria and fungi into the surrounding air [3]. Addi-

tional sources include outdoor air, medical procedures, and contact with contaminated surfaces [4].

Antimicrobial resistance (AMR) poses a serious and growing threat to global public health. In 2019, an estimated 1.27 million deaths were directly attributable to bacterial AMR. The highest mortality rates were reported in sub-Saharan Africa, while the lowest were observed in Australasia. The greatest burden of antimicrobial resistance was associated with lower respiratory tract infections, followed by bloodstream and intra-abdominal infections. Furthermore, it is estimated that approximately 39 million people may die as a result of AMR between 2025 and 2050 [5,6].

The AMR is applied throughout the manuscript when referring to the broader public health, policy, surveillance, and stewardship context of resistance, including World Health Organization (WHO) initiatives and international monitoring frameworks. The term antibiotic resistance is used specifically in relation to bacterial pathogens, resistance to antibacterial agents, laboratory susceptibility testing, and antibiotic-resistant bacteria identified in healthcare environments. This terminology was adopted to ensure consistency with WHO definitions and to distinguish between the general AMR framework and bacterial antibiotic resistance discussed in the reviewed studies.

1.1. Prevalence and Characteristics of Airborne Antibiotic-Resistant Bacteria in Healthcare Environments

A key mechanism in the spread of microorganisms is aerosolization, defined as the suspension of small particles (bioaerosols) in the air (Figure 1). Activities such as breathing, speaking, coughing, performing medical procedures, changing bedding, cleaning, or operating ventilation systems generate particles that can become airborne. Some of these particles remain suspended, while others settle on surrounding surfaces [7]. This dynamic process enables microorganisms to disperse over varying distances, deposit on surfaces, and, importantly, be inhaled by patients and healthcare staff, making airborne transmission a significant pathway for the spread of pathogens.

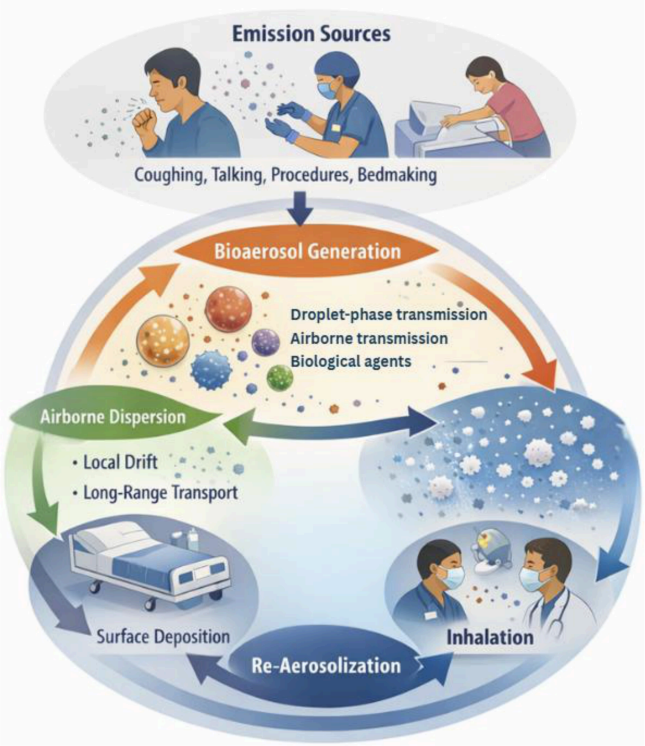

Figure 1. Aerosolization of microorganisms in healthcare settings.

In healthcare settings, the frequent use of broad-spectrum antibiotics, the presence of immunocompromised patients, invasive procedures, and intensive contact between staff, patients, and medical equipment promote the selection, proliferation, and spread of antibiotic-resistant bacteria [5]. These microorganisms are characterized by their ability to persist in the environment, including through spore formation, as well as by their capacity to transfer resistance genes between cells.

1.2. Survival Mechanisms and Antibiotic Resistance Assessment

The persistence and dissemination of antibiotic-resistant bacteria in healthcare environments depend not only on the presence of microorganisms in the air but also on their ability to survive environmental stressors and maintain resistance traits. Several biological mechanisms enhance bacterial survival under adverse conditions and contribute to the persistence and spread of resistance determinants.

Airborne antibiotic-resistant bacteria detected in healthcare environments may persist and spread due to several survival and adaptation mechanisms including:

- Coccoid forms—metabolic slowdown associated with morphological changes, resulting in reduced susceptibility to antimicrobial agents and environmental stress [8].
- Dormant cells (persisters)—reduced metabolic activity due to transition into a dormant state, enabling survival under adverse conditions [8–10].
- Biofilm formation—limitation of antibiotic penetration and protection against host immune responses through the development of a protective extracellular matrix [8,10,11].
- Stress response systems—mitigation of antibiotic-induced DNA and protein damage through activation of specific cellular response mechanisms [8–10].
- Horizontal gene transfer—dissemination of resistance genes through conjugation, transformation, or transduction [8,10,11].

Furthermore, reliable assessment of antibiotic resistance requires standardized laboratory methods that enable the identification and characterization of resistant bacterial isolates. The most commonly used methods for determining antibiotic resistance in airborne bacterial isolates include:

- Disk diffusion method—the oldest technique, based on the diffusion of an antibiotic into a culture medium; it is characterized by low cost and ease of use [12,13].
- Dilution methods—involve determining the minimum inhibitory concentration (MIC) of an antibiotic that prevents bacterial growth in broth or on agar through a series of dilutions; these methods are considered the gold standard [13,14].
- Gradient method—combines features of diffusion and dilution techniques, typically using antibiotic gradient strips on agar [13].
- Automated systems—offer rapid and standardized analysis with high efficiency [12,13].
- Molecular methods—involve genotypic analysis, including the detection of resistance genes (e.g., electrophoresis, PCR) [13].

In healthcare settings, samples may be collected from clinical specimens (e.g., blood, respiratory swabs), surfaces and medical equipment, water sources (e.g., taps), or air, with appropriate analytical methods selected depending on the sample type. Bacterial load is typically expressed as colony-forming units (CFU) per unit volume of the analyzed sample.

1.3. Research Gap and Study Objectives

Although antimicrobial resistance (AMR) has been recognized as one of the most significant global public health challenges, research on its airborne environmental dimension remains considerably less developed than studies focused on clinical infections, wastewater, soil, or aquatic environments [15]. In particular, knowledge regarding antibiotic-resistant bacteria present in healthcare bioaerosols remains fragmented and dispersed across in-

dividual studies conducted in different types of healthcare facilities, geographic regions, and epidemiological settings. Existing investigations vary substantially in sampling strategies, microbiological methods, susceptibility testing approaches, and reporting standards, making direct comparisons difficult and limiting the interpretation of resistance patterns across studies.

Furthermore, while environmental and behavioral determinants of airborne bacterial contamination in healthcare facilities have been increasingly investigated, there is still no comprehensive synthesis linking these factors with the occurrence and dissemination of antibiotic-resistant bacteria in healthcare bioaerosols. Previous studies have primarily focused either on airborne microbial concentrations or on clinical antimicrobial resistance surveillance, whereas the potential role of air as a transmission pathway for antibiotic-resistant bacteria has received considerably less attention. In addition, the clinical relevance of airborne bacterial isolates is rarely interpreted within broader antimicrobial stewardship and surveillance frameworks, such as the WHO AWaRe classification and the Tracking Antimicrobial Resistance Country Self-Assessment Survey (TrACSS).

Therefore, the aim of this narrative review was to critically synthesize current knowledge regarding antibiotic-resistant bacteria detected in healthcare bioaerosols, with particular emphasis on the bacterial species identified, their resistance profiles, analytical methodologies used for their assessment, and the environmental and behavioral factors influencing their occurrence. Additionally, this review sought to contextualize the available evidence within established international AMR frameworks and to identify methodological limitations, research gaps, and priorities for future studies supporting infection prevention and control strategies in healthcare environments.

2. Materials and Methods

2.1. Literature Search Strategy

This study is a narrative review that provides a structured synthesis assessing the clinical relevance of antibiotic-resistant bacteria in healthcare settings. The following section provides a review of the literature on antibiotic resistance in airborne bacteria in healthcare settings. Relevant literature was identified through searches conducted in PubMed, Web of Science, and ScienceDirect databases. Additional relevant publications were identified through manual screening of reference lists from selected articles. The literature search included peer-reviewed articles published in English from January 2010 to April 2026. The selected timeframe was intended to reflect recent advances in environmental microbiology, bioaerosol monitoring, and antimicrobial resistance surveillance methodologies. The search strategy combined the following terms: ("antibiotic-resistant bacteria" OR "antimicrobial resistance") AND ("bioaerosols" OR "airborne bacteria") AND ("healthcare facilities" OR "hospital environment"). The same conceptual search strategy was applied across all databases, with minor adaptations to the database-specific search interfaces. The complete search strategies are provided in Appendix A. Studies meeting the following criteria were included:

- Addressed antibiotic-resistant bacteria,
- Focused on the airborne environment,
- Provided empirical data,
- Were conducted in healthcare facility settings.

Studies not related to healthcare environments, non-airborne transmission pathways, conference abstracts, editorials, and non-peer-reviewed publications were excluded. Titles and abstracts were screened for relevance, followed by full-text evaluation of eligible articles. The final selection of studies was based on their relevance to airborne antimicrobial

resistance in healthcare-associated environments and their contribution to the current understanding of environmental AMR dissemination.

The study selection process followed PRISMA principles and included identification of records, removal of duplicates, screening of titles and abstracts, full-text eligibility assessment, and final inclusion of studies meeting all predefined criteria. The complete selection process and the number of records retained at each stage are presented in the PRISMA flow diagram (Figure 2).

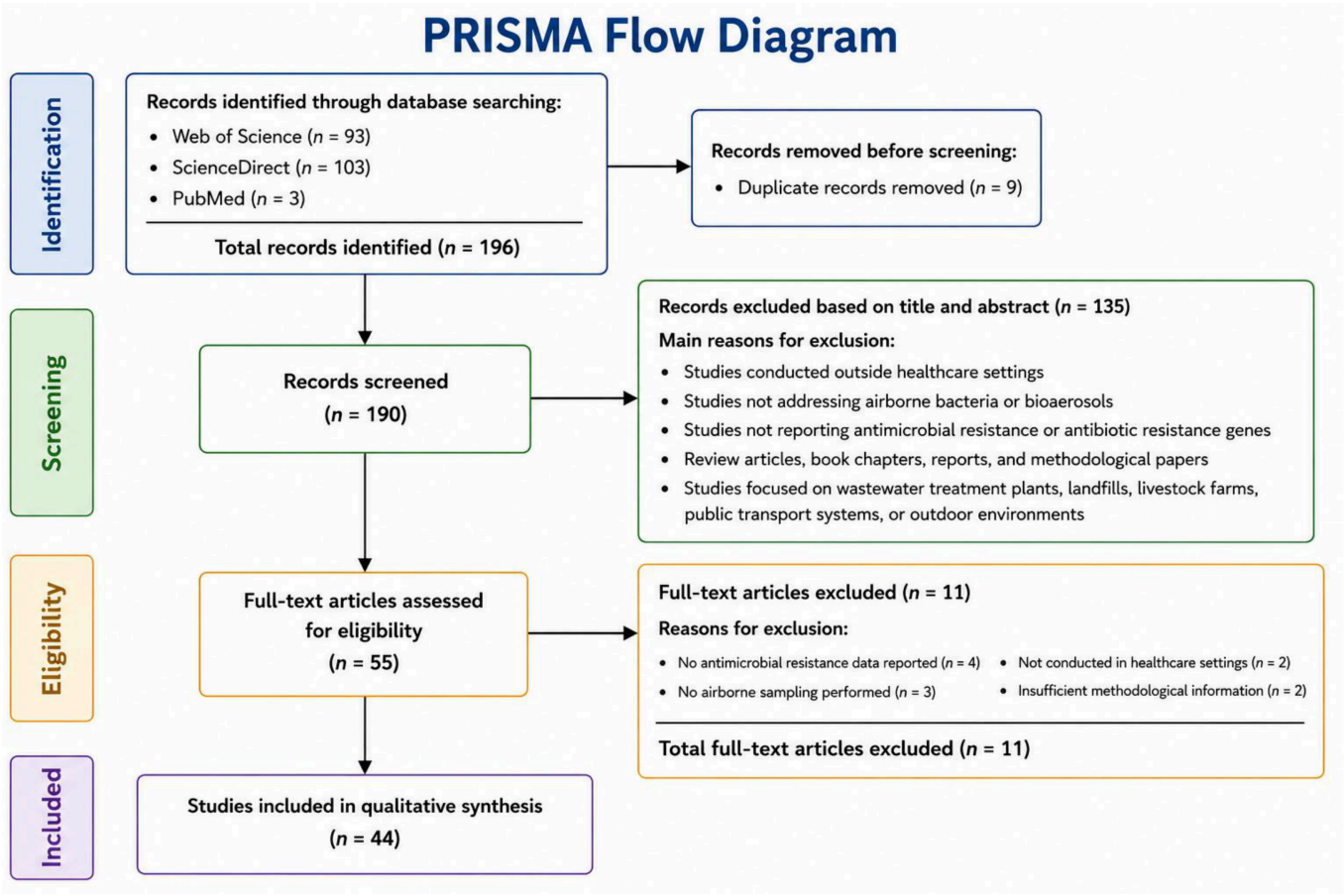

Figure 2. PRISMA flow diagram of the literature search and study selection process.

2.2. Analytical Framework for Addressing the Research Questions

The analysis was further contextualized using the World Health Organization Access–Watch–Reserve (AWaRe) classification and the Tracking Antimicrobial Resistance Country Self-Assessment Survey (TrACSS) framework. In this review, the AWaRe classification was used to contextualize antibiotics associated with the reported resistance patterns, while the TrACSS framework was used to discuss the implementation of antimicrobial resistance surveillance, stewardship, and infection prevention strategies. These frameworks were applied as interpretative tools rather than as primary analytical methods. The review was designed to address the following research questions:

- How can the WHO AWaRe antibiotic classification and TrACSS framework be used to contextualize antimicrobial resistance patterns in healthcare settings?—The combined use of this framework enables the interpretation of resistance data within a broader clinical and policy context, supporting a more structured understanding of antimicrobial resistance in healthcare environments.
- Which sources of airborne antibiotic-resistant bacterial contamination have been identified in healthcare environments, and how do environmental and behavioral factors

influence their occurrence and dissemination? This question was formulated to identify the main sources of airborne bacterial contamination in healthcare settings and to evaluate the extent to which environmental and behavioral determinants contribute to the spread of antibiotic-resistant bacteria.

- Which analytical methods are most commonly used to assess airborne antibiotic-resistant bacterial isolates in healthcare settings, and how comparable are the results obtained across different studies? This question was formulated to identify the analytical methods most commonly used to assess airborne antibiotic-resistant bacterial isolates in healthcare environments and to evaluate the comparability of results reported across studies. It also addresses the issue of heterogeneity in laboratory and analytical methods, which may affect the comparability and interpretability of results from different studies.
- Which antibiotic-resistant bacteria are most common in healthcare environments?—Identification of the most frequently detected pathogens provides a basis for understanding the microbiological composition of hospital bioaerosols and supports prioritization of clinically relevant resistant organisms in infection control and surveillance strategies.

These questions serve as a supporting framework for identifying research gaps and formulating implications for infection control and directions for future research.

3. Results and Discussion

3.1. Antimicrobial Resistance Mitigation in Healthcare Environments

Inappropriate use of antimicrobial medications and inadequate infection control facilitate the persistence of resistant bacteria in the environment and their airborne spread within healthcare settings. Clinical strains are often more resistant than environmental strains due to strong selective pressure in healthcare facilities [16]. Because healthcare environments represent important reservoirs and dissemination points for airborne antibiotic-resistant bacteria, antimicrobial stewardship and infection prevention strategies play a critical role in limiting their spread.

The One Health concept, which has evolved over time, is defined as an interdisciplinary approach aimed at improving health by recognizing the interconnections between humans, animals, plants, and the environment [17]. It emphasizes the need for countries to develop and implement effective national strategies to address antibiotic resistance and to ensure the availability of adequate human and financial resources. Forecasts suggest that, without effective interventions, the number of deaths associated with antimicrobial resistance could reach 10 million annually by 2050 [18].

Progress in the implementation of national action plans against antibiotic resistance is monitored worldwide using TrACSS [19]. In this review, TrACSS indicators are used as a structured tool to interpret the level of implementation of antimicrobial resistance policies and surveillance systems across healthcare settings. Table 1 presents an analysis of the 2025 survey results for the human health category. Colors are used to indicate the level of implementation, where red denotes areas requiring urgent intervention or poor implementation, yellow indicates partial implementation or the need for strengthening, and green represents relatively well-functioning systems.

Table 1. Analysis of data from the TrACSS 2025 study (Adapted from the WHO Global Database for Tracking Antimicrobial Resistance Country Self-Assessment Survey [19]).

Refers to	Result	Interpretation	Finding
Training and professional education on antimicrobial resistance in the human health sector	Formalized	Globally education exists but not fully developed/integrated	High likelihood of antibiotic overuse due to insufficient knowledge
National system for national antimicrobial use in humans	Globally—collaborative Regions—from integrated to none	Substantial differences between regions	Uneven levels of control over antibiotic use
Monitoring system for facility-level/hospital antimicrobial use in human health	Globally and regions—established or collaborative	Considerable global differences	Lack of a uniform level
National monitoring and reporting system for substandard and falsified antimicrobials in humans	Globally—from formalized to none Regions—from collaborative to none	Weak area between countries—no system or in initial phase	Selection of resistant strains
National surveillance system for AMR in humans	Collaborative	Well-developed resistance monitoring systems identify resistant bacteria and their locations	Probably the system includes only clinical samples, not environmental ones
Capacity to perform Antimicrobial Susceptibility Testing (AST) for critically important bacteria	Collaborative	Laboratory diagnostics functional	Countries can detect resistance
Continuity of services for clinical bacteriology laboratories	None Only western pacific region no implementation reported or collaborative	Laboratories exist, but their operation remains unstable	Serious issue—continuity of service not ensured
Infection Prevention and Control (IPC) in human health care	Globally—formalized Regions—from integrated to established	Systems exist but rather at intermediate level	Infection control partially effective = higher risk of infections
Optimizing antimicrobial use in hospitals	Globally—none Regions—from established to none	Almost no effective measures	Inappropriate use of antibiotics in hospitals
Optimizing antimicrobial use in primary care	Globally—collaborative Regions—from integrated to formalized	Better antibiotic control outside hospital	Probably fewer severe infections; treatment standards easier to implement
Adoption of "AWaRe" classification of antibiotics in the National Essential Medicines List	Globally—established Regions—from established to formalized	System partially implemented	Possible misuse of restricted or 'last-resort' antibiotics

Note: Green indicates full implementation, yellow indicates partial implementation, and red indicates limited or no implementation according to the TrACSS report.

Although TrACSS was not developed specifically for airborne antibiotic-resistant bacteria, it provides a useful framework for interpreting healthcare system preparedness to address antimicrobial resistance in healthcare environments.

In summary, the analysis of the TrACSS survey indicators provides insight into systems that are partially implemented as well as those already in operation, while also identifying critical gaps. The TrACSS results presented in Table 1 highlight heterogeneous levels of implementation of antimicrobial resistance strategies across healthcare systems. Ineffective quality control and issues related to the authenticity of antibiotics may reduce the effectiveness of pharmacotherapy and contribute to increased selection of resistant bacteria. Disruptions in the continuity of clinical laboratory services can result in unequal access to diagnostics and inconsistencies in data reporting. Optimizing antibiotic use in hospitals remains a significant challenge. Empirical antibiotic use without susceptibility testing may prolong hospital stays, increase the risk of healthcare-associated infections, and impose an additional economic burden on the healthcare system [20]. However, the control of antibiotic resistance requires an interdisciplinary approach that considers both environmental and systemic factors and is adapted to local conditions and specific bacteria–antibiotic interactions [21].

The data indicate varying levels of preparedness and implementation of AMR measures across different sectors worldwide. Although TrACSS does not specifically address airborne antibiotic-resistant bacteria, the implementation of antimicrobial stewardship, surveillance systems, infection prevention, and control measures within healthcare settings may indirectly influence the occurrence and dissemination of antibiotic-resistant bacteria, including those detected in bioaerosols. A comparison with data from previous years (2017–2024), although limited to selected regions, suggests a gradual improvement in outcomes; however, the overall pattern of responses has remained largely unchanged.

Beyond system-level implementation assessed through TrACSS, WHO Target Product Profiles further define the desired characteristics of future antimicrobial agents needed to address emerging resistance challenges [22]. These include:

- Indications for use and target population—new antibacterial agents should be intended for the treatment of severe infections, particularly in hospitalized patients, critically ill individuals, and immunocompromised populations.
- Effectiveness and spectrum of action—new antibiotics should be active against priority pathogens. While any mechanism of action may be acceptable, novel or differentiated mechanisms are preferred, as they are associated with a lower risk of resistance development.
- Safety and pharmacokinetics—new drugs should demonstrate predictable pharmacological profiles and enable effective, standardized dosing in most patients without the need for complex individualized regimens.
- Dosage and formulation—new antibiotics should offer flexible treatment options, including both intravenous (for hospital use) and oral forms, allowing for sequential therapy and continuation of treatment in outpatient settings.
- Stability and availability—new drugs should be globally accessible, including in both high- and low-income countries, and should remain stable under diverse environmental conditions, such as high temperature and humidity.

The above recommendations reflect an effort to align antibiotic development with global clinical needs by shifting the focus from the number of new molecules to their clinical relevance and therapeutic value. They also highlight that antimicrobial resistance is not solely a biological issue, but a systemic one, encompassing limited pharmaceutical innovation and inequalities in access to treatment. However, the practical implementation of this approach faces significant challenges. The rate at which bacterial resistance mechanisms evolve often exceeds the pace of new antibiotic development, and meeting multiple stringent requirements simultaneously—such as efficacy, safety, broad-spectrum activity, and global stability—substantially increases the complexity and cost of the research and development process.

As a result, even successful innovative therapeutic strategies may yield benefits only after a delay, which, in the context of rapidly increasing resistance, may be insufficient to effectively limit the scale of the problem.

3.2. Antibiotic Consumption and Stewardship in Healthcare Settings

Estimates from the European Antimicrobial Resistance Surveillance Network for 2020 indicate that over 35,000 people die each year in the EU/EEA as a result of infections caused by multidrug-resistant bacteria [23]. Global antibiotic consumption has reached 49.3 billion DDD [24]. This is particularly significant in healthcare settings, where treatment effectiveness is increasingly challenged not only by resistance but also by the limited availability of new antibiotics. In response to the growing problem of antibiotic resistance, the WHO developed a classification system in 2017 that categorizes antibiotics into three groups:

1. Access—first- or second-line antibiotics with high effectiveness and lower resistance potential.
2. Watch—antibiotics recommended for specific infections, with a higher risk of resistance development.
3. Reserve—last-resort antibiotics, to be used only in severe or multidrug-resistant infections.

According to the latest Surveillance of Antimicrobial Resistance in Europe report, nearly 70% of European countries have achieved the target of at least 60% use of antibiotics from the Access group. Outside Europe, this proportion remains below 30% [25,26].

In Europe, antibiotic resistance is becoming an increasingly serious problem, particularly among Gram-negative bacteria, which are more difficult to treat. Special concern is associated with carbapenem-resistant *Klebsiella pneumoniae* and third-generation cephalosporin-resistant *Escherichia coli*, both of which show rising infection rates. This trend is largely driven by increasing resistance to last-resort antibiotics, which significantly limits treatment options. High transmissibility and widespread occurrence further contribute to this issue [27,28].

In healthcare air environments, commonly detected bacteria include Gram-negative species such as *Enterobacter* spp., *Klebsiella* spp., *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as Gram-positive species such as *Enterococcus* spp., *Staphylococcus aureus*, and *Clostridium difficile* [29].

3.3. Clinically Relevant Airborne Antibiotic-Resistant Bacteria

Table 2 summarizes selected bacterial species commonly detected in airborne samples from healthcare environments in the literature and their clinical relevance based on the WHO bacterial priority pathogens list (BPPL). Antibiotic information reflects commonly used therapeutic options for infections caused by these pathogens, while the AWaRe classification is included as a supplementary interpretative layer to contextualize antibiotic selection rather than to represent direct susceptibility data from bioaerosol isolates.

Table 2. Resistance profiles of common bacterial bioaerosols in healthcare settings and their clinical relevance based on WHO BPPL, with AWaRe classification for antibiotic categorization. Based on Epidemiological Report from 2024—Antimicrobial resistance in the EU/EEA [23].

Genus/Species	WHO BPPL Status	Resistance *	Medicine Used in Treatment/ AWaRe Group
Enterobacterales (*Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp.)	Critical	Carbapenems, third-generation cephalosporins	Ceftazidime-avibactam, Meropenem-vaborbactam/ Reserve
Pseudomonas aeruginosa	High	Carbapenems	Ciprofloxacin; Ceftazidime-avibactam, Ceftolozane-tazobactam/Watch; Reserve
Enterococcus spp. (particularly *E. faecium*)	High	Vancomycin	Linezolid, Daptomycin, Tigecycline/ Reserve
Staphylococcus aureus	High	Methicillin	Vancomycin, Linezolid, Daptomycin/ Reserve

* Resistance refers to the average resistance profile reported for the listed species in the reviewed studies.

The bacterial species presented below have been reported in airborne samples collected from healthcare environments and therefore represent clinically relevant targets for bioaerosol monitoring and antimicrobial resistance surveillance.

The table highlights the challenges associated with combating antibiotic resistance. In this table, the AWaRe classification is used to contextualize the clinical relevance of antibiotics associated with treatment of resistant pathogens, rather than to represent prescribing recommendations. This approach allows interpretation of whether observed resistance patterns are linked to reliance on Watch and Reserve antibiotics in clinical practice. The majority of listed antibiotics recommended for treatment belong to the Reserve group, meaning they should be used only as last-resort options in exceptional circumstances. The limited representation of Access-group antibiotics in the analysed dataset may suggest their reduced applicability for selected airborne or bioaerosol-associated pathogens, which may complicate the implementation of WHO antimicrobial stewardship recommendations in clinical practice.

The World Health Organization's report on integrating antimicrobial resistance prevention into primary care is also highly relevant, as it complements the AWaRe classification by highlighting the level at which most antibiotic prescribing occurs. It is estimated that approximately 80–90% of antibiotics are prescribed in primary care settings, emphasizing the critical role of this level of healthcare in shaping antimicrobial selection pressure and influencing resistance patterns observed in both clinical and environmental contexts. This highlights that effective AMR control requires targeted interventions at the patient's first point of contact with the healthcare system, where decisions regarding antibiotic therapy are made [30].

3.4. Assessment of Airborne Antibiotic-Resistant Bacteria in Healthcare Facilities

This section presents an assessment of antibiotic resistance in airborne bacteria in healthcare facilities, based on a review of the available literature (Table 3). The aim of the review was to map the current state of knowledge regarding research approaches to antibiotic-resistant bacteria in healthcare environments.

Table 3. A review of bacterial bioaerosol research on antibiotic-resistant in healthcare settings.

Facility	Interpretation	Predominant Bacterial Species	Main Resistance Profile	Ref.
Hospitals and health care clinics, Poland	EUCAST * and KORLD guidelines **	Disk diffusion method *Staphylococcus saprophyticus* *Staphylococcus warneri*	Most common—tetracycline and erythromycin	[31]
Nursing home, Denmark	EUCAST guidelines	*Staphylococcus aureus*	Methicillin-Resistant, Methicillin-Susceptible trimethoprim-sulfamethoxazole ciprofloxacin, cefepime, ceftriaxone	[32]
Hospital, Ethiopia	CLSI recommendations ***	*Acinetobacter baumannii* *Pseudomonas aeruginosa*	trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, ceftriaxone	[33]
Hospital, Jordan	CLSI recommendations	Methicillin-resistant *Staphylococcus aureus*	cefoxitin, oxacillin, azithromycin, cefotaxime, penicillin	[34]
Hospital, Nigeria	CLSI recommendations	*Staphylococcus aureus* *Bacillus* spp. *Escherichia coli*, *Klebsiella pneumoniae* *Micrococcus* spp.	ampicillin, penicillin, cefoxitin cefoxitin, ampicillin clindamycin, azithromycin	[35]
Hospital, Malaysia	NA	*Staphylococcus aureus* α- and β-*Streptococcus* spp. *Bacillus* spp. *Clostridium* spp.	The highest resistance was observed to ampicillin	[36]
Dental care unit, Pakistan	CLSI recommendations	Dominant *Staphylococcus aureus*	erythromycin, ceftazidime, cefotaxime	[37]
Hospital, Lebanon	CLSI recommendations	Gram-positive (mainly *Staphylococcus*) Gram-negative (*Pseudomonas*, *Escherichia coli*)	Penicillin, clindamycin, ceftazidime Penicillin, cephalothin	[38]

Table 3. *Cont.*

Facility	Interpretation	Predominant Bacterial Species	Main Resistance Profile	Ref.
		Vitek II system		
Hospital, Turkey	CLSI recommendations	*Acinetobacter baumannii*	Most airborne isolates are resistant to carbapenems	[39]
Hospitals, Bangladesh	CLSI recommendations	*Staphylococcus aureus Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*	Ampicillin, azithromycin, erythromycin, cefixime	[40]
Clinic, Germany	references to internal procedures	*Acinetobacter schindleri Micrococcus luteus*, *Staphylococcus epidermidis*	Methicillin	[41]
Hospital, Russia	NA	*Enterococcus* spp. *Micrococcus* spp. *Sphingomonas* spp.	Multidrug-resistant strains (MDRO)	[42]
		BD Phoenix-100 system		
Hospitals, Taiwan	CLSI recommendations	*Staphylococcus* spp. *Micrococcus* spp. *Bacillus cereus*	β-lactams	[43]
Hospital, Columbia	CLSI recommendations	*Staphylococcus epidermidis Staphylococcus saprophyticus Pseudomonas aeruginosa*	penicillin G, ampicillin, clindamycin ampicillin, penicillin G, erythromycin carbapenem	[44]
		Reverse transcription RT-qPCR (resistance genes analysis)		
Hospitals, China	NA	*Staphylococcus saprophyticus*, *Corynebacterium minutissimum*, *Streptococcus pneumoniae*, *Escherichia coli*, *Arcobacter butzleri*, *Aeromonas veronii*, *Pseudomonas aeruginosa*, *Bacillus cereus*.		[45]
		PCR-DGGE, PCR—resistance genes		
Hospital, Canada	reference to the literature	*Staphylococcus epidermidis Staphylococcus hominis Bacillus* spp. *Micrococcus luteus*	erythromycin, tetracycline	[46]
		Thermo Fisher Scientific Sensititre Aris 2X AST system		
Hospital, Philippines	CLSI recommendations	*Staphylococcus epidermidis*, *Staphylococcus warneri*, *Staphylococcus lugdunensis*	Penicillin, oxacillin	[47]
		MRSA chromogenic agar medium		
Primary health care centers, Qatar	Manufacturer's instructions	*Staphylococcus*, *Acinetobacter*, *Pseudomonas*, *Bacillus*	Methicillin	[48]
		chromID VRE selective agar		
Hospital, Brazil	NA	Most common *Staphylococcus*, *Bacillus* spp.	Vancomycin	[49]
		Agar screening		
Hospitals, Iran	CLSI recommendations	*Acinetobacter baumannii Staphylococcus epidermidis Staphylococcus saprophyticus Staphylococcus hominis Staphylococcus haemolyticus*	oxacillin, ceftazidime and cefazolin	[50]
		Agar dilution method		
Hospital	CLSI recommendations	*Micrococcus* spp., *Staphylococcus* spp.	Streptomycin	[51]
Public and private dental clinics, Italy		Cocci and saprophytic environmental bacteria dominated	Cefuroxime	

NA-not available. * EUCAST, European Committee on Antimicrobial Susceptibility Testing. ** KORLD, Korean Outdoor Air Research Database. *** CLSI, Clinical and Laboratory Standards Institute.

An analysis of the studies summarized in Table 3 reveals considerable methodological consistency in some areas and substantial variability in others. Disk diffusion testing interpreted according to CLSI recommendations was the most frequently applied approach

for antimicrobial susceptibility assessment, whereas automated systems (Vitek II, BD Phoenix, and Sensititre) and molecular techniques were used less frequently. The reviewed studies were conducted across diverse geographical regions, including Europe, Asia, Africa, North America, and South America; however, most investigations were based on individual healthcare facilities and local sampling campaigns. This geographical and methodological heterogeneity limits the generalizability of findings and complicates direct comparison of resistance patterns reported in different healthcare environments. Nevertheless, the recurrent detection of resistant *Staphylococcus* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacterales* across multiple regions suggests that airborne antibiotic-resistant bacteria represent a widespread phenomenon rather than isolated local events.

3.5. Environmental and Behavioral Determinants

Human activities that generate aerosols (e.g., floor cleaning or bed-making) also play a significant role. However, these sources should not be considered in isolation, as they interact and jointly influence emission dynamics. Environmental factors (e.g., outdoor air pollution and dust) and systemic factors (e.g., the operation of HVAC systems) further modulate the distribution and persistence of bioaerosols.

Air parameters such as relative humidity promote the release and growth of bacteria [35,40] and may contribute to the persistence of resistance genes in the air [1]. Temperature is often associated with increased bacterial concentrations [40], likely due to enhanced metabolic activity and biofilm formation [35,52]. Dust particles act as carriers and protective matrices for microorganisms, shielding them from UV radiation and desiccation and enabling their transport over longer distances [40,42,52]. Airflow and ventilation influence the dispersion and accumulation of pathogens within indoor spaces [15,42,52]. Behavioral factors include crowd density, with a strong correlation observed between the number of occupants and airborne bacterial concentrations [37,51]. Specific activities (e.g., medical procedures, bed-making, and patient visits), as well as patient mobility and hygiene practices, also influence the release of microorganisms into the environment [41,50]. The findings indicate that both environmental and behavioral factors, such as ventilation conditions, occupancy levels, patient activity, and cleaning practices, operate at different but interconnected levels to influence the occurrence and airborne dissemination of antibiotic-resistant bacteria in healthcare facilities. Behavioral factors primarily determine the formation of bioaerosols in clinical settings, while environmental parameters influence bacterial survival and aerosol stability, whereas airflow and particulate matter determine transport dynamics [15,35,42,52].

The analyzed literature consistently showed that the most frequently reported resistance was observed against β-lactam antibiotics (e.g., penicillin and ampicillin), which may reflect the clinical importance and extensive use of this drug class in both empirical and targeted treatment of bacterial infections.

The results indicate that the most frequently detected bacterial bioaerosols in healthcare facilities are predominantly Gram-positive microorganisms, particularly *Staphylococcus* spp. and *Bacillus* spp. Their higher abundance may be attributed to their structural characteristics, which enhance survival under adverse environmental conditions, as well as their continuous emission from human skin, reinforcing the role of patients, healthcare workers, and visitors as primary sources of contamination [53]. Clinically relevant Gram-negative pathogens, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and members of the Enterobacterales family, were also identified. These organisms are typically associated with clinical reservoirs [54], and their detection in bioaerosols highlights the relevance of airborne dissemination as a potential transmission pathway in healthcare environments.

The frequent occurrence of resistance to commonly used antibiotics is consistent with the selective pressures exerted by antimicrobial use in these settings.

3.6. Methodological Limitations and Future Perspectives

Several limitations should be considered when interpreting the findings of this review. Studies investigating antibiotic-resistant bacteria in airborne bioaerosols remain relatively scarce compared with research focused on clinical samples or other environmental matrices. Moreover, most available studies were conducted in hospital settings and frequently targeted selected wards, limiting the representativeness of the findings. Airborne bacterial communities and resistance profiles may vary substantially according to ward type, patient population, geographic location, season, and local environmental conditions, including ventilation systems, air conditioning, and outdoor air quality [55].

Additional limitations arise from methodological heterogeneity among studies. Air sampling strategies varied considerably and included both active methods (e.g., impaction and filtration) and passive approaches (e.g., sedimentation onto agar plates), affecting the comparability of reported bacterial concentrations. Similarly, antimicrobial susceptibility testing was performed using different analytical approaches and interpretive criteria, including CLSI and EUCAST guidelines, automated systems, and molecular techniques. Although these methods provide valuable information on resistance phenotypes and mechanisms, differences in methodology and reporting standards may influence resistance classification and limit direct comparison across studies.

From a practical perspective, the limited integration of environmental monitoring with infection prevention and control (IPC) programs represents an important knowledge gap. Future research should prioritize the development of standardized sampling and analytical protocols, multicenter longitudinal studies, and investigations linking airborne contamination with patient outcomes. Greater emphasis should also be placed on evaluating the effectiveness of ventilation systems, indoor air quality management, and environmental surveillance as components of IPC and antimicrobial stewardship strategies. Such efforts are essential to improve the understanding of the clinical significance of airborne antibiotic-resistant bacteria and their role in healthcare-associated infections.

4. Conclusions

The combined use of the AWaRe classification and TrACSS framework enables the contextualization of antimicrobial resistance patterns by linking observed resistance profiles with patterns of antibiotic consumption and stewardship implementation. This approach facilitates the interpretation of microbiological data within a broader clinical and health system context.

Although environmental factors (e.g., temperature, humidity, particulate matter, and ventilation) and behavioral factors (e.g., human activity, occupancy, and clinical procedures) influencing airborne antimicrobial resistance have been widely studied, they are often analyzed in isolation, with limited integration into comprehensive models that account for their combined effects on bioaerosol dynamics in healthcare settings.

Methods for the analysis of airborne antibiotic-resistant bacterial isolates are widely applied in healthcare environments and are generally standardized at the laboratory level. However, differences in interpretive criteria, testing methodologies, and laboratory procedures may result in varying classifications of the same bacterial isolate, thereby limiting the comparability of findings across studies.

The most frequently detected antibiotic-resistant bacteria in hospital environments belong to two major groups: Gram-positive and Gram-negative opportunistic pathogens. These organisms are both natural human colonizers and well-adapted to hospital envi-

ronments, where multidrug-resistant strains can readily emerge under selective pressure. Their presence in bioaerosols suggests that air in healthcare facilities may contribute to the transmission of bacteria associated with healthcare-associated infections, emphasizing the importance of prioritizing clinically relevant resistant organisms in infection control and surveillance strategies.

Air in healthcare facilities should be considered a potentially active component in the dissemination of antimicrobial resistance rather than merely a passive environmental background. Interactions between human and environmental factors, combined with the selective pressure associated with antibiotic use, create a complex system that supports the persistence and spread of resistant pathogens.

The findings of this review have important implications for clinical practice and infection prevention strategies. The recurrent detection of antibiotic-resistant bacteria, including clinically significant multidrug-resistant pathogens, in healthcare bioaerosols supports the incorporation of environmental surveillance into existing infection prevention and control programs. Healthcare facilities should prioritize optimization of ventilation systems, routine assessment of indoor air quality, and targeted environmental monitoring in high-risk areas. Integration of airborne microbiological monitoring with antimicrobial stewardship initiatives may further improve the early detection of emerging resistance threats and support more effective infection control interventions. Future research should prioritize the development of standardized sampling and analytical protocols, multicenter longitudinal studies, and investigations linking airborne contamination with patient outcomes to better assess the clinical significance of airborne antimicrobial resistance transmission.

These measures should be considered complementary components of infection prevention and control (IPC) programs, particularly in high-risk wards where vulnerable patient populations are present.

These findings highlight the need to integrate environmental monitoring, microbiological data, and antimicrobial stewardship strategies within a coherent infection control framework. Multicenter and longitudinal studies are also required to better understand the dynamics and clinical significance of airborne AMR transmission in healthcare settings.

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Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial Resistance
AWaRe	Access–Watch–Reserve (AWaRe) classification framework
BPPL Status	Bacterial Priority Pathogens List status
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
DDD	Defined Daily Dose
DNA	Deoxyribonucleic Acid
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HVAC	Heating, Ventilation and Air Conditioning
KORLD	National Reference Centre for Susceptibility Testing (Poland)
MRSA	Methicillin-Resistant *Staphylococcus aureus*
PCR	Polymerase Chain Reaction
PCR-DGGE	Polymerase Chain Reaction—Denaturing Gradient Gel Electrophoresis
Reverse transcription RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
TrACSS	Tracking Antimicrobial Resistance Country Self-Assessment Survey
UV radiation	Ultraviolet radiation
WHO	World Health Organization

Appendix A

The literature search was conducted using database-specific search strategies based on the following conceptual framework: ("antibiotic-resistant bacteria" OR "antimicrobial resistance") AND ("bioaerosols" OR "airborne bacteria") AND ("healthcare facilities" OR "hospital environment"). The same conceptual search strategy was applied across all databases (PubMed, Web of Science, and ScienceDirect), with adaptations to database-specific syntax where required. Searches were limited to peer-reviewed articles published in English between January 2010 and April 2026.

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