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REVIEW

# Healthcare-Associated Infections: The Role of Microbial and Environmental Factors in Infection Control—A Narrative Review

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## ABSTRACT

Healthcare-associated infections (HAIs), previously known as nosocomial infections, represent a significant threat to healthcare systems worldwide, prolonging patient hospital stays and the duration of antimicrobial therapy. One of the most serious consequences of HAIs is the increase in the rate of antibiotic resistance (AR) generated by the prolonged, frequent, and sometimes incorrect use of antibiotics, which leads to the selection of resistant bacteria, making treatment difficult and expensive, with direct consequences for the safety of patients and healthcare personnel. Therefore, timely and accurate diagnosis of HAIs is mandatory to develop appropriate infection prevention and control practices (IPC) and new therapeutic strategies. This review aimed to present the prevalence, risk factors, current diagnosis, including artificial

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intelligence (AI) and machine learning approaches, future perspectives in combating HAIs causative bacteria (phage therapy, microbiome-based interventions, and vaccination), and HAIs surveillance strategies. Also, we discussed the latest findings regarding the relationships of AR with climate change and environmental pollution in the context of the One Health approach. Phage therapy is an emerging option that can offer an alternative to ineffective antibiotic treatments for antibiotic-resistant bacteria causing HAIs. Clinical trials dealing with vaccine development for resistant bacteria have yielded conflicting results. Two promising strategies, fecal microbiota transplantation and probiotic therapy, proved highly effective against recurrent *Clostridium difficile* infections and have been shown to reduce HAI incidence in hospitalized patients undergoing antibiotic therapy. Artificial intelligence and machine learning systems offer promising predictive capabilities in processing large volumes of clinical, microbiological, and patient data but require robust data integration. Our paper argues that HAIs are still a global challenge, requiring stringent IPC policies, computer vision, and AI-powered tools. Despite promising avenues like integrated One Health approaches, optimized phage therapy, microbiome-based interventions, and targeted vaccine development, several knowledge gaps in clinical efficacy, standardization, and pathogen complexity remain to be answered.

**Keywords:** Hospital-acquired infections; Phage therapy; Vaccines; Microbiome; Dysbiosis; One-Health; Multidrug resistant

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**Key Summary Points**

Healthcare-associated infections (HAIs) development could be seriously influenced by factors like prolonged hospital stays, medical devices, and specific comorbidities.

The combination of molecular diagnostic techniques, microbiome-based solutions, and artificial intelligence-powered systems has significantly improved HAIs management.

Phage therapy is an emerging option that can provide an alternative to ineffective antibiotic treatments for HAIs causative bacteria.

Clinical trials dealing with vaccine development for multidrug-resistant bacteria have yielded conflicting results.

In the era of the climate crisis, life-threatening infectious diseases could still be reduced by implementing One Health strategies.

## INTRODUCTION

Healthcare-associated infections (HAIs), previously known as nosocomial infections, represent a significant challenge for healthcare systems worldwide [1, 2].

These infections are among the most significant complications associated with modern medical therapy, being directly influenced by the high use of invasive medical devices, inappropriate use of antimicrobial therapy, patient age, and immunological status [3–6]. Globally, HAIs are a significant public health problem, contributing to the prolongation of patient hospital stays and antimicrobial therapy. Thus, HAIs have a significant impact on the selection of resistant bacteria, generating high costs for health systems [6–12]. Recent reports have shown that for every 100 hospitalized patients, seven patients in high-income countries and 10 in low- and middle-income countries contract at least one type of HAI, and of these patients, 10% die.

The most prevailing types of HAIs exhibiting increased risk of complications are non-ventilator-associated hospital-acquired pneumonia [13], urinary tract infections [14], and sepsis [15]. These representative HAIs responsible for worldwide death are caused by multidrug-resistant (MDR) bacteria, including MDR Gram-negative bacilli, vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Clostridioides difficile* [6, 11, 16–20]. The global burden produced by HAIs at the individual, community and public levels could be decreased by implementing appropriate infection prevention and control (IPC) policies and strategies [9, 21]. However, in the absence of IPC infrastructure, and due to inappropriate antibiotic use in clinical settings and industrial facilities contributing to environmental contamination, HAIs outbreaks could spread very quickly in healthcare facilities and outside [22].

Human activities such as massive land changes, pollution, and biodiversity loss [23, 24] lead to critical environmental distortions, which could increase the HAI expansion through antibiotic and pesticide emissions [11, 25]. Recent data endorse the close connection between the climate crisis and the rise of heavy metals or biocides concentrations in soil and water, influencing antimicrobial resistance (AMR) by co-/cross-resistance mechanisms [26–31]. Consequently, there is a pressing need to understand the effects of environmental change on the rise of HAIs and to propose solutions to mitigate this severe public health crisis [22, 32].

This review presents the prevalence, risk factors, current diagnosis, including artificial intelligence and machine learning approaches, future perspectives in combating HAIs causative bacteria (phage therapy, microbiome-based interventions, and vaccination), and strategies for preventing and controlling HAIs. Also, we discuss the latest findings regarding the relationships of antibiotic resistance (AR) with climate change and environmental pollution in the context of the One Health approach. Although numerous papers explore these aspects, our paper provides

an integrated perspective by incorporating the latest research, particularly regarding advanced omics approaches in HAI diagnostic and emerging therapeutic strategies. These aspects are essential for providing pathogens comprehensive identification and mitigating life-threatening illnesses caused by HAIs causative bacteria. Unlike prior studies, which often discuss the results from preclinical studies, this review incorporates the recent clinical trials focused on exploring novel therapeutic strategies and addressing current challenges. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

## SEARCH METHODOLOGY AND INCLUSION CRITERIA

A structured approach was applied to ensure coverage of key studies rather than exhaustive systematic screening. For this purpose, relevant publications were searched from 2005 to 2025 using the databases ScienceDirect, PubMed, Springer Nature, and Google Scholar. The search strategy involved the use of keywords and medical subject heading (MeSH) terms, such as "healthcare-associated infections," "antimicrobial therapy," "phage therapy," "vaccines," "dysbiosis," One-Health, "climate change," and "microbiome." Various Boolean operators ("OR" and "AND") were employed to develop the search strategies. The search was conducted from 15 October to 10 March 2025, and all English-published articles available online until the day of data collection were considered. The search terms were refined based on preliminary searches, and duplicate studies were removed.

## PREVALENCE OF HAIS AT THE INTERNATIONAL LEVEL

An observational study by the World Health Organization (WHO) [33] between 1995 and 2010 reported a 7.6% prevalence in developed countries. In Europe, it is estimated that annually, over 4 million patients are affected by HAIs. The same study reported a 4.5% incidence in the USA, while in low-income countries, the incidence is much higher (up to 88.9%) [33]. In 2016, WHO reported a prevalence rate of HAIs in low- and middle-income countries (LMIC) of 5.7–19.1%. A more recent study reports a HAIs prevalence variation from 7% in developed countries to 30% in LMIC, a difference that could be explained, at least partially, by the insufficient training of healthcare personnel, limited resources, and non-compliance with infection control measures [34]. However, these data could be biased by inadequate data storage infrastructure and resources [9, 11, 35, 36].

The HAIs prevalence is higher in intensive care units (ICUs). In a multicenter study conducted by WHO in 2010, the prevalence of HAIs in ICUs was 51%, which led to prolonged hospitalization and an increased risk of other infections and complications [37]. Hazard and collaborators reported a rate of 17 cases of HAIs per 1000 days of hospitalization in ICUs in developed countries [38].

In neonates, the prevalence of HAIs caused by extended-spectrum beta-lactamase (ESBL)-producing bacteria has been reported to be as high as 11% (cumulative prevalence was 15% in Africa, 12% in South America, 11% in India, 7% in the rest of Asia, 4% in Europe and 0% in Oceania) and is increasing by approximately 3.2% per year. HAIs caused by ESBLs is associated with a mortality rate twice as high as HAIs caused by other organisms, 36% versus 18% [39].

In the European Area, over 2.5 million new cases of HAIs are reported annually [7, 9, 11]. According to the last report of the European Centre for Disease Prevention and Control (ECDC) [40], approximately 4.3 million patients acquired at least one HAI per year in European acute care hospitals in 2022–2023. The most common HAIs cases reported were respiratory tract infections, urinary tract infections, surgical wound infections, sepsis, and gastrointestinal infections. The report provides data supporting the expansion of resistance due to the abusive use of antibiotics, which has led to limited treatment options. It is estimated that at least

20% of HAIs could be prevented by sustained and comprehensive IPC programs, including simple measures such as appropriate hand hygiene [40].

In Italy, the HAIs overall prevalence has been 8.4%, with a higher frequency in patients admitted to smaller hospitals, in ICUs, and among patients using invasive medical devices, the HAIs being associated with a significant expansion of AR [6]. While a study reported an overall prevalence of HAIs of 7.6% [41] in Italy, a significant decrease from 5% in 2009 to 2.1% in 2019 has been reported [42].

Annually, HAIs are diagnosed in approximately 1.7 million people in the United States, with a prevalence of 4.5% and 90,000–99,000 people dead [7].

Goh and collaborators led a systematic review based on 31 studies, including 47,666 participants and 7658 HAIs cases from Southeast Asia, disclosing a 21.6% prevalence of these infections with significant disparities across regions. The results unveiled that the highest prevalence was registered in Indonesia (30.4%) and the lowest in Singapore (8.4%) [43].

In South Korea, despite infection control measures [44–46], the COVID-19 pandemic influenced significantly the HAIs management, particularly in the immunosuppressed patients [47–51]. This fact is sustained by the results of Oh and collaborators, who found that the incidence of common respiratory virus (cRV) infections diminished significantly after implementing IPC measures for COVID-19. However, they observed increased C. *difficile* infections and the incidence rates of VRE and carbapenemase-producing *Enterobacterales* (CPE) strains in immunosuppressed patients [52].

A systematic review of 19 studies in Africa indicated a prevalence of HAIs between 2.5% and 14.8%, with surgical wound infections and the involvement of Gram-negative bacteria frequently reported [6, 53]. Other studies reported that *E. coli* (18.3%), *S. aureus* (17.3%), *Klebsiella* spp. (17.2%), *Pseudomonas* spp. (10.3%) and *Acinetobacter* spp. (6.8%) strains were the most common [54], indicating a prevalence of HAI of up to 50% in ICUs. However, due to the significant percentage obtained within specific institutions, these reports may not reflect the situation at the national level [6, 53–55].

In Tanzania, a cross-sectional study of 134 patients from different clinical departments revealed a prevalence of 15.7% of HAI, an unusually high value for a tertiary care clinical unit [34].

In Australia, data analysis from 2017–2019 indicates that 75–80% of HAIs are reported in New South Wales, Victoria, and Queensland, with *S. aureus* bacteremia being the only indicator consistently tracked nationally. With an estimated prevalence of 9.9% according to a 2018 study and approximately 83,096 cases reported annually, this data allows hospitals to evaluate the effectiveness of interventions to reduce HAsI and increase transparency regarding infection rates [56].

In New Zealand, an observational study including 5468 patients indicated a prevalence of HAIs of 6.6% among hospitalized patients. The most common types of HAIs were surgical site infections (25%), urinary tract infections (18%), and sepsis (13%). Approximately 66% of patients had at least one medical device, such as a peripheral intravenous catheter, leading to 16% of HAIs associated with medical devices [57].

In Thailand, research by Moolasart and collaborators revealed a 3.9% prevalence of HAIs in pediatric inpatients and found that more extended hospitalization periods and central venous catheters (CVCs) were the most important risk factors [58].

In Papua New Guinea, based on ECDC protocol, Curtis and collaborators did a point prevalence survey on HAIs and registered a 6.7% prevalence in a national hospital. However, when the authors estimated prevalence through an extended HAI definition that combined infections identified by the ECDC criteria with a physician diagnosis, the HAIs overall prevalence increased to 12.4% This study provides essential baseline data for prioritizing interventions and monitoring future changes [59].

One of the most serious consequences of HAIs is the increase in the rate of AR generated by the frequent and sometimes incorrect use of antibiotics, which leads to the natural selection of resistant bacteria, making treatment difficult

and expensive, with direct consequences for the safety of patients and healthcare personnel. Almost all pathogenic microorganisms have developed resistance to antimicrobial agents [12]. A study conducted in the USA by Teillant et al. estimated that by 2050, the increase in AR could cause a global economic loss of approximately $300 trillion in global gross domestic product [60, 61].

HAIs caused by MDR bacteria are associated with prolonged hospital stay and antibiotic use, additional treatment costs, and increased readmission rates, favoring higher risk of morbidity and mortality [20, 26, 38, 54, 62–65]. In the United States, these costs range from $28.4 billion to $45 billion, thus exerting significant financial pressure on the public health system, considering that such events can be prevented by alignment to workplace conduct, procedures, and IPC guidelines [12, 38, 54, 66–69]. In Africa, MDR bacteria were common: 70.3% of *Enterobacterales* strains were resistant to 3rd generation cephalosporins. In comparison, 70.5% of *S. aureus* strains were methicillin-resistant, and 55% of *P. aeruginosa* strains were resistant to all agents tested [54].

The studies presented before demonstrate that several factors like healthcare infrastructure, socioeconomic factors, IPC practices influence the prevalence of HAIs across different regions, explaining the lower prevalence rates (4.5–9.9%) in high-income countries compared to LIMCs (5.7–21.6%). In resource-limited settings, HAIs are exacerbated by infrastructure issues, such as the lack of safe water in hospitals, inadequate sanitation and sanitation systems, understaffing, lack or failure to enforce health policies on antibiotic use, lack of essential laboratory equipment for diagnosis, failure to follow safety practices by health professionals, low reporting rates, and limited financial resources [54, 70, 71].

HAIs are still a significant global challenge, with various prevalence rates depending on IPC policies, healthcare infrastructure, and socioeconomic factors. High-income countries report lower prevalence (4.5–9.9%), while low- and middle-income countries (LMICs) experience significantly higher rates (5.7–21.6%), resistance percentages varying due to incorrect use of antibiotics, inadequate sanitation, and limited resources. ICUs are particularly affected, and HAI rates lead to increased morbidity, mortality, and financial burdens—ranging from $28.4 billion to $45 billion annually in the U.S. alone. Despite the improvements made by several developed in the long-term reduction of HAI incidence, there are still shortcomings in regions facing infrastructural and financial constraints [54]. Therefore, a coordinated strategy bringing together technological advancements with context-specific interventions is critically needed to bridge these gaps and develop appropriate IPC practices [38, 64].

## RISK FACTORS ASSOCIATED WITH HAIS

Specific comorbidities, such as diabetes mellitus [72], advanced patient age [73], dementia, use of antipsychotics, stroke, length of hospital stay, type of care (especially rehabilitation and short-term care, use of medical devices (urethral catheters, suprapubic catheters, tracheostomies, peripheral venous catheter, peripheral central access catheter, CVC, urinary catheters, nasogastric tubes) and duration of the placement may

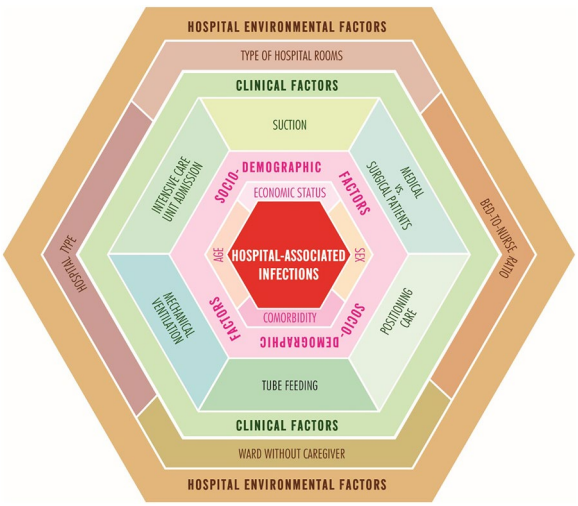


**Fig. 1** Schematic representation of the risk factors linked with HAIs (created with Adobe Illustrator CS6, Adobe Photoshop CS3)

be important risk factors for nosocomial infections [20, 42, 74–78] (Fig. 1).

Diabetes mellitus, in particular, significantly increases the risk of urinary tract infections, while its role as a risk factor for healthcare-associated pneumonia remains a matter of debate [20, 72, 79, 80].

The prevalence of nosocomial infections among elderly patients is higher and the presence of neurological diseases in the elderly is an additional risk factor for contracting HAIs [20, 81, 82]. Related to this, although there is little evidence to support this, some studies suggest that antipsychotic medications, commonly used in neurology, could be an underestimated risk factor for nosocomial infections, especially pneumonia [83]. Taking into account that HAIs and the length of hospital stay are synergistically interconnected, the average length of hospital stay is significantly longer in geriatric patients with neurological diseases compared to the average length of hospital stay of other patient categories [20]. In newborns, HAIs are common and can be extremely dangerous, especially those caused by ESBL bacteria [84]. According to the ECDC, the prevalence of HAIs in children is almost 4%. However, this percentage is more than double in neonatal intensive care units (NICUs), where more than one in ten newborns develop some type of HAI [85]. Unfortunately, current prevention measures could not entirely address these high-risk groups in specific clinical settings like NICUs and geriatric care.

To conclude, various comorbidities (diabetes mellitus, dementia, and stroke), together with the use of medical devices (e.g., catheters, tracheostomies, and central venous catheters), significantly elevate the risk of HAIs. For patients with diabetes mellitus and urinary tract infections, its role in healthcare-associated pneumonia remains debated. In children, the prevalence of HAIs caused by ESBL-producing bacteria is around 4% but could affect over 10% of newborns. High-risk groups such as geriatric and neonatal patients remain particularly vulnerable, requiring the need for targeted IPC strategies and improved monitoring.

## ADVANCED DIAGNOSTIC APPROACHES FOR HAIS

A timely and accurate diagnosis of HAIs is essential for the implementation of effective IPC measures and appropriate antimicrobial therapy, to reduce the burden of HAIs in terms of morbidity, mortality, and healthcare costs.

Despite their widespread use in routine diagnostic laboratories, traditional microbiological methods, such as culture-based techniques, present significant limitations, due to prolonged response time, sensitivity as well as the complexity and costs the required procedures [86].

Recent advancements in molecular diagnostics such as polymerase chain reaction (PCR)-based qualitative or quantitative assays, matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS), and next-generation sequencing (NGS) empowered by artificial intelligence (AI) and machine learning (ML) systems have revolutionized the rapid, accurate, and comprehensive identification of pathogens involved in HAIs [87, 88] (Fig. 2).

### PCR technologies

PCR is used to detect MDR bacterial pathogens involved in the etiology of HAIs, especially those from the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) and *Clostridioides difficile* [86, 89]. However, the epidemiological variations that appeared in the last years unveiled the need to change the initial proposed acronym "ESKAPE" to "ESCAPE" (*E. faecium*, *S. aureus*, *Clostridioides difficile*, *A. baumannii*, *P. aeruginosa*, and *Enterobacteriaceae*) [90].

PCR is also highly effective in detecting nosocomial viral infections, such as cytomegalovirus (CMV), norovirus, and respiratory viruses [91], but also in the rapid detection of *Candida* sp. and *Aspergillus* sp. infections. At the same time, multiplex PCR

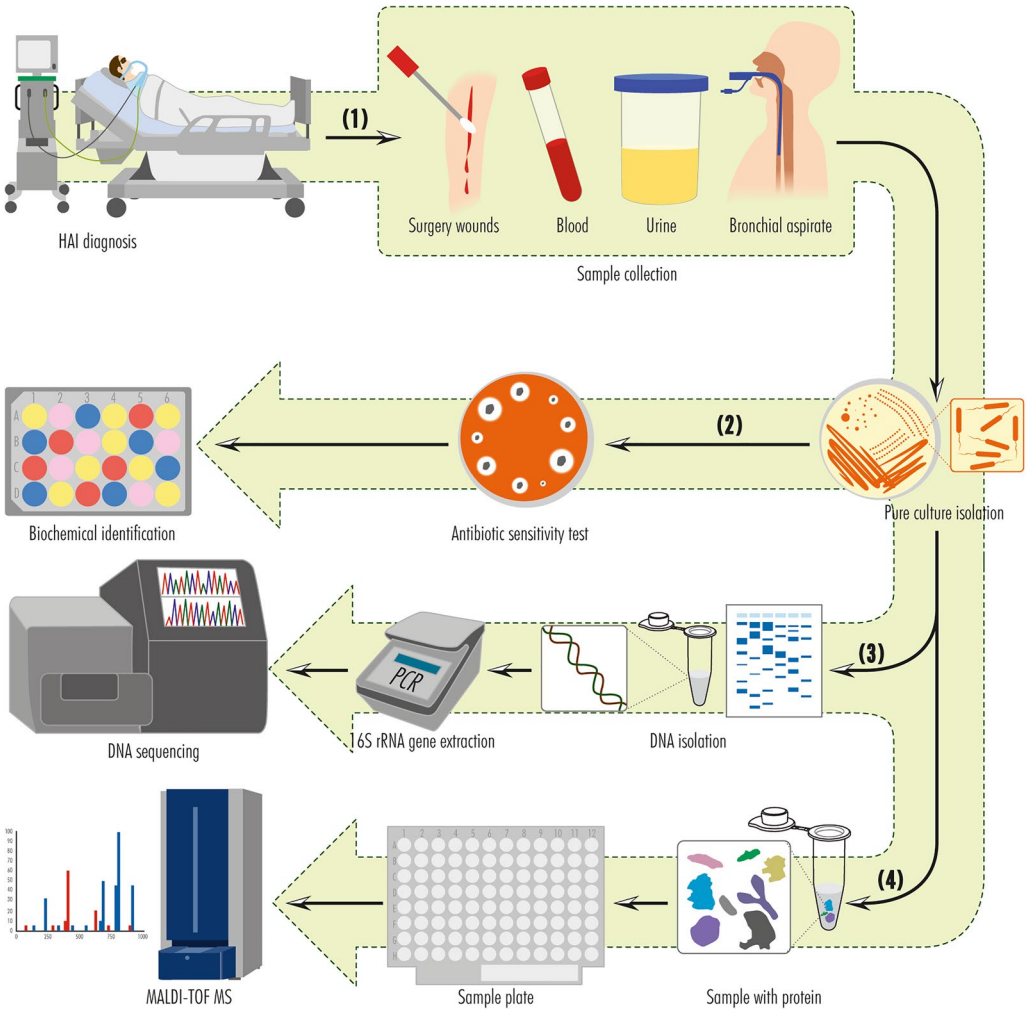


**Fig. 2** Workflow for complete and accurate diagnosis of HAIs. (1) Sample collection in HAI diagnosis (surgical wounds, blood, urine, and bronchial aspirates); (2) Isolation of the pure culture and identification by conventional methods (antibiotic susceptibility testing and biochemical identification); (3) DNA isolation and identification by NGS (bacterial DNA and 16S rRNA isolation and sequencing); (4) MS workflow for identifying microorganisms involves placing a fresh colony or biological samples on a MALDI target microplate, which will then be analyzed by mass spectrometry (created with Adobe Illustrator CS6, Adobe Photoshop CS3)

panels for the rapid detection of sepsis within hours, thus improving the early antimicrobial therapy, are available [92].

Based on PCR technology, real-time PCR (RT-PCR) is one of the most clinically used techniques for diagnosing infectious diseases. This technique allows real-time monitoring of the target amplification using either unspecifically intercalating fluorescent dyes or specific fluorescently labeled probes [93]. Various PCR platforms have focused on the most prevalent carbapenemases, such as $bla_{KPC}$, *bla*OXA-48, *bla*OXA-23, *bla*OXA24/40, *bla*NDM, *bla*VIM, and *bla*IMP [94, 95]. Accordingly, a study by Cerezales and collaborators developed a multiplex PCR to identify the most prevalent carbapenemase-encoding genes in 546 carbapenem-resistant isolates retrieved in Germany. They designed primers for 14 carbapenemases, including *bla* VIM, *bla* OXA-48, *bla* OXA-23, $bla_{KPC}$, $bla_{NDM}$, $bla_{OXA-40}$, $bla_{OXA-58}$, $bla_{IMP}$, $bla_{GIM}$, $bla_{GES}$,

IS*Aba1-bla*$_{\text{OXA-51}}$, *bla*$_{\text{IMI}}$, *bla*$_{\text{FIM}}$, and *bla*$_{\text{DIM}}$, allowing real-time monitoring of the target amplification [95].

Advances in RT-PCR have led to various isothermal amplification techniques, including loop-mediated amplification (LAMP), helicase-dependent amplification (HDA), nucleic acid sequence-based amplification (NASBA), and transcription-mediated amplification (TMA).

Unlike conventional PCR, LAMP uses a DNA polymerase with high strand displacement activity and four primers able to distinguish six–to eight distinct regions of the target DNA [96]. Certain commercial LAMP kits have been developed for the detection of resistance genes, such as *bla*$_{\text{KPC}}$ and *bla*$_{\text{NDM-1}}$, in *K. pneumoniae, A. baumannii, Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enterica, and S. aureus* [97–101].

NASBA and TMA isothermal amplification reactions are based on various mRNAs as target sequences [102]. In contrast, HDA uses the unwinding activity of a helicase to separate dsDNA into two single strands that can serve as a template for new DNA synthesis [103]. HDA, NASBA, and TMA techniques have been successfully applied to detect *Clostridium difficile*, *S. aureus*, *E. coli*, *Chlamydia trachomatis,* and *N. gonorrhea* [103, 104].

Several PCR-based assays have been developed to identify prevalent carbapenemase-encoding genes (e.g., *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$). At the same time, advances in isothermal amplification techniques, such as LAMP, HDA, NASBA, and TMA, and development of commercial kits have improved diagnostic efficiency by enabling rapid and sensitive detection of broad bacterial strains (e.g., *K. pneumoniae, A. baumannii*, *E. coli*, *P. aeruginosa*, *Salmonella enterica*, *S. aureus*, *C. difficile*, *Chlamydia trachomatis*, and *N. gonorrhea*).

## MALDI-TOF

MALDI-TOF MS is a mass spectrometry-based technique that identifies microorganisms based on their protein profiles. The mass spectra of bacterial or fungal isolates are compared to a reference database for species-level identification (Fig. 2) [105, 106].

Since its recent introduction into clinical and microbiological diagnostics, MALDI-TOF MS technology has undergone considerable expansion, emerging as a remarkably accurate tool for microorganism identification [107, 108]. In comparison to traditional methods, it is quick, effective, simple to use, and inexpensive, shortening the time until beginning treatment for a sick patient [107, 109–112]. Both bacterial and fungal pathogens can be successfully identified by MALDI-TOF MS [113].

Rensner and collaborators integrated a deuterium-based labeling method with Bruker MALDI-TOF Biotyper to identify MRSA. They demonstrated that this assay provides a high-sensitivity detection of MRSA after only 3 h of culture time and minimal sample processing [114]. In addition, MALDI-TOF provided promising results in bacterial subtyping for rapidly detecting biomarkers in 229 *S. aureus* isolates retrieved from subclinical bovine mastitis [115]. Smith and collaborators developed a novel method for identifying colistin resistance in *Enterobacter* species and *Klebsiella aerogenes* without performing antimicrobial susceptibility testing. Firstly, they determined broth MICs for colistin and performed killing assays. Subsequently, they used the novel fast lipid analysis technique for lipid A extraction and further analysis via MALDI-TOF. The correlations between MIC, killing efficacy, and predictive lipid A modifications demonstrate the potential utility of MALDI-TOF as a rapid diagnostic platform for colistin resistance in *Enterobacter* species [116].

Recently, Flores-Flores and collaborators conducted a study aiming to identify potential biomarker peaks for resistance or biofilm production in ESKAPE pathogens using MALDI-TOF MS. They detected antimicrobial susceptibility and biofilm production properties in carbapenem-resistant *A. baumannii*, MRSA, *P. aeruginosa*, VRE, and carbapenem-resistant *Enterobacterales* strains. However, despite that, they did not find potential biomarker peaks for biofilm production. Several potential biomarker peaks for drug resistance were detected in VRE, MRSA, and *P. aeruginosa* [117].

Recent studies revealed the utility of MALDI-TOF MS in MRSA detection, colistin resistance

profiling, and AMR markers identification in ESKAPE pathogens, with limited results in identifying biofilm-associated biomarkers. Integrating AI-powered systems for spectral analyses is crucial for enhancing its diagnostic accuracy, and it has the potential to revolutionize clinical and veterinary microbiology.

### NGS Technology

NGS is an advanced technology used for whole genome sequencing (WGS) of microbial pathogens or for metagenomic sequencing [109], providing valuable information regarding complete genome architecture, genetic varieties, gene expression, and epigenetic mechanisms. Current endeavors in NGS concentrate on expanding sequencing accuracy, diminishing costs, and improving bioinformatic computations, opening new perspectives in the development of personalized medicine [89, 109, 118]. NGS can elucidate the etiology of HAIs, including those that are not culturable by established methods, by analyzing specific ribosomal RNA sequences, such as 16S rRNA for bacteria and 18S RNA and the internal transcribed spacer region (ITS) in fungi [109, 119]. Park and collaborators developed a new NGS panel primer set targeting 18 specific virulence factor genes from six target pathogens (*Bacillus cereus*, *Yersinia enterocolitica*, *S. aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*). The NGS panel detected all 18 target genes in a single reaction, yielding almost the same efficiency and specificity as the quantitative real-time PCR (qPCR) analyses [120]. Gaston and collaborators evaluated the performance of a broad targeted NSG panel consisting of a Respiratory Pathogen ID/AMR (RPIP) kit (Illumina, Inc.) with automated Explify bioinformatic analysis (IDbyDNA, Inc.) to identify the AMR markers from bronchoalveolar lavage samples. AMR markers were detected in 136 of 201 samples, and AMR associations were made for various resistant organisms, including ESBL-producing *E. coli*, VRE, MRSA, and *Mycobacterium tuberculosis* [121].

In the context of the influence of the COVID pandemic on HAI management, recent studies demonstrated that WGS could be used to enhance clinical and epidemiological investigations, identify cryptic SARS-CoV-2 transmission pathways, and aid IPC teams in integrating clinical, phenotypic, or epidemiological data for enhanced surveillance [122–124].

### Data-Driven Methodologies

Data-driven methodologies, i.e., artificial intelligence (AI) and machine learning (ML) have become game-changing technologies in the healthcare industry, improving the early detection, prediction, and prevention of HAIs, therefore augmenting patient outcomes and lessening the tension in healthcare systems [125]. Large amounts of clinical, microbiological, and patient data can be processed in real-time by AI and ML algorithms to find early indicators of HAIs. In order to detect abnormalities or patterns suggestive of infections, such as shifts in vital signs (e.g., temperature, heart rate) or laboratory results (e.g., white blood cell counts), AI-powered systems (e.g., automated infection surveillance systems) can examine patient electronic health records (EHRs) [88]. Natural language processing algorithms can extract relevant information from unstructured clinical notes in EHRs, such as symptoms or observations, to flag potential infections [126, 127]. ML algorithms applied to imaging data, such as X-rays or CT scans, can aid in detecting infections like ventilator-associated pneumonia (VAP) [128].

ML aids in analyzing microbial genome sequences to identify virulence factors and resistance genes [129, 130] and can predict future outbreaks by correlating environmental and patient factors with infection patterns [131]. Two recent studies integrated MALDI-TOF MS with ML technologies to evaluate the efficacy of mass spectra for predicting AR and discrimination of vancomycin-susceptible from vancomycin-resistant isolates. They analyzed protein spectra from 178 *E. faecium* and 2229 *S. aureus*, *E. coli*, and *K. pneumoniae* clinical isolates using classification algorithms like support vector machine, random forest, partial least squares–discriminant analysis (PLS-DA), logistic regression, and CatBoost. The

results demonstrated that integrating MALDI-TOF MS with ML technologies could enhance resistance prediction and represent a rapid and effective tool for VRE screening [132, 133].

AI and ML models optimize the allocation of resources, such as staffing and sterilization protocols, to reduce the likelihood of HAIs. AI systems analyze patient flow and room usage to recommend optimized cleaning schedules [134]. Computer vision and AI-powered tools monitor compliance with infection control practices, such as hand hygiene and proper use of personal protective equipment (PPE) [134, 135]. Recent data show that AI and ML can build predictive models to estimate the risk of HAI development for individual patients based on historical and real-time data [136].

## CURRENT PROGRESS AND FUTURE PERSPECTIVES ON ALTERNATIVE APPROACHES FOR COMBATING AND TREATING HAIS

Recent studies show that the most frequently isolated microorganisms causing HAIs are MDR pathogen, known as ESKAPE or ESCAPE [11, 36, 86]. These MDR pathogens are a significant public health issue in clinical settings, increasing mortality when the initial antibiotic treatment is inappropriate, especially if the patient is infected with bacterial strains resistant to many antibiotics. In conclusion, new prevention and treatment options for these bacterial strains causing HAIs are urgently required. Among the new strategies, phage therapy, vaccination and microbiome-based interventions are promising alternatives to mitigate life-threatening illnesses caused by these bacteria [137–139].

### Phage Therapy Against HAIs Causative Bacteria

Phage therapy is an emerging option that can offer an alternative to ineffective antibiotic treatments for antibiotic-resistant bacteria causing HAIs. This strategy is based on natural viruses called bacteriophages, which can infect, replicate, and, theoretically, destroy the bacterial population in an infected patient [140].

In 2016, the first successful clinical use of intravenous bacteriophage therapy occurred, treating severe MDR *A. baumannii* infection. From this point, the interest in bacteriophage therapy renews [141, 142]. Wright and collaborators conducted a randomized, double-blind, placebo-controlled phase I/II clinical trial including 24 patients to evaluate the efficacy and safety of a therapeutic bacteriophage preparation (Biophage-PA) targeting *P. aeruginosa* in chronic otitis. The patients received either a single dose of Biophage-PA or a placebo. The results showed that *P. aeruginosa* counts were significantly lower only in the phage-treated group, and no treatment-related adverse event was reported [143]. In another phase 1 clinical trial, the efficiency of a cocktail containing eight bacteriophages (WPP-201) targeting *P. aeruginosa*, *S. aureus*, and *E. coli* was tested in 39 patients with chronic venous leg ulcers who completed the trial. The authors concluded that the percentages of healed participants were not significantly different between the treatment and control groups, and no adverse events occurred [144]. Terwilliger and collaborators reported using a four-phage cocktail to treat urinary tract infections caused by *E. coli* in a 56-year-old male. The patient tolerated the phage cocktail without any adverse effects, and no bacteria were detected in the urine after the first dose of the phage [145]. The results of the recent clinical trials aiming to use phage therapy targeting HAIs causative bacteria are summarized in Table 1.

The results of the clinical studies indicate a variable efficacy of bacteriophage therapy [144, 148, 149]. One reason can be that most clinical trials have different designs, using different bacteriophage variants, treating different conditions (chronic venous leg ulcers, chronic otitis, acute bacterial diarrhea, urinary tract infections) and bacteria (*P. aeruginosa*, *A. baumannii*, *S. aureus*, *E. coli*, *P. mirabilis*) and using different concentrations of bacteriophage (Table 1). Subsequently, given these differences, it is troublesome to extract accurate conclusions. However, several clinical trials reported great safety

**Table 1** Clinical trials based on bacteriophage therapy against HAIs causative bacteria

| Phages | Study design | Dose and treatment duration | Target | Route of administration | Patients | Expected results/outcomes | Clinical trial registration | Phase and status | References |
|---|---|---|---|---|---|---|---|---|---|
| Biophage-PA | Randomized, double-blind, placebo-controlled | 200 µL; single dose | PA | Topical | 24 | Clinical indicators improvement (ulceration/granulation/polyps, discharge quantity, discharge type and odour) in chronic otitis | EUDRACT 2004–001691-39 | 1/2, completed | [143] |
| WPP-201 | Safety trial | 4 mL; 12 weeks | PA, SA, and EC | Topical | 39 | No adverse events; no significant differences between test and control groups regarding rate and healing frequency | WIRB, protocol#20,061,649 | 1, completed | [144] |
| HP3, HP3.1, ES17, and ES19 | Case report | 1.0 × 109 PFU/mL; 2 weeks | EC | Intravenous | 1 (case report) | Safety and bactericidal action after the first dose; revealed the utility of rationally designed phage cocktails with antibiotics for controlling *E. coli* infection | NA | NA, completed | [145] |

**Table 1** continued

| Phages | Study design | Dose and treatment duration | Target | Route of administration | Patients | Expected results/outcomes | Clinical trial registration | Phase and status | References |
|---|---|---|---|---|---|---|---|---|---|
| LBP-EC01 | Randomized, uncontrolled, open-label | $2 \times 10^{12}$ PFU; 1 mL of $1 \times 10^{9}$–1 mL of $1 \times 10^{11}$; 3 days, twice daily | EC | Intraurethral, intravenous | 39 | Well tolerated, consistent pharmacokinetic profiles in urine and blood, rapid and durable reduction of EC | NCT05488340 | 2, ongoing | [146] |
| Phage cocktail, *Siphoviridae Cystoviridae* and *Podoviridae* family | Double-blind, placebo-controlled, randomized study | 10 mL; 7 days, every 12 h | SA, PA, AB | Inhalation, mesh nebulizer | 60 | Well tolerated, improvement of clinical signs; potential effect on secondary infection and in the outcome of COVID-19 patients | IRCT20111224008507N6 | 2, completed | [147] |
| T4-like coliphages | A prospective, single center, randomized, placebo-controlled, parallel group clinical trial | 106 PFU/mL; 4 days | EC | Oral | 120 | Safe gut transit, fail to improve diarrhea outcome possibly due to insufficient phage coverage and too low *E. coli* pathogen titers | NCT00937274 | NA, completed | [148] |
| BCF-1, *Myoviridae* and *Podoviridae* family | Clinical study | 1 mL per 50 $cm^2$; single dose | PA, SA | Topical | 9 | Well tolerated, low bactericidal action | NA | NA, completed | [149] |

**Table 1** continued

| Phages | Study design | Dose and treatment duration | Target | Route of administration | Patients | Expected results/outcomes | Clinical trial registration | Phase and status | References |
|---|---|---|---|---|---|---|---|---|---|
| PP1131 | Randomized, Multicentric, Open Label, Standard of Care (Silver Sulfadiazine) Controlled Clinical Trial | 1 × 106 PFU/mL; 7 days | PA | Topical | 27 | PP1131 decreased bacterial burden in burn wounds at a slower pace than standard of care | NCT02116010 | 1/2, completed | [150] |
| Pyo bacteriophage cocktail | Randomized, placebo-controlled, clinical trial | 20 mL; 7 days, twice daily | EC, PA, PM | Intravesical | 97 | Favorable safety profile, no evidence of bacteria in 5 of 28 patients in the Pyophage group | NCT03140085 | 2/3, completed | [151, 152] |
| TP-102 | Double-Blind and Randomized Study | $10^9$ PFU/mL/$cm^3$; 1 week for non-infected diabetic foot ulcers and 28 days for infected ones | PA, SA, AB | Topical | 20 | Decrease of target bacteria counts, well-tolerated | NCT04803708 | 1/2, completed | [153] |
| WRAIR-PAM-CF1 | Randomized, placebo-controlled, double-blind study | $4 \times 10^7$ PFU; single dose | PA | Intravenous | 72 | Evaluation of safety of a single dose and microbicidal activity | NCT05453578 | 1b/2, ongoing | [154] |

*EUDRACT* European Union Drug Regulating Authorities Clinical Trials Database, *PA P. aeruginosa*; *SA S. aureus*, *EC E. coli*, *AB Acinetobacter baumannii*, *PM Proteus mirabilis*, *WIRB* Western Institutional Review Board, *NA* not applicable

profiles, improvement of clinical signs after the first doses, and decrease of targeted bacteria counts [145–147, 150, 152–154]. Future clinical trials are needed to test the efficiency of specific bacteriophage strains and the accurate dosage and to enroll more patients caring for different HAIs-causative bacteria.

## Harnessing Microbiome and Host–Pathogen Interactions to Mitigate HAIs

Commensal microorganisms, collectively called the microbiome, are essential to host biology, profoundly influencing immune responses and infection susceptibility [155]. The microbiome supports immune system homeostasis and can affect the progression of infections caused by pathogenic agents [156]. A balanced microbiome is fundamental to maintaining host health, protecting against infections through multiple mechanisms, including competition for resources and ecological niches, modulation of immune responses, regulation of epithelial barrier function, interaction with pathogen signaling mechanisms, induction of antiviral responses, and the production of antimicrobial metabolites [156–161]. The significance of this role varies depending on the host context, justifying the exploration of microbiota-based therapeutic strategies for HAIs prevention and treatment.

An important proportion of HAIs results from the transition of the microbiota from a balanced state to dysbiosis, increasing susceptibility to opportunistic infections. This alteration is influenced by multiple factors, including hospitalization conditions and reasons, environmental influences (both internal and external), and the hygiene status of the healthcare settings [162, 163]. Therefore, the integrated and multidimensional approach required for controlling HAIs should include microbiota preservation [163, 164].

A well-known and illustrative example is that of *C. difficile*, an opportunistic, spore-forming, anaerobic, Gram-positive microorganism capable of colonizing the gastrointestinal tract [165]. Although it is considered part of the normal intestinal microbiota, its proliferation is typically suppressed by dominant anaerobic microorganisms. The colonization rate of the human intestine with *C. difficile* varies with age, being higher in infants and gradually decreasing with age [166, 167]. A critical opponent against the colonization of the intestinal epithelium by *C. difficile* is represented by the integrity of the intestinal microbiota's barrier. The disruption of this barrier, whether due to antibiotic administration, leads to the loss of protective microbiota and creates ecological niches that favor *C. difficile* colonization [168, 169]. In addition to antibiotic exposure, prolonged hospitalization, a low-fiber diet, advanced age, and proton pump inhibitor therapy can contribute to dysbiosis and increase the risk of *C. difficile* infection. Thus, on the one hand, gut microbiota influences infection susceptibility, while on the other hand, alterations caused by external factors can promote *C. difficile* proliferation and disease onset [160]. Moreover, dysbiosis can also lead to an exacerbated inflammatory response, promoting intestinal damage induced by *C. difficile* toxins [157, 170]. Alongside *C. difficile*, an imbalanced microbiome can facilitate infections with other opportunistic pathogens, such as *K. pneumoniae, P. aeruginosa,* and *E. faecium*, frequently involved in HAIs. Therefore, restoring a healthy intestinal microbiome may be an effective strategy for preventing and treating these infections [171].

Various microbiota-based interventions are being studied and clinically applied to prevent and control HAIs. Among the most significant are fecal microbiota transplantation (FMT) and probiotics [167, 172].

FMT is one of the most effective therapies for recurrent *C. difficile* infections, with success rates exceeding 80% in restoring intestinal microbiome balance. The procedure involves transferring microbiota from a healthy donor to the patient [173]. FMT can be administered orally (in capsule form), via the digestive tract (through nasogastric, nasoduodenal, or nasojejunal tubes), or rectally (via enema or colonoscopy). Each approach has specific advantages and disadvantages. The effectiveness of FMT relies on reintroducing a diverse microbial community that suppresses *C. difficile* growth

through competition for nutrients and the production of antimicrobial metabolites [174].

Additionally, the administration of probiotics, such as *Saccharomyces, Lactobacillus,* and *Bifidobacterium*, can help prevent HAIs by colonizing the intestine with beneficial species that inhibit pathogens through various mechanisms. Numerous studies have demonstrated that probiotics can effectively reduce the incidence of *C. difficile* infections in hospitalized patients undergoing antibiotic therapy [175].

### Vaccination Strategies Against HAIs Causative Bacteria

Vaccination is one of the most important approaches in the fight against AMR, which is critical in reducing antibiotic consumption and infection rates with resistant strains [176]. Currently, the efforts are concentrated on exploring novel technologies that can be harnessed to identify and address current challenges impeding advances in antigen and vaccine discoveries [177].

Different research directions in vaccine development are inactivated whole cells (IWC) [178], outer membrane vesicles (OMVs) [179], outer membrane complex (WTO) [180], outer membrane proteins (OMP) including OmpA [181], and passive immunization through monoclonal antibodies (mAbs) [182]. These molecules were evaluated in several clinical trials aiming to discover feasible vaccines against pathogens, especially those from the ESKAPE group which are one of the most prevalent MDR bacteria responsible for HAIs in clinical settings [183–187].

Several preclinical studies investigated the efficiency of vaccines using semi-synthetic glycoconjugates against *K. pneumoniae* [185], OMPs against *P. aeruginosa* [187], and a live attenuated *A. baumannii* strain deficient in thioredoxin against *A. baumannii* strains [186].

In two clinical trials conducted by the same research group, the authors used a vaccine containing the capsular polysaccharide serotypes 5 and 8 conjugated to the nontoxic mutant form of diphtheria toxin (CRM197), a recombinant mutant clumping factor A (ClfA) and a recombinant manganese transporter C (MntC), named SA4Ag to achieve immunity against *S. aureus*. The vaccine was well tolerated, inducing antibody synthesis and supporting immune responses 12 months after vaccination [183] and 36 months after vaccination [184] (Table 2).

Other three clinical trials (NCT02388165, NCT00071214, NCT01160172), including a total of 7138 patients, investigated the efficiency and immunogenicity of a staphylococcal vaccine based on capsular polysaccharides type 5 and 8 (CPS5/8) against *S. aureus* strains. Levy and collaborators observed staphylococcal vaccine-induced functional antibodies with no safety concerns and robust humoral immune responses [188]. In the double-blind trial conducted by Fattom and collaborators, 3359 patients received the CPS5/8 vaccine, inducing a robust immune response and an acceptable safety profile [189]. More recently, Hassanzadeh and collaborators led a multicenter, double-blind trial in which patients received a single dose of SA4Ag (Table 2). While inducing robust functional immune responses, the vaccine did not exhibit efficiency in preventing postoperative *S. aureus* infection [190]. Although two studies [188, 189] consistently confirmed the immunogenicity and the favorable safety profile of the SA4Ag vaccine, the recent study by Hassanzadeh and collaborators demonstrates the failure in preventing postoperative *S. aureus* infections [190]. The results of these clinical trials are summarized in Table 2.

Recently, Fierro and collaborators conducted a study (NCT03819049) showing that the ExPEC10V (VAC52416) vaccine was well tolerated, exhibited a robust antibody-mediated immunogenic response, functional opsonophagocytic killing activity across all measured serotypes, and had a favorable safety profile in participants with a history of UTI (Table 2) [192].

In *P. aeruginosa*-driven HAIs infection, several trials focused on the evaluation of the safety, immunogenicity, and efficiency of various mAbs (NCT01695343, NCT02696902, NCT03027609, NCT03494959, NCT04763759) and OMP (NCT00876252, NCT01563263) as vaccines for preventing or treating infections caused by this pathogen. These clinical trials are conducted

across phases ranging from I to III, involving diverse patient populations, including those with cystic fibrosis, ventilator-associated pneumonia, and burn wounds (Table 2).

The vaccines based on mAbs target specific P. *aeruginosa* components, such as exotoxins or surface polysaccharides. Several mAbs demonstrate promising results regarding safety and immunogenicity. However, other trials show limited efficacy in clinical settings, mainly due to variability in patient responses (low levels of the type III secretion proteins), and the complexity of the pathogen's virulence mechanisms [193, 194] (Table 2).

In two clinical trials, (NCT01563263, NCT00876252), the vaccines based on OMP produced a significant immunogenic effect against *P. aeruginosa*. However, no clinical benefits were registered in terms of mortality and preventing *P. aeruginosa* infection (Table 2). In conclusion, although OMP-based vaccines have shown potential in eliciting immune responses, their clinical efficacy in preventing *P. aeruginosa* infections has yet to be firmly established.

Reportedly, no vaccine candidates for *A. baumannii* have stepped into clinical trials [199–201]. One reason could be the easy degradation of subunit vaccines in vivo and weak immunogenicity. Several *A. baumannii* subunit vaccines have been investigated in vivo regarding immunogenicity and protective effects [202–206]. The most studied components are OMPs (OmpA, Omp33-36, Omp22, OmpW) [207], OMVs, fimbrial proteins (CsuA/B and FimA), capsular polysaccharide [208], and antigens predicted by reverse vaccinology (Pfsr, LptE, OmpH, CarO and FimF) [209]. Choosing the appropriate adjuvants, immunization routes, or delivery vehicles to prepare subunit vaccines can slow down degradation and improve immunogenicity [201].

Future strategies for vaccine development are currently being investigated, including reverse vaccinology [210], systems immunology and serology [211], mass spectrometry-based immunopeptidomics [212], computational protein structure prediction and modeling [213–216], and computational tools for T cell epitope-based vaccines [177, 217].

## HOSPITAL ENVIRONMENT AND IPC MEASURES IN HAIS SURVEILLANCE

Surveillance of HAIs remains a critical challenge in both resource-rich and resource-limited settings, yet it is essential for informing policymakers and improving IPC strategies. Pathogens associated with HAIs are typically transmitted via two primary routes: airborne (droplet) transmission, occurring during natural processes such as breathing, talking, sneezing, or coughing, and indirect transmission through contaminated surfaces (fomites) [32, 54, 218–220]. Certain microorganisms, including MRSA, vancomycin-resistant *S. aureus*, *Clostridioides* spp., *Pseudomonas* spp., *Enterococcus* spp., and viruses such as SARS-CoV, influenza virus, and norovirus, can persist on surfaces for hours to months, significantly increasing the risk of outbreaks [221, 222].

Up to 40% of HAIs have been attributed to cross-contamination by healthcare workers, where pathogens spread via contaminated clothing, gloves, and frequently touched surfaces such as medical equipment, tables, and beds [220, 223]. The hospital environment itself plays a pivotal role in sustaining and transmitting extended-spectrum beta-lactamase (ESBL)-producing bacteria [224]. Enhanced infection control measures, including the use of UV-C light for surface disinfection, have been shown to reduce HAIs caused by these microorganisms and are regarded as highly effective sanitization strategies [225].

The persistence of pathogens on surfaces is influenced by material properties such as roughness, porosity, electrical charge, and hydrophobicity. Proteins or other biological substances in body fluids (sweat, mucus, saliva) may stabilize these pathogens, prolonging their viability. A documented outbreak of carbapenemase-producing *Enterobacteriaceae* (CPE) in two hospitals was linked to contamination by floor-cleaning equipment, underscoring the risks associated with inadequate environmental hygiene [226]. Proper disinfection protocols significantly reduce HAIs, as evidenced by research indicating a higher risk of pathogen colonization in patients

occupying rooms previously used by individuals infected with MDR bacteria [227]. Adhering to international recommendations for cleaning and disinfection, such as those in the National Infection Prevention and Control Manual (NIPCM) of England, is vital for mitigating infection risks [228].

Recently, a study conducted in a tertiary care hospital aimed to evaluate the biofilm eradication properties of several commercially available disinfectants bleach, including Optizan, Virkon, and Clinell against ESBL-producing bacteria. The authors isolated the ESBL strains from wastewater pipes, determined the antibiotic susceptibility profiles, and assessed their ability to form biofilms. The results showed that all disinfectants displayed strain-dependent biofilm eradication [229].

The European Cooperation in Science and Technology (COST) established the 'AMiCI—Antimicrobial Coating Innovations to prevent infectious diseases' action, aiming to investigate the influence of antimicrobial coatings (AMCs), specifically for surfaces in healthcare settings [230]. Recent studies emphasize the need for broad-spectrum antimicrobial coatings to prevent infectious diseases transmission, aligning with recommendations from the ECDC [231]. Novel nitric oxide (NO)-releasing polymeric coatings, designed for medical applications, have demonstrated substantial antibacterial activity against *P. aeruginosa* and MRSA, achieving complete bacterial reduction within 24 h and significant SARS-CoV-2 elimination within 10 min [32]. Integrating copper-based adhesives with UV-C disinfection has also been linked to reduced HAIs rates and the elimination of ESBL-producing bacterial infections, reinforcing its potential as a key strategy against MDR pathogens [84]. While the antimicrobial properties of copper have been demonstrated, studies assessing its impact on MDR bacterial infections, particularly in neonatal care, remain limited [232, 233]. Preventive applications of copper have shown promise in decreasing infection rates, reducing antibiotic usage, and shortening hospital stays [234, 235]. Copper and silver ion applications have shown promise in disrupting biofilms, decreasing infection rates, reducing antibiotic usage, and shortening hospital stays [230, 234, 235]. Contact-active antimicrobial coatings containing chitosan, quaternary ammonium compounds, polyaniline, and biocide-releasing antimicrobial coatings have exhibited good results in HAIs reduction [220]. Recently, Cheng and collaborators conducted a pilot randomized controlled trial to investigate the effectiveness of an antimicrobial surface coating randomized trial on 96 stretcher rails with the highest touch frequency in an emergency department. They used a modified acrylate and silane, producing biocidal activity against bacterial cell membrane and murein layer. Bacterial isolation (MRSA) was performed pre- and post-treatment at 24 h, 7, and 180 days. The results showed that total aerobic bacteria found on antimicrobial-coated patient transport stretcher rails was significantly lower than placebo rails at 24 h [236].

Approximately 35–50% of HAIs are attributed to five primary healthcare practices: hand hygiene, urinary catheter use, intravenous (IV) devices, pulmonary supports (e.g., ventilation), and surgical procedures. Addressing these aspects can lead to significant improvements in patient safety and infection control [237]. Hand hygiene, a fundamental preventive measure, remains the most effective strategy against HAIs [12]. Historical research by Labarraque, Semmelweis, and Wendell Holmes established the role of hand hygiene in preventing nosocomial infections, highlighting the transmission of pathogens via healthcare workers' hands [238–240]. Proper implementation of hand hygiene protocols, along with protective gown and glove usage, has been shown to reduce HAIs in transplant recipients in pediatric ICUs [241]. A surveillance study aimed to evaluate hand hygiene practices (hand rubbing with alcohol-based hand rubs) in 342 nursing students. The study was based on an electronic questionnaire to gather data regarding hand hygiene after contact with body fluid, before a clean or aseptic procedure, before touching a patient, after touching a patient, and after touching patients' surroundings. Even though practices were generally positive, 16% of students were unaware of the clinical contraindications for using alcohol-based hand rub, and 9% did not know when to use soap and water or alcohol-based hand rub

**Table 2** Clinical trials evaluating the efficiency of vaccines targeting HAIs causative bacteria

| Vaccine | Study design | Dose and treatment duration | Target | Route of administration | Patients Enrollment | Expected results/Outcomes | Clinical trial registration | Phase and status | References |
|---|---|---|---|---|---|---|---|---|---|
| SA4Ag, SA3Ag | Placebo-controlled, randomized, double-blind trial | 0.5 mL; single dose | *SA* | Intramuscular | 738 | Well tolerated, rapid and robust functional immune responses | NCT01643941, NCT01364571 | 1/2, completed | [183, 184] |
| CPS5-TT/CPS8-TT/AT/ClfA | Partially blind study | 5/5/10/10 μg; 10/10/30/30 μg; months 0, 1, 6 | *SA* | Intramuscular | 88 | Robust immune response, acceptable safety profile | NCT01160172 | 1, completed | [188] |
| CPS5/8 | Multicenter, randomized, placebo-controlled, double-blinded study | Not provided; week 0 and 35 | | | 3359 | Immune response present, acceptable safety profile | NCT00071214 | 3, completed | [189] |
| SA4Ag | Multicenter, site-level, randomized, double-blind trial | 0.5 mL; single dose | | | 3450 | Failure in preventing postoperative *S. aureus* infections | NCT02388165 | 2b, completed | [190] |
| ExPEC9V | Randomized, double-blind, placebo-controlled, multicenter Study | Not provided; single dose | *EC* | Intramuscular | 19,800 | Evaluation for prevention of the invasive extraintestinal pathogenic *E. coli* disease | NCT04899336 | 3, ongoing | [191] |
| ExPEC10V (VAC52416) | Randomized, observer-blind, first-in-human study | 0.5 mL; single dose | *EC* | Intramuscular | 576 | Well tolerated, robust antibody-mediated immunogenic response | NCT03819049 | 1/2a, completed | [192] |

**Table 2** continued

| Vaccine | Study design | Dose and treatment duration | Target | Route of administration | Patients Enrollment | Expected results/Outcomes | Clinical trial registration | Phase and status | References |
|---|---|---|---|---|---|---|---|---|---|
| KB001-A | Randomized, double-blind, placebo-controlled, repeat-dose study | 10 mg/kg; weeks 2, 4, 8, and 16 | *PA* | Intravenous | 182 | Well-tolerated, but low clinical efficiency | NCT01695343 | 2, completed | [193] |
| MEDI3902 500 | Randomized, parallel-group, double-blind, placebo-controlled study | 1500 mg; single dose | | | 188 | Did not reduce nosocomial pneumonia incidence in PA-colonized mechanically ventilated subjects | NCT02696902 | 2, completed | [194] |
| AR-105 (Aerucin, anti-alginate Mab) | Placebo-controlled, double-blind, randomized study | 20 mg/kg; single dose | | | 158 | Evaluation of the efficacy and safety of AR-105against *P. aeruginosa* | NCT03027609 | 2, completed | NA |
| Pentaglobin (PENTALLO) | Exploratory study | 5 mL/kg | *PA; CRE* | | 120 | Through the evaluation of the mortality rate | NCT03494959 | 2, completed | NA |

Table 2 continued

| Vaccine | Study design | Dose and treatment duration | Target | Route of administration | Patients Enrollment | Expected results/Outcomes | Clinical trial registration | Phase and status | References |
|---|---|---|---|---|---|---|---|---|---|
| TRL1068 | Blinded, single ascending dose study to evaluate safety, pharmacokinetics, and activity of TRL1068 | 6, 15, and 30 mg/kg; single dose | *PA, KP, SA, Enterobacter* spp. | Pre-surgical intravenous infusion | 15 | No adverse effects; no relapse of the original infection by the end of the study (day 169) | NCT04763759 | 1, completed | [195] |
| IC43 | Randomized, placebo-controlled, partially blinded pilot study | 100–200 µg; twice in a 7-day interval | *PA* | Intramuscular | 400 | Immunogenicity, but no significant difference between treatment and control groups | NCT00876252 | 2, completed | [196] |
| | Confirmatory, randomized, placebo-controlled, multi-center, double-blinded study | 100 µg; twice in a 7-day interval | | | 803 | Well tolerated, high immunogenicity, but no clinical benefit in terms of mortality | NCT01563263 | 2/3, completed | [197] |
| MV140 | Canadian, Single Centre, Pilot, Open Label Study | 100 µL; daily, 3 months | *EC, KP, Enterobacter* spp. | Sublingual pulverization | 67 | 75.3% reduction in UTI for the 9-month period | NCT04096820 | 2, completed | [198] |

*NA* not available, *PA P. aeruginosa*, *SA S. aureus*, *EC E. coli*, *AB Acinetobacter baumannii*, *KP Klebsiella pneumoniae*, *CRE* carbapenem-resistant *Enterobacteriaceae*

[242]. Another observational study led by the same research group sent a similar questionnaire to 872 nursing and medical students from one university. They observed higher compliance with the WHO's 'My Five Moments for Hand Hygiene' model among nursing students than medical students. As in the first study, 16% of nursing students were not aware of the clinical contraindications to alcohol-based hand rub [243].

Antibiotic stewardship plays a crucial role in HAIs reduction by preserving the efficacy of existing antimicrobial agents [12]. A notable study found that short-term antibiotic use in cardiac arrest patients significantly decreased ventilator-associated pneumonia incidence [244]. Another study from London, United Kingdom, analyzing pediatric antibiotic prescriptions over five years, revealed that 32.5% of hospitalized children received antimicrobial agents, predominantly from the "Watch" category (46.8–70.5%), highlighting the urgent need for stewardship initiatives to optimize prescribing and combat AMR [245]. Even in the Group of Seven (G7) nations (Canada, France, Germany, Italy, Japan, the United Kingdom, and the United States), IPC measures, education programs, and environmental pollution control (e.g., antibiotic and pesticide emissions) require further reinforcement to combat AMR effectively [246].

To enhance IPC practices, the RISK PRINCIPE research project aims to integrate patient data from diverse sources to develop infection risk profiles and validate a semi-automated surveillance system for hospital-acquired bloodstream infections. This platform will automate infection case registration and facilitate the early identification of vulnerable patient groups for targeted interventions [247].

Overall, a combination of IPC protocols, enhanced surface disinfection, prudent antibiotic stewardship, and rigorous surveillance systems could significantly reduce HAIs and curb the spread of AMR. Future research should prioritize the development of innovative materials for infection control and continue strengthening global IPC strategies to enhance patient safety.

## THE LINK BETWEEN CLIMATE CHANGE, AMR, AND HAIS

One Health is an integrated and collaborative strategy aiming to contribute to sustainable development and solve problems related to water, food, energy, and climate change [248]. The intricate interconnection between human, animal, and environmental health forms the cornerstone of the One Health multidisciplinary concept, addressing complex global health challenges including HAIs and AMR. It is well known that climate change acts as a catalyst for the emergence and spread of infectious diseases. Rising temperatures, altered precipitation patterns, and extreme weather events contribute to the geographical redistribution of pathogens and vectors, while also impacting water and air quality. These environmental shifts can exacerbate the prevalence of HAIs by straining healthcare infrastructure, increasing patient vulnerability, and facilitating the proliferation of resistant organisms. Simultaneously, climate-induced changes in ecosystems can further influence the spread of resistant bacteria across different sectors. The increased incidence of HAIs in the context of climate-related disasters and resource-limited settings underscores the urgent need for comprehensive strategies that address both environmental and healthcare factors.

Recent studies suggest that temperature rise due to climate crisis could impact AMR expansion in humans, animals, plants, and the environment [249, 250], due to association with increased bacterial growth rates and horizontal gene transfer [248, 251] (Fig. 3). Several studies demonstrated the link between high-temperature climates and the rise of heavy metals or biocides concentrations in soil and water, influencing AMR by co-resistance mechanisms [27–32].

An investigation of AMR drivers and pathways in surface waters revealed that HAIs are influenced mainly by wastewater, healthcare facilities, agricultural settings, food, wildlife populations, and climate change [252]. Starting from over 900,000 clinical isolates retrieved from several clinical settings in 30 European countries, Kaba and collaborators conducted a cross-sectional study to decode the link between

HAIs causative bacteria and environmental factors. The reported connection between carbapenem-resistant *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and MRSA strains and warm-season changes in temperature (32–36 °C) support the impact of climatic factors on AMR [253]. Similarly, Macfadden and collaborators reported the correlation between the AMR in *E. coli, K. pneumoniae*, and *S. aureus* and the 10 °C difference in average temperature across regions of the US, association consistent across most classes of antibiotics and pathogens [28].

Other findings show that climatic conditions (dew point, humidity) are linked to the severity of respiratory infections among hospitalized children [254, 255].

De Jongh and colleagues recently suggested that honeybees be used as a model organism to better explain the interactions between climate change and AMR based on the One Health perspective. They identified three potential liaisons between environmental pollution and climatic factors and the risk of AMR. Firstly, the AR achieved by honey bee cells through MDR transport upregulation could extend the risk of AMR in symbiotic microbes. Secondly, they found that cell membrane transporters could diminish microbial exposure to the antimicrobial agents administered. Thirdly, a decrease in honey bee immunity could influence the quick spread and pathogens development inside the hive [256].

These findings are an important step in understanding the relationships of AMR with climate change and environmental pollution in the context of One Health. Adopting the One Health approach allows for more effective management of zoonotic and vector-borne diseases and supports the development of health infrastructures that are resilient to climate change and natural disasters. Therefore, this approach could improve disease prevention strategies and

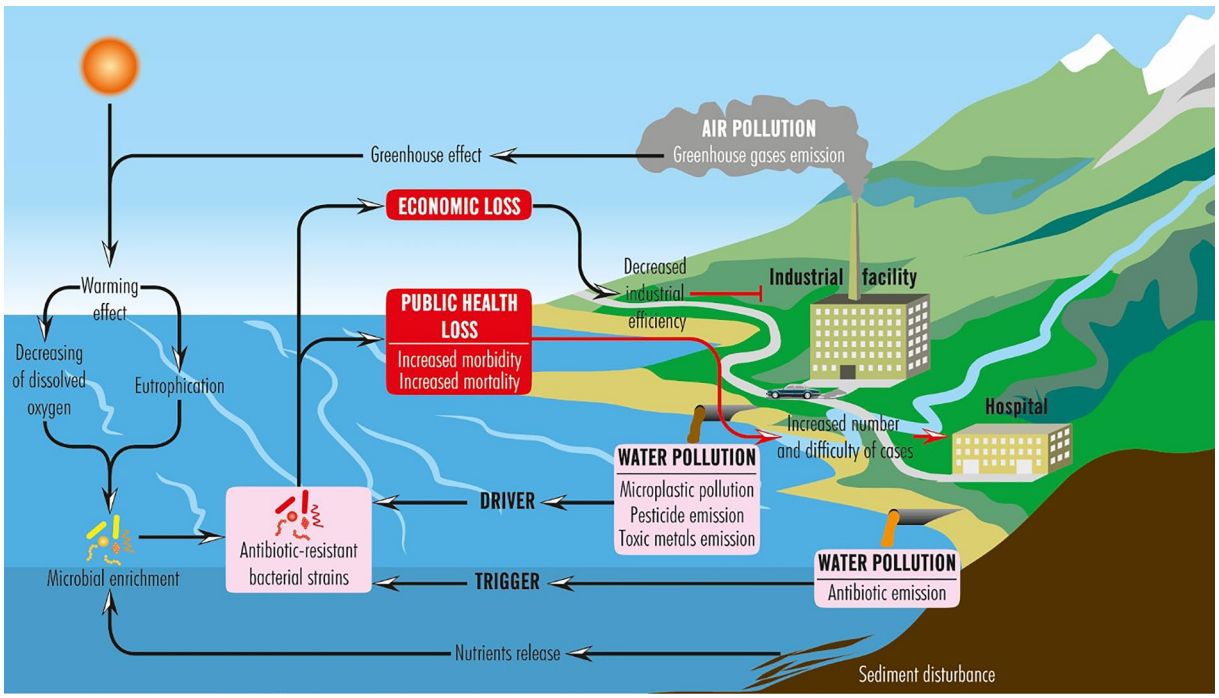


**Fig. 3** Environmental changes connected with HAIs expansion. Temperature rise is linked with increased bacterial growth rates and heavy metals or biocides concentrations in soil and water, influencing AMR by co-resistance mechanisms. In addition, wastewater, healthcare and industrial facilities can lead to water pollution by antibiotic, microplastic, pesticide and toxic metals emission, influencing HAIs expansion (created with Adobe Illustrator CS6, Adobe Photoshop CS3)

address health inequities, facilitating the construction of stronger, safer, and more equitable health systems adapted to global challenges [22]. In health systems, constant monitoring and effective international cooperation policies are essential for reducing the risks associated with climate change [257].

Although studies highlight multiple ways climate change and AMR may intersect and influence each other, research in this area is still in its infancy, with few papers focusing on the intersection of the two topics. Greater attention, based on empirical evidence, is needed to explore knowledge gaps related to the specific risks associated with climate change and MDR bacteria, including fungi, helminths, protists, and viruses [24, 250].

## CONCLUSIONS: MAIN FINDINGS AND FUTURE DIRECTIONS

This review aimed a holistic approach to HAIs by covering: i) their microbial determinants (pathogens involved, prevalence, AR, microbiome disruptions, rapid diagnosis tools, alternative therapeutic and prophylactic strategies); ii) the role of hospital environment (focusing on contamination, infection control strategies, and transmission pathways); iii) climate change (impact on pathogen spread, hospital hygiene challenges, and AMR evolution).

### Main Findings

- The studies dealing with HAIs prevalence demonstrate that while high-income countries report lower prevalence rates (< 10%), low- and middle-income countries exhibit higher rates (up to > 20%), often due to inadequate surveillance and infection prevention measures.
- Although several high-income countries have made advancements in cutting-down HAIs' negative consequences by implementing molecular diagnosis tools, stringent infection control policies and statistical modeling, there are still shortcomings in regions facing infrastructural and financial constraints [54].
- Factors like inappropriate antibiotic use, prolonged hospitalization, and environmental parameters changes can lead to dysbiosis, favoring the rise of opportunistic pathogens like *P. aeruginosa, K. pneumoniae,* and *C. difficile*.
- Phage therapy is an emerging option that can offer an alternative to ineffective antibiotic treatments for antibiotic-resistant bacteria causing HAIs. Although early clinical trials reveal favorable safety profiles and microbiological activity, the discrepancies in study design, bacterial targets, and phage formulations do not allow the formulation of indisputable conclusions, underscoring the need to optimize phage selection and standardize dosages.
- Clinical trials dealing with vaccine development for MDR bacteria have yielded conflicting results. Although several candidates, like SA4Ag and ExPEC10V conjugates, have provided consistent pharmacokinetic profiles, their clinical efficacy in preventing infections remains questionable. For instance, vaccines against *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* exhibited low efficiency due to pathogen complexity and host variability. In addition, subunit vaccine stability and immunogenicity shortcomings have stopped the progress of *A. baumannii* vaccines into clinical trials.
- Recent advances in microbiome research have spurred interest in therapeutic interventions aimed at restoring microbial balance. Two promising strategies, FMT and probiotic therapy, proved highly effective against recurrent *C. difficile* infections and have been shown to reduce HAI incidence in hospitalized patients undergoing antibiotic therapy [167, 172].
- AI and ML offer promising predictive capabilities in processing large volumes of clinical, microbiological, and patient data but require robust data integration. Logistical, ethical, and economic constraints must be

addressed to accomplish and adopt a multidisciplinary approach.

## Future Directions

- By integrating preventive and therapeutic strategies based on biological factors (e.g., vaccination, phage therapy or microbiome-based strategies) into HAIs prevention and treatment protocols, healthcare systems may reduce reliance on antibiotics and lower infection rates.
- Advances in metagenomics and AI may allow patient-specific microbiome assessments to identify those at high risk for HAIs.
- In the era of the climate crisis, life-threatening infectious diseases could still be reduced by implementing One Health strategies and recognizing the intimate connection between human health, animals, and the environment.

To conclude, HAIs are still a demanding global challenge, requiring stringent IPC policies, computer vision, and AI-powered tools. Despite promising avenues like integrated One Health approaches, optimized phage therapy, microbiome-based interventions, and targeted vaccine development, several knowledge gaps in clinical efficacy, standardization, and pathogen complexity remained to be answered.

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***Data Availability.*** Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

## *Declarations*

***Conflict of Interest.*** Andreea Mihaela Sandu, Mariana Carmen Chifiriuc, Corneliu Ovidiu Vrancianu, Roxana-Elena Cristian, Cristina Florentina Alistar, Marian Constantin, Mihaela Paun, Alexandru Alistar, Loredana Gabriela Popa, Mircea Ioan Popa, Ana Catalina Tantu, Manuela Elisabeta Sidoroff, Mara Madalina Mihai, Andreea Marcu, George Popescu, and Monica Marilena Tantu declare that they have no competing interests.

***Ethical Approval.*** This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.



## REFERENCES

1. Storr J, Twyman A, Zingg W, et al. Core components for effective infection prevention and control programmes: new WHO evidence-based recommendations. Antimicrob Resist Infect Control. 2017;6:6. https://doi.org/10.1186/s13756-016-0149-9.

2. Out A, Wellbelove Z, Samson A, et al. Use of low-dose, high-frequency in situ simulation for preventing healthcare-associated infections - the STOP-HCAI pilot project. Infect Prev Pract. 2024;6(2): 100368. https://doi.org/10.1016/j.infpip.2024.100368.

3. Raad I, Hanna H, Maki D. Intravascular catheter-related infections: advances in diagnosis, prevention, and management. Lancet Infect Dis. 2007;7(10):645–57. https://doi.org/10.1016/S1473-3099(07)70235-9.

4. Percival SL, Suleman L, Vuotto C, et al. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol. 2015;64(Pt 4):323–34. https://doi.org/10.1099/jmm.0.000032.

5. Murni IK, Duke T, Kinney S, et al. Risk factors for healthcare-associated infection among children in a low- and middle-income country. BMC Infect Dis. 2022;22(1):406. https://doi.org/10.1186/s12879-022-07387-2.

6. Antonelli A, Ales ME, Chiecca G, et al. Healthcare-associated infections and antimicrobial use in acute care hospitals: a point prevalence survey in Lombardy, Italy, in 2022. BMC Infect Dis. 2024;24(1):632. https://doi.org/10.1186/s12879-024-09487-7.

7. Klevens RM, Edwards JR, Richards CL Jr, et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep. 2007;122(2):160–6. https://doi.org/10.1177/003335490712200205.

8. Danasekaran R, Mani G, Annadurai K. Prevention of healthcare-associated infections: protecting patients, saving lives. Int J Community Med Public Health. 2014;1:67. https://doi.org/10.5455/2394-6040.ijcmph20141114.

9. Guidelines on core components of infection prevention and control programmes at the national and acute health care facility level. Geneva: World Health Organization; 2016. Licence: CC BY-NC-SA 3.0 IGO.

10. Khan HA, Baig FK, Mehboob R. Nosocomial infections: epidemiology, prevention, control, and surveillance. Asian Pac J Trop Biomed. 2017;7(5):478. https://doi.org/10.1016/j.apjtb.2017.01.019.

11. Haque M, Sartelli M, McKimm J, et al. Healthcare-associated infections - an overview. Infect Drug Resist. 2018;11:2321–33. https://doi.org/10.2147/IDR.S177247.

12. Haque M, McKimm J, Sartelli M, et al. Strategies to prevent healthcare-associated infections: a narrative overview. Risk Manag Healthc Policy. 2020;13:1765–80. https://doi.org/10.2147/RMHP.S269315.

13. Shafiqa N, Aston S, Howard A, et al. HAP-FAST: a feasibility study incorporating qualitative, mechanistic and costing sub-studies alongside a randomised pilot trial comparing chest x-ray to low-dose CT scan and empirical antibiotics to antibiotics guided by the BIOFIRE® FILM ARRAY® pneumonia plus panel in adults with suspected non-ventilator-associated hospital-acquired pneumonia. BMJ Open. 2024;14(7): e088490. https://doi.org/10.1136/bmjopen-2024-088490.

14. Rosenthal VD, Duszynska W, Ider BE, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 45 countries for 2013–2018, adult and pediatric units,

device-associated module. Am J Infect Control. 2021;49(10):1267–74. https://doi.org/10.1016/j.ajic.2021.04.077.

15. Kern WV, Rieg S. Burden of bacterial bloodstream infection—a brief update on epidemiology and significance of multidrug-resistant pathogens. Clin Microbiol Infect. 2020;26(2):151–7. https://doi.org/10.1016/j.cmi.2019.10.031.

16. Al-Tawfiq JA, Tambyah PA. Healthcare associated infections (HAI) perspectives. J Infect Public Health. 2014;7(4):339–44. https://doi.org/10.1016/j.jiph.2014.04.003.

17. Vos T, Lim SS, Abbafati C, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1204–22. https://doi.org/10.1016/s0140-6736(20)30925-9.

18. Liu JY, Dickter JK. Nosocomial infections. Gastrointest Endosc Clin N Am. 2020;30(4):637–52. https://doi.org/10.1016/j.giec.2020.06.001.

19. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55. https://doi.org/10.1016/S0140-6736(21)02724-0.

20. Biscetti L, Cameriere V, Rossi T, et al. Dementia, stroke, age, use of medical devices, and antipsychotic drugs may increase the risk of nosocomial infections among elderly patients hospitalized at neurology clinics. Sci Rep. 2023;13(1):18687. https://doi.org/10.1038/s41598-023-46102-2.

21. World Alliance for Patient Safety. The global patient safety challenge, 2005–2006. Clean care is safer care; 2005. WHO PDF. Accessed 16 Oct 2024.

22. Graham SB, Machalaba C, Baum SE, et al. Applying a One Health lens to understanding the impact of climate and environmental change on healthcare-associated infections. Antimicrob Steward Healthc Epidemiol. 2023;3(1): e93. https://doi.org/10.1017/ash.2023.159.

23. Watts N, Amann M, Arnell N, et al. The 2020 report of The Lancet Countdown on health and climate change: responding to converging crises. Lancet. 2021;397(10269):129–70. https://doi.org/10.1016/S0140-6736(20)32290-X.

24. Goniewicz K, Burkle FM, Khorram-Manesh A. Transforming global public health: climate collaboration, political challenges, and systemic change. J Infect Public Health. 2025;18(1): 102615. https://doi.org/10.1016/j.jiph.2024.102615.

25. Vora NM, Hannah L, Lieberman S, et al. Want to prevent pandemics? Stop spillovers Nature. 2022;605(7910):419–22. https://doi.org/10.1038/d41586-022-01312-y.

26. Singer AC, Shaw H, Rhodes V, et al. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. Front Microbiol. 2016;7:1728. https://doi.org/10.3389/fmicb.2016.01728.

27. Coates-Marnane J, Olley J, Burton J, et al. The impact of a high magnitude flood on metal pollution in a shallow subtropical estuarine embayment. Sci Total Environ. 2016;569–70:716–31. https://doi.org/10.1016/j.scitotenv.2016.06.193.

28. MacFadden DR, McGough SF, Fisman D, et al. Antibiotic resistance increases with local temperature. Nat Clim Chang. 2018;8:510–4. https://doi.org/10.1038/s41558-018-0161-6.

29. Chen J, McIlroy SE, Archana A, et al. A pollution gradient contributes to the taxonomic, functional, and resistome diversity of microbial communities in marine sediments. Microbiome. 2019;7:104. https://doi.org/10.1186/s40168-019-0714-6.

30. Zhao W, Ye C, Li J, et al. Increased risk of antibiotic resistance in surface water due to global warming. Environ Res. 2024;263(Pt 2): 120149. https://doi.org/10.1016/j.envres.2024.120149.

31. Furlan JPR, Sellera FP, Lincopan N, et al. Catastrophic floods and antimicrobial resistance: interconnected threats with wide-ranging impacts. One Health. 2024;19: 100891. https://doi.org/10.1016/j.onehlt.2024.100891.

32. Aveyard J, Richards S, Li M, et al. Nitric oxide releasing coatings for the prevention of viral and bacterial infections. Biomater Sci. 2024;12(18):4664–81. https://doi.org/10.1039/d4bm00172a.

33. World Health Organization. Report on the burden of endemic health care-associated infection worldwide. World Health Organization; 2011. WHO IRIS. Accessed 7 Oct 2024.

34. Chuwa AH. Healthcare-associated infections in a tertiary care hospital: significance of patient referral practices. East Afr Health Res J. 2024;8(1):111–5. https://doi.org/10.24248/eahrj.v8i1.756.

35. Cassini A, Plachouras D, Eckmanns T, et al. Burden of six healthcare-associated infections on European population health: estimating incidence-based disability-adjusted life years through a population prevalence-based modelling study. PLoS Med. 2016;13(10): e1002150. https://doi.org/10.1371/journal.pmed.1002150.

36. Suetens C, Latour K, Kärki T, et al. Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. Euro Surveill. 2018;23(46):1800516. https://doi.org/10.2807/1560-7917.ES.2018.23.46.1800516.

37. World Health Organization. The burden of health care-associated infection worldwide: a summary. Patient safety. A world alliance for safer health care. Saves lives. Clean your hands; 2010. WHO PDF. Accessed 16 Oct 2024.

38. Hazard D, von Cube M, Kaier K, et al. Predicting potential prevention effects on hospital burden of nosocomial infections: a multistate modeling approach. Value Health. 2021;24(6):830–8. https://doi.org/10.1016/j.jval.2021.02.002.

39. Flokas ME, Karanika S, Alevizakos M, et al. Prevalence of ESBL-producing *Enterobacteriaceae* in pediatric bloodstream infections: a systematic review and meta-analysis. PLoS ONE. 2017;12(1):e0171216. https://doi.org/10.1371/journal.pone.0171216.

40. European Centre for Disease Prevention and Control, Suetens C, Kärki T, et al. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals, 2022–2023. ECDC. 2024. https://doi.org/10.2900/88011.

41. Pellizzer G, Mantoan P, Timillero L, et al. Prevalence and risk factors for nosocomial infections in hospitals of the Veneto region, north-eastern Italy. Infection. 2008;36(2):112–9. https://doi.org/10.1007/s15010-007-7092-x.

42. Halonen K, van der Kooi T, Hertogh C, et al. Prevalence of healthcare-associated infections in Dutch long-term care facilities from 2009 to 2019. J Hosp Infect. 2019;143:150–9. https://doi.org/10.1016/j.jhin.2023.06.008.

43. Goh LPW, Marbawi H, Goh SM, et al. The prevalence of hospital-acquired infections in Southeast Asia (1990–2022). J Infect Dev Ctries. 2023;17(2):139–46. https://doi.org/10.3855/jidc.17135.

44. Lai S, Ruktanonchai NW, Zhou L, et al. Effect of non-pharmaceutical interventions to contain COVID-19 in China. Nature. 2020;585(7825):410–3. https://doi.org/10.1038/s41586-020-2293-x.

45. COVID-19 National Emergency Response Center, Epidemiology & Case Management Team, Korea Centers for Disease Control & Prevention. Contact transmission of COVID-19 in South Korea: novel investigation techniques for tracing contacts. Osong Public Health Res Perspect. 2020;11(1):60–3. https://doi.org/10.24171/j.phrp.2020.11.1.09.

46. Cho SY, Park SS, Lee JY, et al. Successful prevention and screening strategies for COVID-19: focus on patients with hematologic diseases. Br J Haematol. 2020;190(1):e33–7. https://doi.org/10.1111/bjh.16818.

47. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–33. https://doi.org/10.1056/NEJMoa2001017.

48. Hu LQ, Wang J, Huang A, et al. COVID-19 and improved prevention of hospital-acquired infection. Br J Anaesth. 2020;125(3):e318–9. https://doi.org/10.1016/j.bja.2020.05.037.

49. Cerulli Irelli E, Orlando B, Cocchi E, et al. The potential impact of enhanced hygienic measures during the COVID-19 outbreak on hospital-acquired infections: a pragmatic study in neurological units. J Neurol Sci. 2020;418: 117111. https://doi.org/10.1016/j.jns.2020.117111.

50. Wee LEI, Conceicao EP, Tan JY, et al. Unintended consequences of infection prevention and control measures during COVID-19 pandemic. Am J Infect Control. 2021;49(4):469–77. https://doi.org/10.1016/j.ajic.2020.10.019.

51. Baccolini V, Migliara G, Isonne C, et al. The impact of the COVID-19 pandemic on healthcare-associated infections in intensive care unit patients: a retrospective cohort study. Antimicrob Resist Infect Control. 2021;10(1):87. https://doi.org/10.1186/s13756-021-00959-y.

52. Oh S, Sung YS, Jang M, et al. Impact of the coronavirus disease 2019 pandemic on the incidence of other infectious diseases in the hematology hospital in Korea. Korean J Intern Med. 2024;39(3):513–23. https://doi.org/10.3904/kjim.2023.508.

53. Bagheri Nejad S, Allegranzi B, Syed SB, et al. Healthcare-associated infection in Africa: a systematic review. Bull World Health Organ. 2011;89(10):757–65. https://doi.org/10.2471/BLT.11.088179.

54. Bunduki GK, Masoamphambe E, Fox T, et al. Prevalence, risk factors, and antimicrobial resistance of endemic healthcare-associated infections in Africa: a systematic review and meta-analysis. BMC Infect Dis. 2024;24(1):158. https://doi.org/10.1186/s12879-024-09038-0.

55. Pittet D. Burden of endemic healthcare-associated infection in Africa. Int J Infect Dis. 2014;21S:1–460.

56. Shaban RZ, Mitchell BG, Russo PL, et al. Epidemiology of healthcare-associated infections in Australia: new data and challenges. Infect Dis Health. 2021;26(1):S1–2. https://doi.org/10.1016/j.idh.2021.09.006.

57. Grae N, Singh A, Jowitt D, et al. Prevalence of healthcare-associated infections in public hospitals in New Zealand, 2021. J Hosp Infect. 2023;131:164–72. https://doi.org/10.1016/j.jhin.2022.10.002.

58. Moolasart V, Srijareonvijit C, Charoenpong L, et al. Prevalence and risk factors of healthcare-associated infections among hospitalized pediatric patients: point prevalence survey in Thailand 2021. Children (Basel). 2024;11(6):738. https://doi.org/10.3390/children11060738.

59. Curtis SJ, Barnabas R, Cairns KA, et al. Healthcare-associated infections and antimicrobial use at a major referral hospital in Papua New Guinea: a point prevalence survey. Lancet Reg Health West Pac. 2024;48: 101120. https://doi.org/10.1016/j.lanwpc.2024.101120.

60. Teillant A, Gandra S, Barter D, et al. Potential burden of antibiotic resistance on surgery and cancer chemotherapy antibiotic prophylaxis in the USA: a literature review and modelling study. Lancet Infect Dis. 2015;15(12):1429–37. https://doi.org/10.1016/S1473-3099(15)00270-4.

61. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations The review on antimicrobial resistance. London: Government of the United Kingdom; 2016.

62. Barrasa-Villar JI, Aibar-Remón C, Prieto-Andrés P, et al. Impact on morbidity, mortality, and length of stay of hospital-acquired infections by resistant microorganisms. Clin Infect Dis. 2017;65(4):644–52. https://doi.org/10.1093/cid/cix411.

63. Ohannessian R, Gustin MP, Bénet T, et al. Estimation of extra length of stay attributable to hospital-acquired infections in adult ICUs using a time-dependent multistate model. Crit Care Med. 2018;46(7):1093–8. https://doi.org/10.1097/CCM.0000000000003131.

64. Nuckchady DC. Incidence, risk factors, and mortality from hospital-acquired infections at a hospital in Mauritius. Cureus. 2021;13(11): e19962. https://doi.org/10.7759/cureus.19962.

65. Costantino M, Piazza O, Coscioni E, et al. Analyzing pre-operative hospital stay and incidence of hospital-acquired infection: a retrospective study. Transl Med UniSa. 2024;26(1):46–51. https://doi.org/10.37825/2239-9747.1051.

66. Stone PW. Economic burden of healthcare-associated infections: an American perspective. Expert Rev Pharmacoecon Outcomes Res. 2009;9(5):417–22. https://doi.org/10.1586/erp.09.53.

67. Burnham CD, Leeds J, Nordmann P, et al. Diagnosing antimicrobial resistance. Nat Rev Microbiol. 2017;15(11):697–703. https://doi.org/10.1038/nrmicro.2017.103.

68. Liu X, Spencer A, Long Y, et al. A systematic review and meta-analysis of disease burden of healthcare-associated infections in China: an economic burden perspective from general hospitals. J Hosp Infect. 2022;123:1–11. https://doi.org/10.1016/j.jhin.2022.02.005.

69. Gidey K, Gidey MT, Hailu BY, et al. Clinical and economic burden of healthcare-associated infections: a prospective cohort study. PLoS ONE. 2023;18(2): e0282141. https://doi.org/10.1371/journal.pone.0282141.

70. Bunduki GK, Feasey N, Henrion MYR, et al. Healthcare-associated infections and antimicrobial use in surgical wards of a large urban central hospital in Blantyre, Malawi: a point prevalence survey. Infect Prev Pract. 2021;3(3): 100163. https://doi.org/10.1016/j.infpip.2021.100163.

71. Kanyangarara M, Allen S, Jiwani SS, et al. Access to water, sanitation, and hygiene services in health facilities in sub-Saharan Africa 2013–2018: results of health facility surveys and implications for COVID-19 transmission. BMC Health Serv Res. 2021;21(1):601. https://doi.org/10.1186/s12913-021-06515-z.

72. Ozer B, Ozbakıs Akkurt BC, Duran N, et al. Evaluation of nosocomial infections and risk factors in critically ill patients. Med Sci Monit. 2011;17(3):PH17–22. https://doi.org/10.12659/msm.881434.

73. Cristina ML, Spagnolo AM, Giribone L, et al. Epidemiology and prevention of healthcare-associated infections in geriatric patients: a narrative review. Int J Environ Res Public Health. 2021;18(10):5333. https://doi.org/10.3390/ijerph18105333.

74. Meyers BR, Sherman E, Mendelson MH, et al. Bloodstream infections in the elderly. Am J Med. 1989;86(4):379–84. https://doi.org/10.1016/0002-9343(89)90333-1.

75. Mitchell SL, Teno JM, Kiely DK, et al. The clinical course of advanced dementia. N Engl J Med. 2009;361(16):1529–38. https://doi.org/10.1056/NEJMoa0902234.

76. Reunes S, Rombaut V, Vogelaers D, et al. Risk factors and mortality for nosocomial bloodstream

infections in elderly patients. Eur J Intern Med. 2011;22(5):e39-44. https://doi.org/10.1016/j.ejim.2011.02.004.

77. Westendorp WF, Nederkoorn PJ, Vermeij JD, et al. Post-stroke infection: a systematic review and meta-analysis. BMC Neurol. 2011;11:110. https://doi.org/10.1186/1471-2377-11-110.

78. Detweiler K, Mayers D, Fletcher SG. Bacteriuria and urinary tract infections in the elderly. Urol Clin North Am. 2015;42(4):561–8. https://doi.org/10.1016/j.ucl.2015.07.002.

79. Setia U, Serventi I, Lorenz P. Nosocomial infections among patients in a long-term care facility: spectrum, prevalence, and risk factors. Am J Infect Control. 1985;13(2):57–62. https://doi.org/10.1016/0196-6553(85)90083-5.

80. Vardakas KZ, Siempos II, Falagas ME. Diabetes mellitus as a risk factor for nosocomial pneumonia and associated mortality. Diabet Med. 2007;24(10):1168–71. https://doi.org/10.1111/j.1464-5491.2007.02234.x.

81. Zhao X, Wang L, Wei N, et al. Epidemiological and clinical characteristics of healthcare-associated infection in elderly patients in a large Chinese tertiary hospital: a 3-year surveillance study. BMC Infect Dis. 2020;20(1):121. https://doi.org/10.1186/s12879-020-4840-3.

82. Stewart S, Robertson C, Pan J, et al. Epidemiology of healthcare-associated infection reported from a hospital-wide incidence study: considerations for infection prevention and control planning. J Hosp Infect. 2021;114:10–22. https://doi.org/10.1016/j.jhin.2021.03.031.

83. Rajamaki B, Hartikainen S, Tolppanen AM. Psychotropic drug-associated pneumonia in older adults. Drugs Aging. 2020;37(4):241–61. https://doi.org/10.1007/s40266-020-00754-1.

84. De la Rosa-Zamboni D, Villa-Guillén M, Bustos-Hamdan A, et al. Effect of UV-C disinfection and copper plating on healthcare-associated infections in a NICU with high ESBL infections. Enferm Infecc Microbiol Clin (Engl Ed). 2024. https://doi.org/10.1016/j.eimce.2024.02.014.

85. Saleem Z, Godman B, Hassali MA, et al. Point prevalence surveys of healthcare-associated infections: a systematic review. Pathog Glob Health. 2019. https://doi.org/10.1080/20477724.2019.1632070.

86. Szabó S, Feier B, Capatina D, et al. An overview of healthcare-associated infections and their detection methods caused by pathogen bacteria in Romania and Europe. J Clin Med. 2022;11(11):3204. https://doi.org/10.3390/jcm11113204.

87. Hilt EE, Ferrieri P. Next-generation and other sequencing technologies in diagnostic microbiology and infectious diseases. Genes (Basel). 2022;13(9):1566. https://doi.org/10.3390/genes13091566.

88. Radaelli D, Di Maria S, Jakovski Z, et al. Advancing patient safety: the future of artificial intelligence in mitigating healthcare-associated infections—a systematic review. Healthcare (Basel). 2024;12(19):1996. https://doi.org/10.3390/healthcare12191996.

89. Cason C, D'Accolti M, Soffritti I, et al. Next-generation sequencing and PCR technologies in monitoring the hospital microbiome and its drug resistance. Front Microbiol. 2022;13: 969863. https://doi.org/10.3389/fmicb.2022.969863.

90. De Rosa FG, Corcione S, Pagani N, et al. From ESKAPE to ESCAPE, from KPC to CCC. Clin Infect Dis. 2015;60(8):1289–90. https://doi.org/10.1093/cid/ciu1170.

91. Sancha Dominguez L, Cotos Suárez A, Sánchez Ledesma M, et al. Present and future applications of digital PCR in infectious diseases diagnosis. Diagnostics (Basel). 2024;14(9):931. https://doi.org/10.3390/diagnostics14090931.

92. Miringu G, Musyoki A, Muriithi B, et al. Development of two multiplex PCR assays for rapid detection of eleven Gram-negative bacteria in children with septicemia. Trop Med Health. 2024;52(1):40. https://doi.org/10.1186/s41182-024-00606-3.

93. Gerace E, Mancuso G, Midiri A, et al. Recent advances in the use of molecular methods for the diagnosis of bacterial infections. Pathogens. 2022;11(6):663. https://doi.org/10.3390/pathogens11060663.

94. McClure JA, Conly JM, Obasuyi O, et al. A novel assay for detection of methicillin-resistant *Staphylococcus aureus* directly from clinical samples. Front Microbiol. 2020;11:1295.

95. Cerezales M, Biniossek L, Gerson S, et al. Novel multiplex PCRs for detection of the most prevalent carbapenemase genes in Gram-negative bacteria within Germany. J Med Microbiol. 2021. https://doi.org/10.1099/jmm.0.001310.

96. Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother Off J Jpn Soc Chemother. 2009;15:62–9.

97. Poirier AC, Kuang D, Siedler BS, et al. Development of loop-mediated isothermal amplification rapid diagnostic assays for the detection of *Klebsiella pneumoniae* and carbapenemase genes in clinical samples. Front Mol Biosci. 2022;9(8): 794961. https://doi.org/10.3389/fmolb.2021.794961.

98. Khosravi AD, Khoshnood S, Abbasi Montazeri E, et al. The application of the loop-mediated isothermal amplification method for rapid detection of methicillin-resistant *Staphylococcus aureus*. New Microbes New Infect. 2022;23(45): 100960. https://doi.org/10.1016/j.nmni.2022.100960.

99. Kim EJ, Lee J, Yoon Y, et al. Development of a novel loop-mediated isothermal amplification assay for ß-lactamase gene identification using clinical isolates of Gram-negative bacteria. Front Cell Infect Microbiol. 2023;12(12):1000445. https://doi.org/10.3389/fcimb.2022.1000445.

100. Hassan MM, van Vliet AHM, Higgins O, Burke LP, Chueiri A, O'Connor L, Morris D, Smith TJ, La Ragione RM. Rapid culture-independent loop-mediated isothermal amplification detection of antimicrobial resistance markers from environmental water samples. Microb Biotechnol. 2023;16(5):977–89. https://doi.org/10.1111/1751-7915.14227.

101. Lee JE, Chang JY, Shim WB. Multiplex fluorescence loop-mediated isothermal amplification with lateral flow assay for rapid simultaneous detection of mecA and nuc genes in methicillin-resistant *Staphylococcus aureus*. Anal Chim Acta. 2024;29(1319): 342984. https://doi.org/10.1016/j.aca.2024.342984.

102. Muralidhar S. Molecular methods in the laboratory diagnosis of sexually transmitted infections. Indian J Sex Transm Dis AIDS. 2015;36:9–17.

103. Barreda-Garcia S, Miranda-Castro R, de-Los-Santos-Alvarez N, Miranda-Ordieres AJ, Lobo-Castanon MJ. Helicase-dependent isothermal amplification: a novel tool in the development of molecular-based analytical systems for rapid pathogen detection. Anal Bioanal Chem. 2018;410:679–93.

104. Kim U, Lee SY, Oh SW. Thermophilic helicase-dependent amplification-based CRISPR/Cas12a system: detection of stx2 in *Escherichia coli* O157:H7 by controlling primer dimers. Anal Chim Acta. 2023;25(1239): 340679. https://doi.org/10.1016/j.aca.2022.340679.

105. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev. 2012;36(2):380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x.

106. Sarkar S, Squire A, Diab H, et al. Effect of tryptic digestion on sensitivity and specificity in MALDI-TOF-based molecular diagnostics through machine learning. Sensors. 2023;23:8042. https://doi.org/10.3390/s23198042.

107. Anwer R, Darami H, Almarri FK, et al. MALDI-TOF MS for rapid analysis of bacterial pathogens causing urinary tract infections in the Riyadh Region. Diseases. 2022;10(4):78. https://doi.org/10.3390/diseases10040078.

108. Czeszewska-Rosiak G, Adamczyk I, Ludwiczak A, et al. Analysis of the efficacy of MALDI-TOF MS technology in identifying microorganisms in cancer patients and oncology hospital environment. Heliyon. 2025;11(2): e42015.

109. Wang W, Chauhan V, Luo Y, et al. Comparing NGS-based identification of bloodstream infections to traditional culture methods for enhanced ICU care: a comprehensive study. Front Cell Infect Microbiol. 2024;14:1454549. https://doi.org/10.3389/fcimb.2024.1454549.

110. Sow D, Fall B, Ndiaye M, et al. Usefulness of MALDI-TOF mass spectrometry for routine identification of *Candida* species in a resource-poor setting. Mycopathologia. 2015;180(3–4):173–9. https://doi.org/10.1007/s11046-015-9905-2.

111. Robert MG, Cornet M, Hennebique A, et al. MALDI-TOF MS in a medical mycology laboratory: on stage and backstage. Microorganisms. 2021;9:1283. https://doi.org/10.3390/microorganisms9061283.

112. Elbehiry A, Aldubaib M, Abalkhail A, et al. How MALDI-TOF mass spectrometry technology contributes to microbial infection control in healthcare settings. Vaccines (Basel). 2022;10(11):1881. https://doi.org/10.3390/vaccines10111881.

113. Giraud-Gatineau A, Texier G, Fournier PE, et al. Using MALDI-TOF spectra in epidemiological surveillance for the detection of bacterial subgroups with a possible epidemic potential. BMC Infect Dis. 2021;21(1):1109. https://doi.org/10.1186/s12879-021-06803-3.

114. Rensner JJ, Lueth P, Bellaire BH, Sahin O, Lee YJ. Rapid detection of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* using MALDI-TOF mass spectrometry. Front Cell Infect Microbiol. 2023;23(13):1281155. https://doi.org/10.3389/fcimb.2023.1281155.

115. Lopes T, Fidelis CE, Silva ATF, Mota RA, Rall VLM, Dos Santos MV, Gonçalves JL. MALDI-TOF bacterial subtyping for rapid detection of biomarkers in *Staphylococcus aureus* from subclinical bovine mastitis. J Appl Microbiol. 2023;134(11):lxad249. https://doi.org/10.1093/jambio/lxad249.

116. Smith RD, McElheny CL, Izac JR, Gardner FM, Chandler CE, Goodlett DR, Doi Y, Johnson JK, Ernst RK. A novel lipid-based MALDI-TOF assay for the rapid detection of colistin-resistant *Enterobacter* Species. Microbiol Spectr. 2022;10(1): e0144521. https://doi.org/10.1128/spectrum.01445-21.

117. Flores-Flores AS, Vazquez-Guillen JM, Bocanegra-Ibarias P, Camacho-Ortiz A, Tamez-Guerra RS, Rodriguez-Padilla C, Flores-Treviño S. MALDI-TOF MS profiling to predict resistance or biofilm production in gram-positive ESKAPE pathogens from healthcare-associated infections. Diagn Microbiol Infect Dis. 2025;111(1): 116562. https://doi.org/10.1016/j.diagmicrobio.2024.116562.

118. Satam H, Joshi K, Mangrolia U, et al. Next-generation sequencing technology: current trends and advancements. Biology (Basel). 2023;12(7):997. https://doi.org/10.3390/biology12070997.

119. Nafea AM, Wang Y, Wang D, Salama AM, Aziz MA, Xu S, Tong Y. Application of next-generation sequencing to identify different pathogens. Front Microbiol. 2024;29(14):1329330. https://doi.org/10.3389/fmicb.2023.1329330.

120. Park DG, Kwon JG, Ha ES, Kang B, Choi I, Kwak JE, Choi J, Lee W, Kim SH, Kim SH, Park J, Lee JH. Novel next generation sequencing panel method for the multiple detection and identification of foodborne pathogens in agricultural wastewater. Front Microbiol. 2023;13(14):1179934. https://doi.org/10.3389/fmicb.2023.1179934.

121. Gaston DC, Miller HB, Fissel JA, Jacobs E, Gough E, Wu J, Klein EY, Carroll KC, Simner PJ. Evaluation of metagenomic and targeted next-generation sequencing workflows for detection of respiratory pathogens from bronchoalveolar lavage fluid specimens. J Clin Microbiol. 2022;60(7): e0052622. https://doi.org/10.1128/jcm.00526-22.

122. Hare D, Dembicka KM, Brennan C, Campbell C, Sutton-Fitzpatrick U, Stapleton PJ, De Gascun CF, Dunne CP. Whole-genome sequencing to investigate transmission of SARS-CoV-2 in the acute healthcare setting: a systematic review. J Hosp Infect. 2023;140:139–55. https://doi.org/10.1016/j.jhin.2023.08.002.

123. Rader TS 4th, Srinivasa VR, Griffith MP, Waggle K, Pless L, Chung A, Wagester S, Harrison LH, Snyder GM. The utility of whole-genome sequencing to inform epidemiologic investigations of SARS-CoV-2 clusters in acute-care hospitals. Infect Control Hosp Epidemiol. 2024;45(2):144–9. https://doi.org/10.1017/ice.2023.274.

124. Struelens MJ, Ludden C, Werner G, Sintchenko V, Jokelainen P, Ip M. Real-time genomic surveillance for enhanced control of infectious diseases and antimicrobial resistance. Front Sci. 2024;2:1298248. https://doi.org/10.3389/fsci.2024.1298248.

125. Choudhury A, Asan O. Role of artificial intelligence in patient safety outcomes: systematic literature review. JMIR Med Inform. 2020;8(7): e18599. https://doi.org/10.2196/18599.

126. Hossain E, Rana R, Higgins N, et al. Natural language processing in electronic health records in relation to healthcare decision-making: a systematic review. Comput Biol Med. 2023;155: 106649. https://doi.org/10.1016/j.compbiomed.2023.106649.

127. Gurav P. Natural language processing in electronic health records: a review. Artif Intell Health. 2024;1(1):16–31. https://doi.org/10.36922/aih.2147.

128. Zhang F. Application of machine learning in CT images and X-rays of COVID-19 pneumonia. Medicine (Baltimore). 2021;100(36): e26855. https://doi.org/10.1097/MD.0000000000026855.

129. Kim JI, Maguire F, Tsang KK, et al. Machine learning for antimicrobial resistance prediction: current practice, limitations, and clinical perspective. Clin Microbiol Rev. 2022;35(3): e0017921. https://doi.org/10.1128/cmr.00179-21.

130. Gao Y, Liu M. Application of machine learning-based genome sequence analysis in pathogen identification. Front Microbiol. 2024;15:1474078. https://doi.org/10.3389/fmicb.2024.1474078.

131. Zhao AP, Li S, Cao Z, et al. AI for science: predicting infectious diseases. J Saf Sci Resilience. 2024;5(2):130–46.

132. Candela A, Arroyo MJ, Sánchez-Molleda Á, Méndez G, Quiroga L, Ruiz A, Cercenado E, Marín M, Muñoz P, Mancera L, Rodríguez-Temporal D, Rodríguez-Sánchez B. Rapid and reproducible MALDI-TOF-based method for the detection of vancomycin-resistant *Enterococcus faecium* using classifying algorithms. Diagnostics (Basel). 2022;12(2):328. https://doi.org/10.3390/diagnostics12020328.

133. Feucherolles M. Integrating MALDI-TOF mass spectrometry with machine learning techniques for rapid antimicrobial resistance screening of foodborne bacterial pathogens. Methods Mol Biol. 2025;2852:85–103. https://doi.org/10.1007/978-1-0716-4100-2_6.

134. Godbole AA, Paras Mehra M, Banerjee S, et al. Enhancing infection control in ICUs through AI: a literature review. Health Sci Rep. 2025;8(1): e70288. https://doi.org/10.1002/hsr2.70288.

135. Wang C, Jiang W, Yang K, et al. Electronic monitoring systems for hand hygiene: systematic review of technology. J Med Internet Res. 2021;23(11): e27880. https://doi.org/10.2196/27880.

136. Abhadiomhen SE, Nzeakor EO, Oyibo K. Health risk assessment using machine learning: systematic review. Electronics. 2024;13:4405.

137. Choi YJ, Kim S, Shin M, et al. Isolation and characterization of novel bacteriophages to target carbapenem-resistant *Acinetobacter baumannii*. Antibiotics (Basel). 2024;13(7):610. https://doi.org/10.3390/antibiotics13070610.

138. Kelly L, Jameson E. Bacteriophage cocktail shows no toxicity and improves the survival of *Galleria mellonella* infected with Klebsiella spp. J Virol. 2024;98(6): e0027224. https://doi.org/10.1128/jvi.00272-24.

139. Shein AMS, Wannigama DL, Hurst C, et al. Phage cocktail amikacin combination as a potential therapy for bacteremia associated with carbapenemase-producing colistin-resistant *Klebsiella pneumoniae*. Sci Rep. 2024;14(1):28992. https://doi.org/10.1038/s41598-024-79924-9.

140. Teney C, Poupelin JC, Briot T, et al. Phage therapy in a burn patient colonized with extensively drug-resistant *Pseudomonas aeruginosa* responsible for relapsing ventilator-associated pneumonia and bacteremia. Viruses. 2024;16(7):1080. https://doi.org/10.3390/v16071080.

141. Hitchcock NM, Devequi Gomes Nunes D, Shiach J, et al. Current clinical landscape and global potential of bacteriophage therapy. Viruses. 2023;15(4):1020. https://doi.org/10.3390/v15041020.

142. Palma M, Qi B. Advancing phage therapy: a comprehensive review of the safety, efficacy, and future prospects for the targeted treatment of bacterial infections. Infect Dis Rep. 2024;16(6):1127–81. https://doi.org/10.3390/idr16060092.

143. Wright A, Hawkins CH, Anggård EE, et al. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin Otolaryngol. 2009;34(4):349–57. https://doi.org/10.1111/j.1749-4486.2009.01973.x.

144. Rhoads DD, Wolcott RD, Kuskowski MA, et al. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J Wound Care. 2009;18(6):237–8. https://doi.org/10.12968/jowc.2009.18.6.42801. (**240–243**).

145. Terwilliger A, Clark J, Karris M, et al. Phage therapy-related microbial succession associated with successful clinical outcome for a recurrent urinary tract infection. Viruses. 2021;13(10):2049. https://doi.org/10.3390/v13102049.

146. Kim P, Sanchez AM, Penke TJR, et al. Safety, pharmacokinetics, and pharmacodynamics of LBP-EC01, a CRISPR-Cas3-enhanced bacteriophage cocktail, in uncomplicated urinary tract infections due to *Escherichia coli* (ELIMINATE): the randomized, open-label, first part of a two-part phase 2 trial. Lancet Infect Dis. 2024;24(12):1319–32. https://doi.org/10.1016/S1473-3099(24)00424-9.

147. Samaee HR, Eslami G, Rahimzadeh G, et al. Inhalation phage therapy as a new approach to preventing secondary bacterial pneumonia in patients with moderate to severe COVID-19: a double-blind clinical trial study. J Drug Deliv Sci Technol. 2023;84: 104486. https://doi.org/10.1016/j.jddst.2023.104486.

148. Sarker SA, Sultana S, Reuteler G, et al. Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. EBioMedicine. 2016;4:124–37. https://doi.org/10.1016/j.ebiom.2015.12.023.

149. Rose T, Verbeken G, Vos DD, et al. Experimental phage therapy of burn wound infection: difficult first steps. Int J Burns Trauma. 2014;4(2):66–73.

150. Jault P, Leclerc T, Jennes S, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomized, controlled, double-blind phase 1/2 trial. Lancet Infect Dis. 2019;19(1):35–45. https://doi.org/10.1016/S1473-3099(18)30482-1.

151. Leitner L, Sybesma W, Chanishvili N, et al. Bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. BMC Urol. 2017;17(1):90. https://doi.org/10.1186/s12894-017-0283-6.

152. Leitner L, Ujmajuridze A, Chanishvili N, et al. Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. Lancet Infect Dis. 2021;21(3):427–36. https://doi.org/10.1016/S1473-3099(20)30330-3.

153. Nir-Paz R, Onallah H, Dekel M, et al. Randomized double-blind study on safety and tolerability of TP-102 phage cocktail in patients with infected and non-infected diabetic foot ulcers. Med. 2024. https://doi.org/10.1016/j.medj.2024.11.018.

154. Tamma PD, Souli M, Billard M, et al. Safety and microbiological activity of phage therapy in persons with cystic fibrosis colonized with *Pseudomonas aeruginosa*: study protocol for a phase 1b/2, multicenter, randomized, double-blind, placebo-controlled trial. Trials. 2022;23(1):1057. https://doi.org/10.1186/s13063-022-07047-5.

155. Maciel-Fiuza MF, Muller GC, Campos DMS, et al. Role of gut microbiota in infectious and inflammatory diseases. Front Microbiol. 2023;14:1098386. https://doi.org/10.3389/fmicb.2023.1098386.

156. Alwin A, Karst SM, Yu JJ, et al. The influence of microbiota-derived metabolites on viral infections. Curr Opin Virol. 2021;49:151–6. https://doi.org/10.1016/j.coviro.2021.05.006.

157. McKenney ES, Kendall MM. Microbiota and pathogen 'pas de deux': setting up and breaking down barriers to intestinal infection. Pathog Dis. 2016;74(5):ftw051. https://doi.org/10.1093/femspd/ftw051.

158. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30(6):492–506. https://doi.org/10.1038/s41422-020-0332-7.

159. Segura Munoz RR, Mantz S, Martínez I, et al. Experimental evaluation of ecological principles to understand and modulate the outcome of bacterial strain competition in gut microbiomes. ISME J. 2022;16(6):1594–604. https://doi.org/10.1038/s41396-022-01208-9.

160. Vasilescu IM, Chifiriuc MC, Pircalabioru GG, et al. Gut dysbiosis and *Clostridioides difficile* infection in neonates and adults. Front Microbiol. 2022;12:651081. https://doi.org/10.3389/fmicb.2021.651081.

161. Schreiber F, Balas I, Robinson MJ, et al. Border control: the role of the microbiome in regulating epithelial barrier function. Cells. 2024;13(6):477. https://doi.org/10.3390/cells13060477.

162. Turner NA, Anderson DJ. Hospital infection control: *Clostridioides difficile*. Clin Colon Rectal Surg. 2020;33(2):98–108. https://doi.org/10.1055/s-0040-1701234.

163. Tozzo P, Delicati A, Caenazzo L. Human microbiome and microbiota identification for preventing and controlling healthcare-associated infections: a systematic review. Front Public Health. 2022;10: 989496. https://doi.org/10.3389/fpubh.2022.989496.

164. Cruz-López F, Martínez-Meléndez A, Garza-González E. How does hospital microbiota contribute to healthcare-associated infections? Microorganisms. 2023;11(1):192. https://doi.org/10.3390/microorganisms11010192.

165. Spigaglia P. *Clostridioides difficile* and gut microbiota: from colonization to infection and treatment. Pathogens. 2024;13(8):646. https://doi.org/10.3390/pathogens13080646.

166. Bien J, Palagani V, Bozko P, et al. The intestinal microbiota dysbiosis and *Clostridioides difficile* infection: is there a relationship with inflammatory bowel disease? Therap Adv Gastroenterol. 2013;6(1):53–68. https://doi.org/10.1177/1756283X12454590.

167. Piccioni A, Rosa F, Manca F, et al. Gut microbiota and *Clostridioides difficile*: what we know and the new frontiers. Int J Mol Sci. 2022;23(21):13323. https://doi.org/10.3390/ijms232113323.

168. Patangia DV, Anthony Ryan C, Dempsey E, et al. Impact of antibiotics on the human microbiome and consequences for host health. Microbiologyopen. 2022;11(1): e1260. https://doi.org/10.1002/mbo3.1260.

169. Woelfel S, Silva MS, Stecher B. Intestinal colonization resistance in the context of environmental, host, and microbial determinants. Cell Host Microbe. 2024;32(6):820–36. https://doi.org/10.1016/j.chom.2024.05.002.

170. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. Nature. 2016;535(7610):85–93. https://doi.org/10.1038/nature18849.

171. Flury BB, Andrey D, Kohler P. Antibiotics' collateral effects on the gut microbiota in the selection of ESKAPE pathogens. CMI Commun. 2024;1(2):100012. https://doi.org/10.1016/j.cmicom.2024.100012.

172. Pettigrew MM, Johnson JK, Harris AD. The human microbiota: novel targets for hospital-acquired infections and antibiotic resistance. Ann Epidemiol. 2016;26(5):342–7. https://doi.org/10.1016/j.annepidem.2016.02.007.

173. Cheng YW, Fischer M. Fecal microbiota transplantation. Clin Colon Rectal Surg. 2023;36(2):151–6. https://doi.org/10.1055/s-0043-1760865.

174. Verdier C, Denis S, Gasc C, et al. An oral FMT capsule as efficient as an enema for microbiota reconstruction following disruption by antibiotics, as assessed in an in vitro human gut model. Microorganisms. 2021;9(2):358. https://doi.org/10.3390/microorganisms9020358.

175. Valdés-Varela L, Hernández-Barranco AM, Ruas-Madiedo P, et al. Effect of Bifidobacterium upon

*Clostridioides difficile* growth and toxicity when co-cultured in different prebiotic substrates. Front Microbiol. 2016;7:738. https://doi.org/10.3389/fmicb.2016.00738.

176. Vrancianu CO, Gheorghe I, Dobre EG, et al. Emerging strategies to combat β-lactamase producing ESKAPE pathogens. Int J Mol Sci. 2020;21(22):8527. https://doi.org/10.3390/ijms21228527.

177. Sastalla I, Kwon K, Huntley C, et al. NIAID workshop report: systematic approaches for ESKAPE bacteria antigen discovery. Vaccines (Basel). 2025;13(1):87. https://doi.org/10.3390/vaccines13010087.

178. Shu MH, MatRahim N, NorAmdan N, et al. An inactivated antibiotic-exposed whole-cell vaccine enhances bactericidal activities against multidrug-resistant *Acinetobacter baumannii*. Sci Rep. 2016;6:22332. https://doi.org/10.1038/srep22332.

179. Badmasti F, Ajdary S, Bouzari S, et al. Immunological evaluation of OMV(PagL)+Bap(1–487aa) and AbOmpA(8–346aa)+Bap(1–487aa) as vaccine candidates against *Acinetobacter baumannii* sepsis infection. Mol Immunol. 2015;67:552–8. https://doi.org/10.1016/j.molimm.2015.07.031.

180. Pulido MR, García-Quintanilla M, Pachón J, et al. Immunization with lipopolysaccharide-free outer membrane complexes protects against *Acinetobacter baumannii* infection. Vaccine. 2018;36:4153–6. https://doi.org/10.1016/j.vaccine.2018.05.113.

181. Zhang X, Yang T, Cao J, et al. Mucosal immunization with purified OmpA elicited protective immunity against infections caused by multidrug-resistant *Acinetobacter baumannii*. Microb Pathog. 2016;96:20–5. https://doi.org/10.1016/j.micpath.2016.04.019.

182. Elmassry MM, Colmer-Hamood JA, Kopel J, et al. Anti-*Pseudomonas aeruginosa* vaccines and therapies: an assessment of clinical trials. Microorganisms. 2023;11(4):916. https://doi.org/10.3390/microorganisms11040916.

183. Creech CB, Frenck RW Jr, Sheldon EA, et al. Safety, tolerability, and immunogenicity of a single dose 4-antigen or 3-antigen *Staphylococcus aureus* vaccine in healthy older adults: results of a randomized trial. Vaccine. 2017;35:385–94. https://doi.org/10.1016/j.vaccine.2016.11.032.

184. Frenck RW Jr, Creech CB, Sheldon EA, et al. Safety, tolerability, and immunogenicity of a 4-antigen *Staphylococcus aureus* vaccine (SA4Ag): results from a first-in-human randomized, placebo-controlled phase 1/2 study. Vaccine. 2017;35(2):375–84. https://doi.org/10.1016/j.vaccine.2016.11.010.

185. Seeberger PH, Pereira CL, Khan N, et al. A semi-synthetic glycoconjugate vaccine candidate for carbapenem-resistant *Klebsiella pneumoniae*. Angew Chem Int Ed Engl. 2017;56:13973–8. https://doi.org/10.1002/anie.201700964.

186. Ainsworth S, Ketter PM, Yu JJ, et al. Vaccination with a live attenuated *Acinetobacter baumannii* deficient in thioredoxin provides protection against systemic Acinetobacter infection. Vaccine. 2017;35:3387–94. https://doi.org/10.1016/j.vaccine.2017.05.017.

187. Hoggarth A, Weaver A, Pu Q, et al. Mechanistic research holds promise for bacterial vaccines and phage therapies for *Pseudomonas aeruginosa*. Drug Des Devel Ther. 2019;13:909–24. https://doi.org/10.2147/DDDT.S189847.

188. Levy J, Licini L, Haelterman E, et al. Safety and immunogenicity of an investigational 4-component *Staphylococcus aureus* vaccine with or without AS03B adjuvant: results of a randomized phase I trial. Hum Vaccin Immunother. 2015;11(3):620–31. https://doi.org/10.1080/21645515.2015.1011021.

189. Fattom A, Matalon A, Buerkert J, et al. Efficacy profile of a bivalent Staphylococcus aureus glycoconjugated vaccine in adults on hemodialysis: phase III randomized study. Hum Vaccin Immunother. 2015;11(3):632–41. https://doi.org/10.4161/hv.34414.

190. Hassanzadeh H, Baber J, Begier E, et al. Efficacy of a 4-antigen *Staphylococcus aureus* vaccine in spinal surgery: the STaphylococcus aureus suRgical Inpatient Vaccine Efficacy (STRIVE) randomized clinical trial. Clin Infect Dis. 2023;77(2):312–20. https://doi.org/10.1093/cid/ciad218.

191. Doua J, Ekkelenkamp M, Verheij T, et al. Feasibility of tracking invasive Escherichia coli disease among older adults in a community setting: a prospective observational study. Eur J Clin Microbiol Infect Dis. 2024;43(3):541–50. https://doi.org/10.1007/s10096-023-04738-y.

192. Fierro CA, Sarnecki M, Spiessens B, et al. A randomized phase 1/2a trial of ExPEC10V vaccine in adults with a history of UTI. npj Vaccines. 2024;9:106. https://doi.org/10.1038/s41541-024-00885-1.

193. Jain R, Beckett VV, Konstan MW, et al. KB001-A, a novel anti-inflammatory, found to be safe and well-tolerated in cystic fibrosis patients infected with *Pseudomonas aeruginosa*. J Cyst Fibros. 2018;17(4):484–91. https://doi.org/10.1016/j.jcf.2017.12.006.

194. Chastre J, François B, Bourgeois M, et al. Safety, efficacy, and pharmacokinetics of gremubamab

(MEDI3902), an anti-*Pseudomonas aeruginosa* bispecific human monoclonal antibody, in *P. aeruginosa*-colonized, mechanically ventilated intensive care unit patients: a randomized controlled trial. Crit Care. 2022;26(1):355. https://doi.org/10.1186/s13054-022-04204-9.

195. Conway J, Delanois RE, Mont MA, et al. Phase 1 study of the pharmacokinetics and clinical proof-of-concept activity of a biofilm-disrupting human monoclonal antibody in patients with chronic prosthetic joint infection of the knee or hip. Antimicrob Agents Chemother. 2024;68(8): e0065524. https://doi.org/10.1128/aac.00655-24.

196. Rello J, Krenn CG, Locker G, et al. A randomized placebo-controlled phase II study of a *Pseudomonas* vaccine in ventilated ICU patients. Crit Care. 2017;21(1):22. https://doi.org/10.1186/s13054-017-1601-9.

197. Adlbrecht C, Wurm R, Depuydt P, et al. Efficacy, immunogenicity, and safety of IC43 recombinant *Pseudomonas aeruginosa* vaccine in mechanically ventilated intensive care patients—a randomized clinical trial. Crit Care. 2020;24(1):74. https://doi.org/10.1186/s13054-020-2792-z.

198. Nickel JC, Kelly KL, Griffin A, et al. MV140 sublingual vaccine reduces recurrent urinary tract infection in women: results from the first North American clinical experience study. Can Urol Assoc J. 2024;18(2):25–31. https://doi.org/10.5489/cuaj.8455.

199. Ma C, Chen W. Where are we and how far is there to go in the development of an Acinetobacter vaccine? Expert Rev Vaccines. 2021;20(3):281–95. https://doi.org/10.1080/14760584.2021.1887735.

200. Tan YC, Lahiri C. Promising *Acinetobacter baumannii* vaccine candidates and drug targets in recent years. Front Immunol. 2022;13: 900509. https://doi.org/10.3389/fimmu.2022.900509.

201. Yang N, Jin X, Zhu C, et al. Subunit vaccines for *Acinetobacter baumannii*. Front Immunol. 2023;13:1088130. https://doi.org/10.3389/fimmu.2022.1088130.

202. Jahangiri A, Owlia P, Rasooli I, et al. Specific egg yolk immunoglobulin as a promising non-antibiotic biotherapeutic product against *Acinetobacter baumannii pneumonia* infection. Sci Rep. 2021;11(1):1914. https://doi.org/10.1038/s41598-021-81356-8.

203. Pazoki M, Darvish Alipour Astaneh S, Rameznalizadeh F, et al. Immunoprotectivity of valine-glycine repeat protein G, a potent mediator of pathogenicity, against Acinetobacter baumannii. Mol Immunol. 2021;135:276–84. https://doi.org/10.1016/j.molimm.2021.04.026.

204. Mesbahi Moghaddam M, Rasooli I, Ghaini MH, et al. Immunoprotective characterization of egg yolk immunoglobulin raised to loop 3 of outer membrane protein 34 (Omp34) in a murine model against *Acinetobacter baumannii*. Mol Immunol. 2022;149:87–93. https://doi.org/10.1016/j.molimm.2022.06.010.

205. Raoufi Z, Abdollahi S, Armand R. DcaP porin and its epitope-based subunit promise effective vaccines against *Acinetobacter baumannii*; in-silico and in-vivo approaches. Microb Pathog. 2022;162: 105346. https://doi.org/10.1016/j.micpath.2021.105346.

206. Zhu Z, Zhou Z, Zhu T, et al. Klebsiella pneumoniae ghosts serve as a novel vaccine formulation to enhance immune responses of *Acinetobacter baumannii* subunit vaccine in mice. Microb Pathog. 2025;199: 107226. https://doi.org/10.1016/j.micpath.2024.107226.

207. Singh R, Capalash N, Sharma P. Vaccine development to control the rising scourge of antibiotic-resistant *Acinetobacter baumannii*: a systematic review. 3 Biotech. 2022;12(3):85. https://doi.org/10.1007/s13205-022-03148-9.

208. Gellings PS, Wilkins AA, Morici LA. Recent advances in the pursuit of an effective *Acinetobacter baumannii* vaccine. Pathogens. 2020;9(12):1066. https://doi.org/10.3390/pathogens9121066.

209. Piri-Gharaghie T, Doosti A, Mirzaei SA. Identification of antigenic properties of *Acinetobacter baumannii* proteins as novel putative vaccine candidates using reverse vaccinology approach. Appl Biochem Biotechnol. 2022;194(10):4892–914. https://doi.org/10.1007/s12010-022-03995-5.

210. Rosini R, Nicchi S, Pizza M, et al. Vaccines against antimicrobial resistance. Front Immunol. 2020;11:1048. https://doi.org/10.3389/fimmu.2020.01048. (**Erratum in: Front Immunol. 11: 1578. 10.3389/fimmu.2020.01578**).

211. Weng T, Chen G, Li N, et al. Identifying the in vivo-induced antigenic genes is a strategy to develop DNA vaccine against Nocardia seriolae in hybrid snakehead (Channa maculata ♀ × Channa argus ♂). Fish Shellfish Immunol. 2024;147: 109410. https://doi.org/10.1016/j.fsi.2024.109410.

212. Mayer RL, Verbeke R, Asselman C, et al. Immunopeptidomics-based design of mRNA vaccine formulations against Listeria monocytogenes. Nat Commun. 2022;13(1):6075. https://doi.org/10.1038/s41467-022-33721-y.

213. Callaway E. What's next for AlphaFold and the AI protein-folding revolution. Nature. 2022;604(7905):234–8. https://doi.org/10.1038/d41586-022-00997-5.

214. Schmitz S, Schmitz EA, Crowe JE Jr, et al. The human antibody sequence space and structural design of the V, J regions, and CDRH3 with Rosetta. MAbs. 2022;14(1):2068212. https://doi.org/10.1080/19420862.2022.2068212.

215. Abramson J, Adler J, Dunger J, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature. 2024;630(8016):493–500. https://doi.org/10.1038/s41586-024-07487-w.

216. Nasir S, Anwer F, Ishaq Z, et al. VacSol-ML(ESKAPE): machine learning empowering vaccine antigen prediction for ESKAPE pathogens. Vaccine. 2024;42(22): 126204. https://doi.org/10.1016/j.vaccine.2024.126204.

217. De Groot AS, Moise L, Terry F, et al. Better epitope discovery, precision immune engineering, and accelerated vaccine design using immunoinformatics tools. Front Immunol. 2020;11:442. https://doi.org/10.3389/fimmu.2020.00442.

218. Russo PL, Shaban RZ, Macbeth D, et al. Impact of electronic healthcare-associated infection surveillance software on infection prevention resources: a systematic review of the literature. J Hosp Infect. 2018;99(1):1–7. https://doi.org/10.1016/j.jhin.2017.09.002.

219. Wilson AM, Sleeth DK, Schaefer C, et al. Transmission of respiratory viral diseases to health care workers: COVID-19 as an example. Annu Rev Public Health. 2022;43:311–30. https://doi.org/10.1146/annurev-publhealth-052120-110009.

220. Jose A, Gizdavic-Nikolaidis M, Swift S. Antimicrobial coatings: reviewing options for healthcare applications. Appl Microbiol. 2023;3(1):145–74. https://doi.org/10.3390/applmicrobiol3010012.

221. Kramer A, Assadian O. Use of biocidal surfaces for reduction of healthcare acquired infections. Cham: Springer; 2014.

222. Teoh TK, Powell J, Kelly J, McDonnell C, Whelan R, O'Connell NH, Dunne CP. Outcomes of point-of-care testing for influenza in the emergency department of a tertiary referral hospital in Ireland. J Hosp Infect. 2021;110:45–51. https://doi.org/10.1016/j.jhin.2021.01.004.

223. Weber DJ, Rutala WA, Miller MB, et al. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridioides difficile*, and Acinetobacter species. Am J Infect Control. 2010;38(5 Suppl 1):S25-33. https://doi.org/10.1016/j.ajic.2010.04.196.

224. Huslage K, Rutala WA, Gergen MF, et al. Microbial assessment of high-, medium-, and low-touch hospital room surfaces. Infect Control Hosp Epidemiol. 2013;34(2):211–2. https://doi.org/10.1086/669092.

225. Weber DJ, Rutala WA, Anderson DJ, et al. Effectiveness of ultraviolet devices and hydrogen peroxide systems for terminal room decontamination: focus on clinical trials. Am J Infect Control. 2016;44(5):e77–84. https://doi.org/10.1016/j.ajic.2015.11.015.

226. Benbow A, Clarke M, Yates C, et al. Hospital-wide healthcare-associated carbapenemase-producing Enterobacterales outbreak: risks of electric floor scrubbers in catering facilities and kitchens. J Hosp Infect. 2024;146:59–65. https://doi.org/10.1016/j.jhin.2024.01.016.

227. Mitchell BG, McDonagh J, Dancer SJ, et al. Risk of organism acquisition from prior room occupants: an updated systematic review. Infect Dis Health. 2023;28(4):290–7. https://doi.org/10.1016/j.idh.2023.06.001.

228. NHS England. National infection prevention and control manual (NIPCM) for England. Publication reference: PRN00908, Version 2.7, 25 October 2023. Accessed 20 Oct 2024.

229. Wu J, Thompson TP, O'Connell NH, McCracken K, Powell J, Gilmore BF, Dunne CP, Kelly SA. Extended-spectrum β-lactamase-producing bacteria from hospital wastewater pipes: isolation, characterization and biofilm control using common disinfectants. J Hosp Infect. 2025;156:34–49. https://doi.org/10.1016/j.jhin.2024.11.013.

230. Dunne CP, Askew PD, Papadopoulos T, Gouveia IC, Ahonen M, Modic M, Azevedo NF, Schulte S, Cosemans P, Kahru A, Murzyn K, Keevil CW, Riool M, Keinänen-Toivola MM, AMiCI Consortium. Antimicrobial coating innovations to prevent infectious disease: a consensus view from the AMiCl COST Action. J Hosp Infect. 2020;105(2):116–8. https://doi.org/10.1016/j.jhin.2020.04.006.

231. Deruelle T, Engeli I. The COVID-19 crisis and the rise of the European Centre for Disease Prevention and Control (ECDC). West Eur Polit. 2021;44(5–6):1376–400. https://doi.org/10.1080/01402382.2021.1930426.

232. Efstathiou P, Anagnostakou M, Kouskouni E, et al. O068: Implementation of antimicrobial copper in neonatal intensive care unit (NICU). Antimicrob Resist Infect Control. 2013. https://doi.org/10.1186/2047-2994-2-S1-O68.

233. Von Dessauer B, Navarrete MS, Benadof D, et al. Potential effectiveness of copper surfaces in reducing health care–associated infection rates in a pediatric intensive and intermediate care unit: a nonrandomized controlled trial. Am J Infect Control. 2016;44(8):e133–9. https://doi.org/10.1016/j.ajic.2016.03.053.

234. Dunne CP, Keinänen-Toivola MM, Kahru A, et al. Anti-microbial coating innovations to prevent infectious diseases (AMiCI): cost action ca15114. Bioengineered. 2017;8(6):679–85. https://doi.org/10.1080/21655979.2017.1323593.

235. Butler JP. Effect of copper-impregnated composite bed linens and patient gowns on healthcare-associated infection rates in six hospitals. J Hosp Infect. 2018. https://doi.org/10.1016/j.jhin.2018.05.013.

236. Cheng L, Low SY, Boon Y, Goh C, Ng A, Ng AJY, et al. Antimicrobial surface coating in the emergency department as protective technology for infection control (ASEPTIC): a pilot randomized controlled trial. Antimicrob Resist Infect Control. 2024;13:129. https://doi.org/10.1186/s13756-024-01481-7.

237. NHS Saving Lives—Quality assurance and audit tools. Medical Audits. Accessed 5 Nov 2024.

238. Labarraque AG, Porter J. Instructions and observations concerning the use of the chlorides of soda and lime. 1831.

239. Semmelweis I. Etiology, concept, and prophylaxis of childbed fever. 1st Carter KC. Madison: The University of Wisconsin Press; 1983.

240. Lane HJ, Blum N, Fee E. Oliver Wendell Holmes (1809–1894) and Ignaz Philipp Semmelweis (1818–1865): preventing the transmission of puerperal fever. Am J Public Health. 2010;100(6):1008–9. https://doi.org/10.2105/AJPH.2009.185363.

241. Rickard H, Cloutman-Green E, Ciric L. A microbiological survey approach to understanding the virulence factors of *Pseudomonas* species in healthcare sinks. J Hosp Infect. 2024;151:84–91. https://doi.org/10.1016/j.jhin.2024.06.012.

242. Kingston LM, O'Connell NH, Dunne CP. Survey of attitudes and practices of Irish nursing students towards hand hygiene, including handrubbing with alcohol-based hand rub. Nurse Educ Today. 2017;52:57–62. https://doi.org/10.1016/j.nedt.2017.02.015.

243. Kingston LM, O'Connell NH, Dunne CP. A comparative study of hand hygiene and alcohol-based hand rub use among Irish nursing and medical students. Nurse Educ Today. 2018;63:112–8. https://doi.org/10.1016/j.nedt.2018.01.022.

244. François B, Cariou A, Clere-Jehl R, et al. Prevention of early ventilator-associated pneumonia after cardiac arrest. N Engl J Med. 2019;381(19):1831–42. https://doi.org/10.1056/NEJMoa1812379.

245. Meesters K, Chappell F, Demirjian A. Trends in antibiotic use in a large children's hospital in London (United Kingdom): 5 years of point prevalence surveys. Antibiotics (Basel). 2024;13(2):172. https://doi.org/10.3390/antibiotics13020172.

246. Sabbatucci M, Ashiru-Oredope D, Barbier L, et al. Tracking progress on antimicrobial resistance by the quadripartite country self-assessment survey (TrACSS) in G7 countries, 2017–2023: opportunities and gaps. Pharmacol Res. 2024;204: 107188. https://doi.org/10.1016/j.phrs.2024.107188.

247. Marschollek M, Marquet M, Reinoso Schiller N, et al. Automatisierte Surveillance und Risikovorhersage mit dem Ziel einer risikostratifizierten Infektionskontrolle und -prävention (RISK Prediction for Risk-stratified Infection Control and Prevention). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2024;67(6):685–92. https://doi.org/10.1007/s00103-024-03882-w. (**German**).

248. Magnano San Lio R, Favara G, Maugeri A, et al. How antimicrobial resistance is linked to climate change: an overview of two intertwined global challenges. Int J Environ Res Public Health. 2023;20(3):1681. https://doi.org/10.3390/ijerph20031681.

249. McGough SF, MacFadden DR, Hattab MW, et al. Rates of increase of antibiotic resistance and ambient temperature in Europe: a cross-national analysis of 28 countries between 2000 and 2016. Euro Surveill. 2020;25:1900414. https://doi.org/10.2807/1560-7917.ES.2020.25.45.1900414.

250. van Bavel B, Berrang-Ford L, Moon K, et al. Intersections between climate change and antimicrobial resistance: a systematic scoping review. Lancet Planet Health. 2024;8(12):e1118–28. https://doi.org/10.1016/S2542-5196(24)00273-0.

251. Philipsborn R, Ahmed SM, Brosi BJ, et al. Climatic drivers of diarrheagenic *Escherichia coli* incidence: a systematic review and meta-analysis. J Infect Dis. 2016;214:6–15. https://doi.org/10.1093/infdis/jiw081.

252. Kusi J, Ojewole CO, Ojewole AE, et al. Antimicrobial resistance development pathways in surface waters and public health implications. Antibiotics. 2022;11:821. https://doi.org/10.3390/antibiotics11060821.

253. Kaba HEJ, Kuhlmann E, Scheithauer S. Thinking outside the box: association of antimicrobial resistance with climate warming in Europe—a 30

country observational study. Int J Hyg Environ Health. 2020;223(1):151–8. https://doi.org/10.1016/j.ijheh.2019.09.008.

254. Paynter S, Ware RS, Weinstein P, et al. Childhood pneumonia: a neglected, climate-sensitive disease? Lancet. 2010;376(9755):1804–5. https://doi.org/10.1016/S0140-6736(10)62141-1.

255. Canela LNP, Magalhães-Barbosa MC, Raymundo CE, et al. Viral detection profile in children with severe acute respiratory infection. Braz J Infect Dis. 2018;22(5):402–11. https://doi.org/10.1016/j.bjid.2018.09.001.

256. De Jongh EJ, Harper SL, Yamamoto SS, et al. One Health, One Hive: a scoping review of honey bees, climate change, pollutants, and antimicrobial resistance. PLoS ONE. 2022;17(2): e0242393. https://doi.org/10.1371/journal.pone.0242393.

257. Pavia G, Branda F, Ciccozzi A, et al. The issue of climate change and the spread of tropical diseases in Europe and Italy: vector biology, disease transmission, genome-based monitoring and public health implications. Infect Dis (Lond). 2025;57(2):121–36. https://doi.org/10.1080/23744235.2024.2437027.