

Review Article

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Emerging Antibiotic Resistance in Opportunistic Microorganisms: Integrated Surveillance and Next-Generation Therapeutics

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ABSTRACT

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Antimicrobial resistance (AMR) in opportunistic microorganisms is a growing global health concern, especially in hospitalized and immunocompromised persons. These pathogens display a variety of resistance strategies, such as efflux pump activity, target modification, and enzymatic inactivation. Resistance development is accelerated by contributing factors such as improper antibiotic use, extended hospital stays, and insufficient infection control. Treatment results are further complicated by the emergence of extensively drug-resistant (XDR), pan-drug-resistant (PDR), and multidrug-resistant (MDR) pathogens worldwide. Early detection has been enhanced by developments in surveillance and quick testing, but there are still few treatment choices. Promising options include CRISPR-based methods, phage treatment, and antimicrobial peptides. To slow the emergence of resistance, it is essential to improve antimicrobial stewardship and infection control procedures.

Introduction

The ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to survive and keep growing despite the presence of medications intended to eradicate them is known as the process of antimicrobial resistance. Antimicrobial agents come in a variety of forms, such as antibiotics, antifungals, antivirals, disinfectants, and food preservatives, which either kill or inhibit the growth and multiplication of microorganisms. Antibiotics are a class of antimicrobials that are used far more frequently than any other class to treat bacterial infections (Md. Abdus Salam *et al.*, 2023). Methicillin-resistant *Staphylococcus aureus* (MRSA), a well-known example of the first "superbug," is related to a significant death rate from antimicrobial-resistant illnesses worldwide. Currently, MDR-TB (multidrug-resistant tuberculosis) contributes to 3.5% of active TB infections and 18% of previously treated TB patients globally. Among many MDR-TB cases, there is growing concern for XDR-TB (extensively drug-resistant tuberculosis). Microorganisms like bacteria, fungi, viruses, and parasites that normally do not cause disease in healthy individuals but can become pathogenic when the host's immune system is compromised are known as opportunistic infections (OIs). OIs, intestinal parasites and bacteria that more readily infect immunocompromised patients, are responsible for more than 80% of AIDS (acquired immunodeficiency syndrome)-related deaths. There were 39.9 million HIV-positive individuals worldwide in 2023, and 630,000 people died from HIV-related causes, including 1.4 million children (under the age of 15) (Aleka Aemiro *et al.*, 2025). In Europe, East China, and other low- and middle-income countries, the most common causes of hospital admissions among all HIV-related OIs for children living with HIV on antiretroviral therapy (ART) are pneumonia and tuberculosis (TB). Bacteria, fungi, and, on occasion, protozoa are examples of opportunistic microorganisms that exploit host weaknesses. *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecium* are among the most common bacterial opportunists. Fungal opportunists including *Candida albicans*, *Candida auris*, and *Aspergillus fumigatus* are also becoming more prevalent in clinical settings. These bacteria are linked to ventilator-associated pneumonia, bloodstream infections, urinary tract infections, catheter-related infections,

surgical site infections, meningitis, and invasive fungal diseases.

Integrated approaches are desperately needed to address this growing crisis. Strong surveillance systems, quick lab testing, antimicrobial stewardship initiatives, stringent infection control procedures, and the creation of innovative treatments are a few of these. Future therapeutic paradigms may be altered by cutting-edge strategies including bacteriophage therapy, nanomedicine, CRISPR-based antimicrobials, and AI-guided drug discovery. This review article main focus on emergence of antibiotic resistance in opportunistic microorganisms, key resistance mechanisms, epidemiological trends, surveillance tactics, diagnostic advancements, existing treatments, and next-generation control methods.

Opportunistic microorganisms of clinical importance

Staphylococcus aureus, an asymptomatic colonizer of human nares, chronically colonizes about 30% of individuals. In addition, it is the primary cause of infections of the skin and soft tissues, particularly in those who have already been colonized. Although local skin infections typically resolve on their own, they can occasionally serve as a gateway for this pathogen to enter deeper tissues and the circulation. *Staphylococcus* infections can occur as an opportunistic infection in people with heart conditions, diabetes, and HIV patients.

Sepsis, a systemic inflammatory reaction to infection, can develop as a result of *S. aureus* in the blood stream which is known as (bacteremia). The paradoxical immunosuppressive response, which occasionally occurs together with inflammation, is a common sign of sepsis (Jakub M Kwiecinski *et al.*, 2020). *Pseudomonas* (Gamma-Proteobacteria subclass, Pseudomonadales order, Pseudomonadaceae family) are non-sporulating rods with motility (one or multiple polar agella), a Gram-negative bacteria and a GC content of 58-69%. They are the most diversified class of proteobacteria (Isoken H. Igbiosa *et al.*, 2015).

A frequent opportunistic pathogen that frequently causes nosocomial and community-acquired illnesses is *Pseudomonas aeruginosa*. Moreover, up to 70% of adult CF patients have *P. aeruginosa* infections, making it a major cause of chronic lung infections in CF patients.

Additionally, it is mentioned that the quick spread of carbapenem-resistant *P. aeruginosa* (CRPA) poses a serious risk in clinical settings, jeopardizing the few available treatments (Yu Shi *et al.*, 2023).

The gastrointestinal tract microbiome of healthy humans and animals contains *Klebsiella pneumoniae*, a member of the Enterobacteriaceae family. Gram-negative, encapsulated, non-motile, rod-shaped *Klebsiella* bacteria. Hospitals are often host to this opportunistic pathogen. Approximately one-third of all Gram-negative infections are caused by it. However, it can also result in other extra-intestinal diseases, including endocarditis and septicemia, as well as potentially fatal infections including pneumonia and septic shock. It is one of the most frequently identified organisms in intensive care units (ICUs) and can cause pneumonia, UTIs, and surgical site infections. Treatment for *K. pneumoniae* has become increasingly challenging due to the rise in antibiotic-resistant strains. As a result, this microorganism is now recognized as a nosocomial infection that has the potential to be devastating and cause a pandemic. Furthermore, because they complicate treatment and raise morbidity, mortality, hospital stays, and healthcare costs, multidrug-resistant (MDR) bacteria like *K. pneumoniae* provide serious challenges to global healthcare systems (Jehad A. Aldali *et al.*, 2025).

C. auris, like other fungi, is a eukaryotic, heterotrophic, and primarily aerobic organism with chitin in its cell walls. This organism is commonly found around organic debris. *Candida auris* has emerged as a serious issue in critical care medicine due to the growing number of immunocompromised patients, and candidiasis is the most common cause of fungal infection. Candidemias are the most deadly infections in individuals with immunosuppression or major underlying conditions, including diabetes mellitus, chronic renal disease, HIV infection, solid tumors and hematological malignancies, and neutropenia are few cases have even been observed in infants (Maria Luisa Cristina *et al.*, 2023). Aspergilli are saprophytes that thrive on rotting plant material. They may use a wide range of organic substrates and adapt to several kinds of environmental conditions. *Aspergillus fumigatus* is currently the most common airborne fungal pathogen, causing a variety of diseases depending on the host's immune system response. The majority of cases of invasive aspergillosis are related with haematological malignancies, specifically haematopoietic stem cell transplantation, leukemia, or lymphoma. Patients with prolonged neutropenia or graft-versus-host disease are at a greater chance of invasive

Aspergillus infection (Allison McCormick *et al.*, 2010). Gram-negative bacillus *Acinetobacter baumannii* is pleomorphic, aerobic, and non-motile. *A. baumannii* is an opportunistic infection that is highly prevalent in immunocompromised people, especially those who have spent more than ninety days in the hospital. It has been demonstrated to colonize the skin and to be isolated in large quantities from the respiratory and oropharyngeal secretions of sick patients. It is frequently linked to aquatic habitats. *Acinetobacter baumannii* is mostly responsible for nosocomial bloodstream infections, hospital-acquired pneumonia, and ventilator-associated pneumonia (VAP). *Acinetobacter* is a well-known burn unit pathogen that is challenging to treat in patients with serious burns (Aoife Howard *et al.*, 2012).

Mechanisms of antibiotic resistant

All of the offspring cells of a bacteria that is resistant to a particular antimicrobial agent would also be resistant, unless other mutations took place in the interim. Bacterial cells that are resistant to the medication yet lack resistance genes are referred to as persistent. The persistence is definitely caused by the possibility that certain bacterial cells remain latent, or in a stationary growth phase, and the majority of antimicrobial drugs have no effect on cells that are not actively proliferating and dividing. The frequency of these persister cells in a stationary phase culture is roughly 1%. Antimicrobial resistance mechanisms are grouped into four categories: (1) restricting drug absorption, (2) altering drug targets, (3) inactivating a drug, and (4) active drug efflux. Gram-negative bacteria use all four major processes, but gram-positive bacteria use limited drug absorption (they lack an LPS outer membrane) and do not have the capacity for certain types of drug efflux pathways (Scott T Chancey *et al.*, 2012). Conjugation, transposition, and transformation are a few acquired resistance mechanisms. The most frequent method of acquiring external genetic material is by plasmid-mediated resistance gene transmission; bacteriophage-borne transmission is relatively uncommon. Because they are natively competent, some bacteria, like *Acinetobacter* species, can directly get genetic material from their external surroundings. Genetic material may be moved internally via insertion sequences and integrins, and genetic changes are frequently brought on by stressors on the bacteria, such as hunger, UV light, chemicals, etc (Bogdan-Ioan Coculescu *et al.*, 2009).

Modification of drug resistant- PBPs are

transpeptidases that help the cell wall form peptidoglycan. The number of drug that can bind to that target is affected by changes in the number of PBPs (either a drop in PBPs with normal drug binding or an increase in PBPs with decreased drug binding ability). A structural alteration (such as PBP2a in *S. aureus* due to the acquisition of the *mecA* gene) may reduce or completely prevent drug binding. Drugs that target the ribosomal subunits may lead resistance through ribosomal mutation (oxazolidinones, aminoglycosides), ribosomal subunit methylation (aminoglycosides, macrolides), and gram positive bacteria (oxazolidinones, streptogramins), most often involving *erm* genes (Wanda C Reygaert *et al.*, 2018).

Efflux pumps- Drug efflux pumps, which are integral membrane transporters, mediate the process of active efflux in bacteria that exhibit resistance to inhibitory chemicals like antibiotics. Ag^{2+} , Cu^{2+} , Co^{2+} , Zn^{2+} , Cd^{2+} , and Ni^{2+} are among the metal ions that efflux pumps can export to provide heavy metal resistance. Efflux pumps aid in lowering both the cytoplasmic and periplasmic concentrations of heavy metal ions in Gram-negative bacteria; since periplasmic metal ions can re-enter the cytoplasm, several efflux pumps cooperate to avoid heavy metal toxicity. Depending on the energy source they use to pump out the substrates, efflux pumps are divided into primary and secondary categories. The ATP-binding cassette (ABC) superfamily, major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE), resistance nodulation cell division (RND) family, small multidrug resistance (SMR) family, and proteobacterial antimicrobial compound efflux (PACE) are the six foremost families of efflux pumps that have been identified in bacteria (Sanath Kumar *et al.*, 2013).

Drug inactivation- Bacteria can inactivate drugs in two primary ways: either by transferring a chemical group to the drug or by actually degrading the molecule. A wide class of enzymes that hydrolyze drugs is known as β -lactamases. Tetracycline is another medication that can be rendered inactive by hydrolysis through the *tetX* gene. Acetyl, phosphoryl, and adenylyl groups are the most frequently transferred chemical groups used in drug inactivation. The most versatile mechanism is acetylation, which is known to be effective against fluoroquinolones, streptogramins, aminoglycosides, and chloramphenicol. It has been identified that aminoglycosides are the main targets of phosphorylation and adenylylation (Wubetu Yihunie Belay *et al.*, 2024).

β -lactamase- The β -lactamases, formerly known as cephalosporinases and penicillinases, hydrolyze a particular location in the β -lactam ring structure to cause the ring to open, rendering β -lactam medicines inactive. The target PBP proteins cannot be bound by the opening medications. The group of enzymes known as β -lactamases is widely distributed and has the capacity to render any of the existing β -lactam drugs inactive. The most prevalent defense strategy employed by gram-negative bacteria against β -lactam medications and the most significant defense mechanism against penicillin and cephalosporin medications is the development of β -lactamases. All four structural families of β -lactamases can be produced by gram-negative bacteria. Gram-positive bacteria primarily include β -lactamases from group A, with a small number from group B. These enzymes can be obtained through a plasmid or are naturally present on the bacterial chromosome. Most gram-negative bacteria belonging to the Enterobacteriaceae family have chromosomal β -lactamase genes. *Aeromonas species*, *Acinetobacter species*, and *Pseudomonas species* are other gram-negative bacteria that have these. The Enterobacteriaceae are the most prevalent group of bacteria that carry β -lactamase genes on plasmids (Maria Sargianou *et al.*, 2025).

Drivers and risk factors for resistance development

Antibiotic use is the primary cause of antibiotic resistance. Some bacteria are killed by antibiotics, while resistant bacteria can endure and even grow. Antibiotic usage increases the number of cases of resistant bacteria. The likelihood that microorganisms will develop resistance to antibiotics increases each time we use more antibiotics medicines as a result antibiotics won't be effective in the future. Antibiotics may once again more be efficient in killing bacteria if antibiotics used reduced.

Antibiotics work only for bacterial infections don't work for viral infection like cold, flu. Hand cleanliness is the fundamental method for preventing the transmission of illnesses, even those resistant to antibiotics (Madeline E. Graham *et al.*, 2025). Animal-related practices include the widespread use of antibiotics in livestock and aquaculture, transmission through food chains, and direct contact. Factors associated with wildlife, such as habitat encroachment and transmission through interaction with wildlife.

Animals undoubtedly contribute to the spread of antimicrobial resistance (AMR) because human antimicrobials are utilized in animals both therapeutically and as growth promoters, which has a major impact on the development of AMR. Globalization, international travel and interchange, and interaction with wildlife are other factors that promote the spread of AMR in animals. In the meantime, many ABR and ARGs that can be shared by humans, animals, and the environment have been caused by the improper or excessive use of antibiotics in animal husbandry. People can contract these ARBs and ARGs that have surfaced on farms by inhaling bioaerosol that contains them or by direct or indirect contact with animals, manure, or products (Habtamu Endale *et al.*, 2023). Drug resistance can arise by abuse of medications, including self-medication, over prescription, antibiotic administration without a prescription, patient prospects, and liability pressure. By eliminating the susceptible bacteria, these procedures encourage the survival and growth of antimicrobial-resistant bacteria, which results in the development of AMR. Antimicrobials can eradicate helpful bacteria that shield our bodies from diseases, but they can also kill some pathogenic bacteria. Because of this, the remaining antimicrobial-resistant microbes continue to exist and proliferate, transferring resistance features to other germs through their DNA. The problem of AMR is further exacerbated by the overuse of broad-spectrum antibiotics due to delayed or incorrect identification of the ailment's origin. One of the ways that antibiotics can spread in water and other areas of the environment where microorganisms can come into contact is through the careless disposal and/or handling of unused medications. This includes the discharge of sewage from urban areas, industries, animal husbandry, plant production, and homes. There are a lot of unused or expired medications since many items that are provided to patients or clients are not used to their full potential. Pharmaceutical inappropriate disposal is a serious problem since users frequently flush or discard their unwanted and expired medications because they don't know how to properly dispose of them. This ultimately gets up in drains, landfills, and water sources, contaminating the surrounding area.

Epidemiology and global trends

Bacteria classified as XDR are epidemiological relevant due to their resistance to several antimicrobial drugs, with a high possibility of being resistant to all or almost

all approved antimicrobial agents. In medical literature, XDR refers to many phrases, including 'extreme drug resistance', 'extensive drug resistance', 'very drug resistant', and 'extensively drug resistant'. XDR MTB was initially defined as resistance to first-line agents isoniazid and rifampicin, a fluoroquinolone, and one of three second-line parenteral drugs (amikacin, kanamycin, or capreomycin) (Muluneh Assefa *et al.*, 2025). The term "pandrug resistant" (PDR) refers to resistance to all antimicrobial agents, derived from the Greek prefix "pan" meaning "all." The term "PDR" refers to a bacterial isolate or species that has been screened and found to be resistant to all approved and helpful treatments. Current criteria include resistant to practically all commercially available antimicrobials and resistant to all antimicrobials commonly evaluated. MDR refers to resistance to several antimicrobial agents (classes or subclasses). The most common criterion for Gram-positive and Gram-negative bacteria that is MDR is resistance to three or more antimicrobial classes (A.-P. Magiorakos *et al.*, 2012). *Acinetobacter baumannii*, carbapenem, and third-generation cephalosporin-resistant Enterobacterales are listed as critical in the WHO's 2024 priority bacterial pathogens list. *Staphylococcus aureus*, *Pseudomonas*, *Enterococcus faecium*, *Shigella species*, and *Salmonella species* are considered high priority pathogens.

HCAIs- Health care-associated infections (HCAIs) are diseases that individuals contract while receiving healthcare. The term "HCAIs" used to refer to infections associated with acute-care hospital admissions (previously known as nosocomial infections). However, it now encompasses infections acquired in a variety of healthcare settings, including long-term care, family medicine clinics, home care, and ambulatory care. HCAIs are infections that emerge 48 hours or more after hospitalization or within 30 days of receiving medical care. *S. aureus*, *Enterococcus species* (e.g., *faecalis*, *faecium*), *E. coli*, *coagulase-negative Staphylococci*, *Candida species* (e.g., *albicans*, *glabrata*), *K. pneumoniae* and *Klebsiella oxytoca*, *P. aeruginosa*, *A. baumannii*, *Enterobacter species*, *Proteus species*, *Yeast NOS*, *Bacteroides species*, and other pathogens (Mainul Haque *et al.*, 2018). The most common kinds of HCAIs were lower respiratory tract infections (LRTIs), bloodstream infections, UTIs, SSIs, and systemic infections. SSIs (previously known as "wound infections") remain one of the most prevalent adverse events in hospitalized patients undergoing surgery or in outpatient surgical measures, despite advances in

preventive methods. SSI is the most prevalent complication in postoperative surgery patients, resulting in severe both morbidity, and mortality high rates. SSIs are infections that develop up to 30-90 days after surgery in individuals who have received an organ, group of cells, or gadget and impact both the incisional site and deeper tissues surrounding the operation area (Ahmad Reza Rezaei, MD *et al.*, 2025). UTIs are the most frequent HCAs and one of the top infections, causing approximately 40% of all HCAs, with severe consequences for both morbidity and mortality rate. Prolonged catheter use, female sex, advanced age, diabetes mellitus, lack of systemic antibiotics, catheter placement outside of the operating room, and a breach in the closed system of catheter drainage are among the risk variables for CAUTIs, according to multivariate analysis. Pneumonia affects almost 25% of patients in intensive care units, making it the second most prevalent HCAI. VAP and motorized automated ventilation are linked to about 86% of HCAs. This type of pneumonia affects 9% to 27% of individuals on assisted breathing. After endotracheal intubation and mechanical ventilation, the average critical time to develop VAP was two to three days. Patients typically experience fever, altered bronchial sounds, decreased white blood cell counts, and sputum alterations. *S. aureus* (44%) was the most commonly bacteria identified in VAP patients, followed by *A. baumannii* (30%), *P. aeruginosa* (12%), *Stenotrophomonas maltophilia* (7%), *K. pneumoniae* (6%), and *Serratia marcescens* (2%) (Carlos Cabanillas Diez-Madroño *et al.*, 2025).

Surveillance strategies for antibiotic resistance

The hospital antibiogram is periodic updates of the antimicrobial susceptibilities of local bacterial isolates submitted to the hospital's clinical microbiology laboratory. Clinicians frequently utilize antibiograms to determine local susceptibility rates, as a tool for determining presumptive antibiotic therapy, and to track resistance changes within an institution over time. Antibiograms can also be used to compare susceptibility rates between institutions and monitor resistance trends (S Joshi 2010). The data should be examined on an annual basis. However, if there are a big number of isolates, this could be done six months or more frequently. The analysis should include at least 30 isolates.

Only isolates obtained from diagnostic tests should be included, while those obtained via surveillance cultures,

such as MRSA screening, should be excluded. Include the results of antibiotics that are routinely tested. Only the first isolate from each patient, regardless of specimen site, should be included. The cumulative antibiogram should only show the percentage susceptible, not those who are intermediately susceptible. It is beneficial to categorize the antibiogram into outpatient, inpatient, and ICU data. The Global Antimicrobial Resistance and Use Surveillance System is one of the cornerstones of the WHO's global action plan for AMR evidence base. In 2015, the WHO announced its first international cooperative effort to enhance and regulate AMR surveillance. When it was first implemented between 2015 and 2019, it solely tracked and reported AMR rates and trends in common bacteria. By the conclusion of the first data call in 2017, 42 countries had signed up, and by the first data call in 2020, that number had increased to 126. The first worldwide AMC data, which included 65 participating nations, was released in 2018 and was reported independently. The 2022 GLASS report was the first to report AMR and AMC data simultaneously (Jaya Ranjalkar *et al.*, 2019). The WHO noted increased levels of AMR in the 2022 GLASS report, especially in the bloodstream infection caused by *Klebsiella pneumoniae* infections are more resistant to third-generation cephalosporins. the third most common pathogen that causes bloodstream infections, which has led to an increase in the use of last-resort carbapenems, a global spread of *Enterobacterales* that are resistant to carbapenems, and high rates of aminoglycoside and carbapenem resistance in *Acinetobacter* species, all of which are extremely concerning because carbapenem-resistant strains are frequently MDR and have been linked to treatment failures (Samuel Ajulo *et al.*, 2024). An analytical method called mass spectrometry involves ionizing chemical substances into charged molecules and measuring the mass to charge ratio (m/z). Despite being discovered in the early 1900s, MS was only used in the chemical sciences. However, the 1980s saw the advent of matrix aided laser desorption ionization (MALDI) and electron spray ionization (ESI), which made MS more applicable to big biological molecules like proteins. It is useful for identifying toxins and contaminating organisms (Neelja Singhal *et al.*, 2015). The SARS-CoV-2 pandemic highlighted the potential and significance of genomic monitoring for pathogen surveillance and outbreak response. The enormous success of international SARS-CoV-2 sequencing initiatives, which have produced over 17 million genomes, is a testament to the teamwork and commitment of the scientific and public health

communities. In addition to highlighting the benefits of pathogen intelligence collecting, genomic epidemiology will continue to change the landscape of public health and epidemic response.

Diagnostic advances in detecting resistant pathogens

Conventional manual antimicrobial susceptibility tests (ASTs) are labor-intensive and time-consuming, and human error may occur during manual stages like preparing the antimicrobial solution. Automated AST systems have been widely used in an effort to minimize the time and effort needed to examine microorganisms for several antimicrobials at once. Conventional culture-dependent AST techniques, which necessitate colony isolation and identification in order to produce MIC findings, can extend exposure to ineffective or needlessly broad-spectrum drugs and cause delays in making the right therapeutic decisions. These delays are especially crucial in cases of severe infections, illnesses brought on by organisms resistant to drugs, or in patients who are critically ill and immunocompromised, as early medication optimization is substantially linked to better results and lower mortality. The fully automated MicroScan Walk Away plus Microbiology System provides the identification and/or AST of most bacterial species, including GP and GN bacteria (both fermenting and non fermenting). 40–96 microdilution trays can be incubated and analyzed using this sizable, self-contained incubator/reader equipment (Giorgia Piccinini *et al.*, 2026). The VITEK system, which began as an automated system for identification and AST in the 1970s, has developed into the VITEK 2 system, which, once a main inoculum has been generated and standardized, automatically completes all of the procedures needed for identification and AST. Every fifteen minutes, this system reads each test, enabling kinetic analysis. The optical system measures fluorescence, turbidity, and colorimetric signals using a multichannel fluorimeter and photometer (Marco Ligozzi *et al.*, 2002). PCR (Polymerase Chain Reaction) is a laboratory technique for producing millions to billions of copies of a specific DNA sequence. PCR involves selecting a part of the genome to be amplified using short synthetic DNA fragments known as primers. The segment is then amplified many times. Denaturation, annealing, and extension are the three primary stages in PCR. The accuracy and effectiveness of DNA amplification depend on each stage. RT-PCR is one type of PCR that is frequently used for gene expression research and virus detection. qPCR, digital

PCR, touchdown PCR, multiplex PCR, nested PCR, hot-start PCR, and long PCR are others types of PCR. PCR is used to screen for mutations and identify bacterial, viral, and fungal pathogens (Roshan kumar Dubey *et al.*, 2025). An analytical method called mass spectrometry involves ionizing chemical substances into charged molecules and measuring the mass to charge ratio (m/z). Despite being found in the early 1900s, MS was only used in the chemical sciences. However, the 1980s saw the advent of matrix aided laser desorption ionization (MALDI) and electron spray ionization (ESI), which made MS more applicable to big biological molecules like proteins. It is used to early detection of bacteria in blood cultures, urinary tract infections (UTIs), cerebrospinal fluids, respiratory tract infections, stool samples, etc. can be accomplished by MALDI-TOF MS. It has been demonstrated that MALDI-TOF MS is helpful in the early identification of bacterial pathogens that could pollute drinking water (Neelja Singhal *et al.*, 2015). POCT, or point-of-care testing, involves doing diagnostic tests outside of a lab. These tests are used to identify acute infections and chronic illnesses because of their prompt and accurate results. The significance of point-of-care testing using rapid antigen test (RAT) kits to diagnose COVID-19 in household settings was highlighted by the recent COVID-19 pandemic. Given the infectious nature of the virus, RAT kits had a respectable accuracy rate and helped minimize hospital visits during the epidemic. It should be mentioned that there are many different types of point-of-care testing instruments, including simple dipsticks with urine, portable devices like glucose meters, and even sophisticated molecular analyzers for infection detection. Home pregnancy tests, hemoglobin, fecal occult blood, quick strep, and many more, such prothrombin time/international normalized ratio (PT/INR) for people on the anticoagulant warfarin, are some of the most prominent point-of-care tests.

Current therapeutic approaches

Clinical bacterial infections are frequently treated with aminoglycoside antibiotics. AGAs are frequently used together with β -lactam antibiotics for severe hospital-acquired infections caused by multidrug-resistant organisms that causes sepsis, ventilator-associated pneumonia, and acquired pneumonia. One of the most popular medications for treating gram-negative bacteria, particularly *P. aeruginosa*, is polymyxin. On both polymyxin-sensitive and polymyxin-resistant *P. aeruginosa*, the combination of polymyxin and amikacin

demonstrated a synergistic antibacterial action. *P. aeruginosa* that is resistant to polymyxin and *P. aeruginosa* that is sensitive to it. Strong antibacterial activity and a prolonged duration of half-life make azithromycin the most often used macrolide in clinical practice. Azithromycin and AGAs are frequently used to treat *P. aeruginosa* infections, and their combination can lower each antibiotic's single dose (Nuoyan Wang *et al.*, 2022). Daptomycin is a lipopeptide antibiotic that is frequently used as a last resort to treat bacterial infections that are resistant to many antibiotics. It exhibits exceptional efficacy against Gram-positive pathogens. Initially discovered as a secondary metabolite generated by the Gram-positive oil bacteria, daptomycin A 10-membered cyclic lactone core and three exocyclic amino acids connected to a decanoyl fatty acid tail make up the lipodepsipeptide *Streptomyces roseosporus* 5,6. Daptomycin is negatively charged, in contrast to the majority of antibacterial lipopeptides, and its antimicrobial activity is dependent on binding Ca^{2+} ions, which lower the peptide's negative charge and promote oligomerization (Jessica A. Buttress *et al.*, 2025). Antibiotics belonging to the polymyxin category were identified in 1947 from *Bacillus polymyxa*. More than fifteen different types of polymyxins have been identified and isolated so far, with polymyxin B and polymyxin E (also called colistin) being the most well-known. Since polymyxins have a strong inhibitory effect on Gram-negative bacterial pathogens, they are now the best option for treating pathogens that have already developed resistance to frontline antibiotics including aminoglycosides, β -lactams, and fluoroquinolones. Polymyxins are therefore regarded as the antibiotic of last resort for dealing with MDR Gram-negative bacteria. Colistin and polymyxin B are primarily used to treat infections caused by MDR pathogens like *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*. The both antibiotics exhibit equivalent antimicrobial characteristics against Gram-negative bacterial species (SASWAT SMOHAPATRA *et al.*, 2021). Tigecycline is an antibacterial drug belongs to the tetracycline class that originated to treat polymicrobial MDR infections caused by both Gram-positive and Gram-negative bacteria. The Food and Drug Administration (FDA) approved tigecycline, also known as GAR-936 or Tygacil, in 2005. It is the first and only member of the glycylcycline class of semisynthetic medicines that is administered parenterally. The FDA later warned in 2010 that using tigecycline to treat sepsis and serious infections was strongly linked to a higher risk of dying from all causes. Tigecycline is now authorized as a

monotherapy for three indications in adults: community-acquired bacterial pneumonia (CAP), difficult intra-abdominal infections (cIAI), and problematic skin and skin structures infections (cSSTI), excluding diabetic foot infections. The last line of treatment for MDR bacterial infections, particularly carbapenem-resistant *Enterobacteriaceae*, is tigecycline. Tigecycline shown good effectiveness against penicillin-resistant *Streptococcus pneumoniae*, extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae*, *vancomycin-resistant enterococci (VRE)*, and *methicillin-resistant Staphylococcus aureus (MRSA)* (Sajad Yaghoubi *et al.*, 2022).

Triazoles, polyenes, echinocandins, and pyrimidine analogues are the four primary groups of antifungal medications now utilized in clinical practice to treat invasive fungal infections (IFDs). A crucial tactic for preventing fungal infections in high-risk, usually immunocompromised people, such as those receiving chemotherapy for hematologic cancers, recipients of transplants, or people living with HIV, is antifungal prophylaxis. It seeks to stop IFDs brought on by diseases such as *Aspergillus*, *Candida*, *Pneumocystis*, and *Cryptococcus* species. In patients with underlying risk and general clinical signs of infection (such as refractory to broad-spectrum antibiotic therapy), empirical antifungal therapy entails the use of broad-spectrum antifungal drugs based on concerns of fungal infection, but without (ideally while awaiting) a definitive diagnosis. Particularly in cases of severe or resistant fungal infections, combination therapy is essential for improving efficacy and lowering the risk of resistance. The combination of AmB and flucytosine for cryptococcal meningitis serves as an example of this combined therapy, as AmB damages fungal cell membranes while flucytosine increases fungal death. Azole-resistant *Aspergillo*sis can be defeated by combining an echinocandin, such as caspofungin, with an azole, such as fluconazole or voriconazole, which has been successful in treating IC (Norman Van Rhijn *et al.*, 2025).

Novel and emerging therapeutic strategies

Bacteriophages, sometimes known as phages, are bacterial viruses that enter bacterial cells and, in the case of lytic phages, cause the bacterium to lyse by interfering with its metabolism. Bacteriophages are microscopic viruses that lack a complete cell structure and only contain one nucleic acid as genetic

material. Panel B displays the three-dimensional structure of a type of *Staphylococcus aureus* as well as the structure of a phage. Protein capsids and nucleic acids, which mostly consist of single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA), are the main constituents of bacteriophages. A large number of bacteriophages found in nature are dsDNA bacteriophages. The protein shell, which also shields the interior nucleic acids from environmental degradation, determines the morphology and surface features of bacteriophages. In nature, phages are ubiquitous, diverse, and abundant—roughly ten times as many as bacteria. Antimicrobial therapy uses bacteriophages, which can be administered in a variety of ways to treat infectious infections in various systems. During phage therapy, oral, injectable, and local delivery can increase survival and offer protection in animal models. The particular method of administration is determined by the patient's condition as well as what is most effective and quick (Ling Zhao *et al.*, 2025). Bioactive macromolecules known as antimicrobial peptides (AMPs) have immunomodulatory, antiviral, and antibacterial properties. They arise from a variety of sources and are present in all living things, including bacteria, plants, vertebrates, and invertebrates. They are crucial for treating cancers, aiding wound healing, and preventing the spread of diseases. As a result, AMPs have become a viable substitute for antibiotics of the next generation. A type of oligopeptides known as AMPs is resistant to viruses and bacteria. Usually made up of 12–50 residues of amino acids. AMPs are widely utilized in food, medicine, and agriculture because they have broad-spectrum antibacterial abilities and can target various pathogens, reducing the development of drug-resistant strains. Hydrophobicity is important in the interaction with cell membranes because it determines the peptide's ability to partition into the membrane layer. AMPs typically have around 50% hydrophobic residues. Hydrophilic residues, including glycine, histidine, arginine, and aspartic acid. The primary components of AMPs and a factor in their environmental stability are these hydrophilic residues. The variety of AMPs' secondary structures, which include α -helix, β -sheet structures, and $\alpha\beta$ and non- $\alpha\beta$ conformations, is one of their most crucial characteristics. These configurations are necessary for AMPs to interact with and disrupt microbial membranes (XuMa *et al.*, 2024).

The exact elimination of specific bacterial populations using a CRISPR-Cas-based system shows promise as a

novel strategy for the development of novel antimicrobial agents. By specifically inactivating genes linked to antibiotic resistance, biofilm formation, pathogenicity, virulence, and bacterial viability, the CRISPR-Cas targeting system—which is renowned for its high adaptability and specificity—offers a great chance to combat antibiotic resistance in pathogens. By either curing plasmids that encode antibiotic resistance or inactivating chromosomal genes, the CRISPR-Cas method can have antimicrobial effects (AriannaMayorga-Ramos *et al.*, 2023). The arranged, intricate structure of microbial populations known as biofilm has raised concerns for public health. One of the primary ways that bacteria and fungi develop resistance to antibiotics is through the formation of biofilms. Infections, particularly those linked to in-dwelling medical devices, are significantly influenced by biofilms. In addition to biotic surfaces like human teeth, tissues, wounds, and lungs, they can also be found on the surfaces of medical implants including catheters, heart valves, and contact lenses. By altering a number of the organism's regulatory systems, the myxobacterium *Sorangium cellulosum*'s carolacton demonstrated anti-biofilm action against *Streptomyces mutans*. *Paenibacillus species* 139SI bioactive substances shown anti-biofilm properties against both Gram-positive and Gram-negative bacterial isolates (Ibrahim Mohammed Hussaini *et al.*, 2024). Antimicrobial Stewardship programme (AS) is an interprofessional endeavor that focuses on the best and most sensible use of antibiotics for patients in acute, inpatient, long-term care, and outpatient settings. By recognizing documented patterns and outbreaks of epidemiologically relevant organisms and training clinicians about infection prevention strategies, ASPs can help infection prevention and control (IPC) programs. To successfully identify the organism, assess its susceptibility, implement the required precautions, and provide the narrowest-acting antibiotic that will eradicate it, ASPs must use the skills of every member of the healthcare team (Julia Moody *et al.*, 2012).

Infection prevention and control measures

Disinfectants, sanitizing agents, and cleaning chemicals are used to control pathogens and ensure food safety in production facilities. These substances are collectively referred to as "biocides," and their practical application largely determines how they are subdivided into distinct groups, such as sanitizers and disinfectants. They could consist of particular formulations with one or more

active biocidal chemicals that attack bacterial cell structures indiscriminately. Quaternary ammonium compounds (QACs), benzalkonium chloride (BAC), chlorohexidine, chlorine and its derivatives, acid anionic agents, hydrogen peroxide, and peracetic acid are examples of active agents in commercially available biocides used in the food sector (JOHN ANTHONY DONAGHY *et al.*, 2019). There are many different kinds of disinfectants, each having special qualities and uses. The type of bacteria being targeted, the surface or material being disinfected, and the required level of efficiency all influence the disinfectant selection. Isopropanol with ethanol extensively utilized for skin disinfection, household surfaces, and surface disinfection in medical settings. Calcium hypochlorite and sodium hypochlorite (bleach) utilized in a variety of contexts for decontamination, surface disinfection, and water treatment. An important factor is the disinfectant solution's concentration plays important role while too much concentration can cause practical difficulties, safety issues, or material damage, too little concentration can lead to inadequate disinfection. Antimicrobial peptides are being investigated as possible disinfectants. AMPs are naturally occurring compounds with broad-spectrum antibacterial properties. The antibacterial qualities of nanoparticles, like silver nanoparticles, have been investigated and they can effectively interact with microbial structures because of their modest size (Gitesh Bhargava *et al.*, 2024).

Bacterial spores cannot be treated with antiseptics and must be manually removed using soap and water or scrubbing. Antiseptics often used in healthcare include chlorhexidine (CHG) (alone or in together with alcohol), alcohol (alone or in combination with CHG or an iodophor), and iodophors (alone or in combination with alcohol). Antiseptics (10% povidone iodine) were also utilized to clean the operating room floor with minimal cell viability loss after a bonegraft was dropped. "Hands are the main pathways of pathogen transmission during health care," according to the World Health Organization. Additionally, experts predict that proper hand cleanliness could prevent up to 30% of hospital-acquired diseases (James Rickert MD *et al.*, 2023). Vaccines can prevent AMR in a number of ways by boosting the immune system's ability to identify and combat particular invaders, such as bacteria and viruses. First, vaccines lower the prevalence of illnesses caused by drug-susceptible and drug-resistant microorganisms, which lowers the number of cases and fatalities as well as the financial burden of treating infections. Second,

after a first influenza infection, vaccinations can prevent subsequent infections such *Streptococcus pneumoniae*. An excellent example of how vaccination campaigns were used successfully as part of the outbreak response and can impact AMR across all the pathways mentioned is the use of typhoid conjugate vaccine (TCV) to stop the spread of *Salmonella Typhi*, both susceptible and resistant, in Pakistan and Zimbabwe (Mateusz Hasso-Agopsowicz *et al.*, 2024).

Challenges and future perspectives

AMR is one of the concerns that governments must address. Bacterial resistance has increased and susceptibility has decreased as a result of antibiotic abuse and overuse. As a result, medications and antibiotics are now less successful in treating bacterial infections and other illnesses. Many people may not know that they or their livestock may be suffering from a resistant sickness, and those who care for them often have no idea either. This implies that the problem is mostly unidentified and unreported. One persistent problem is the lack of representative AMR data, especially in LMICs. It is difficult to create a compelling national AMR narrative to increase public awareness, financial commitments, and political action because the actual extent of the issue and its possible outcomes are uncertain. Agricultural practices, waste and wastewater management, and pharmaceutical production systems may need to change in order to phase out the use of antimicrobial agents for animal growth enhancement and deploy pollution-control techniques. For the sake of both human health and the environment, ecosystem-based methods of reducing the use of antibiotics in plant cultivation must be controlled (Shikha Sharma *et al.*, 2024). The cost of treating resistant diseases is greatly increased due to prolonged hospital stays, increased medical consultations, and the need for expensive drugs as a final alternative. To improve our knowledge of antimicrobial resistance, multidisciplinary collaboration amongst microbiologists, pharmacologists, epidemiologists, social scientists, and policymakers is essential. In addition to surveillance and diagnostic research, the development of novel antimicrobial medications and alternative therapies is essential to fight against antibiotic resistance. Important developments in this field include discovering new molecular targets, refining existing antibiotics, and exploring unconventional therapeutic approaches like immunomodulation and bacteriophage

therapy. Promoting translational research is essential to speeding up the adoption of novel interventions by tying laboratory findings to practical applications (Sirwan Khalid Ahmed *et al.*, 2024).

In conclusion, Antimicrobial resistance in opportunistic microorganisms has developed as a major worldwide health concern, driven by widespread antibiotic exploitation, fast microbial adaptability, and a growing number of susceptible, immunocompromised individuals. The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) infections has significantly hindered treatment efficacy that resulting in longer hospital stays, higher healthcare expenses, and increased mortality. Opportunistic infections, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Candida species*, are especially concern because of their capacity to thrive in healthcare settings and develop resistance mechanisms. This emphasizes the critical need for comprehensive surveillance systems, enhanced epidemiological tracking, and rapid diagnostic tools to ensure the early detection and control of resistance illnesses.

Addressing this expanding threat necessitates a diverse and coordinated approach that includes antibiotic stewardship, infection prevention and control techniques, and the development of next-generation therapies. Advances in molecular diagnostics, new medication discovery, bacteriophage therapy, and immunomodulatory methods present promising paths for combating resistant pathogens. However, obstacles such as limited access to new therapies, insufficient global coordination, and the slow rate of antibiotic discovery remain.

To slow the emergence of resistance, we must strengthen healthcare infrastructure, promote responsible antibiotic use, and boost international collaboration. Continued research and innovation will be critical in sustaining antibiotic efficacy and ensuring long-term management of opportunistic infections.

Author Contributions

S. Arul Jothy: Investigation, formal analysis, writing—original draft. M. Prakash: Validation, methodology, writing—reviewing. S. Selvasuresh:—Formal analysis, writing—review and editing. K. Arivazhagan: Investigation, writing—reviewing. S. Saranya: Resources, investigation writing—reviewing. S.

Sivakumar: Validation, formal analysis, writing—reviewing. Subasri Prakash: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Consent to Publish Not applicable.

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