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Advancements in nanosensors for detecting pathogens in healthcare environments†

Antonia Bruno, [a] Farida Tripodi,[a] Alice Armanni, [a] Linda Barbieri, [a] Alessandro Colombo,[a] Sara Fumagalli,[a] Hind Moukham, [a] Giulia Tomaino,[a] Ekaterina Kukushkina,[b] Roberto Lorenzi, [b] Letizia Marchesi,[c] Angelo Monguzzi,[bd] Alberto Paleari, [b] Alessandra Ronchi,[b] Valeria Secchi,[b] Laura Sironi[cd] and Miriam Colombo *[ad]

Hospitals serve as critical environments for the management of diverse medical conditions, ranging from routine illnesses to life-threatening emergencies. However, alongside providing healthcare services, hospitals represent reservoirs for the transmission of microbial pathogens. Understanding the distribution and dynamics of pathogens within hospital settings is crucial for effective infection control and prevention strategies. Concurrently, the integration of cutting-edge technologies for the early detection and monitoring of target bacteria stands as a pivotal strategy in this battle against nosocomial infections. This critical review aims to provide a systematic insight into the main threatening microbes in hospitals and the detection of pathogens in different environments, ranging from intensive care units to general wards, including hospital entrances, bathrooms, high-touch surfaces, patient bed rails, medical equipment, and floors, which are often contaminated. We discuss recent scientific and technological advances in pathogen detection by exploring innovative methods that leverage nanotechnology to improve biosensing effectiveness and selectivity. This review is divided into sections focusing on various types of hospital environments, classes of mostly represented pathogens and kinds of available nanobiosensors. We include two comprehensive tables summarizing bacterial contamination in hospital wards and the materials and substrates associated with the nanobiosensors that have been developed. Eventually, we highlight the open challenges and perspectives in nanotechnology-based healthcare-environment monitoring and remediation as a promising solution to counteract pathogen emergence and spread.

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Environmental significance

The COVID-19 pandemic has confronted us with a deeply altered human and environmental scenario that affects both psychological and physical health. In particular, an exponential increase in attention to and care for the cleanliness of habitable spaces and public facilities has been a common experience. This has created a new mentality that requires high environmental control, especially in places and surfaces where pathogens are more likely to accumulate and thrive. This issue is particularly relevant in hospital environments and various hospital wards and departments. In this review, we focused on the various types of pathogens that are most frequently encountered and the most innovative methods based on the recent advances in nanotechnology that are used for their detection and identification, with the aim of making healthcare environments increasingly safe and habitable.

1. Introduction

According to the global report on infection prevention and control by the World Health Organization (WHO) in 2022, the frequency of health care-associated infection (HAI) varies between countries and according to economic conditions, ranging from 3.2% in the US to 6.5–8.9% in the UAE and up to 9–11% in Southeast Asia and Eastern Mediterranean region (**https://iris.who.int/bitstream/handle/10665/354489/9789240051164-eng.pdf?sequence=1**). The consequences of HAIs can range from a prolonged hospital stay to long-term complications and disability and premature deaths. These are often associated with the occurrence of sepsis, which has a global incidence of 15.4 cases per 1000 in adult patients and an incredibly high 112.9 cases per 1000 among neonates (**https://iris.who.int/bitstream/handle/10665/354489/9789240051164-eng.pdf?sequence=1**).

[a] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy. E-mail: miriam.colombo@unimib.it*
[b] *Department of Materials Science, University of Milano-Bicocca, Milan, Italy*
[c] *Department of Physics, University of Milano-Bicocca, Milan, Italy*
[d] *Nanomedicine Center NANOMIB, University of Milano-Bicocca, Milan, Italy*
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Despite great efforts to prevent these hospital-associated infections, there is still a great urgency to implement procedures to limit the risk factors determining these infections. In this context, the development of new technologies to detect pathogens in a fast, cheap and easy-to-use way would be of great help in limiting nosocomial infections.

2. Pathogenic scenario across the main hospital wards

Hospitals are dynamic ecosystems in which patients with diverse medical conditions receive care, ranging from routine treatments to critical interventions. However, amidst the provision of healthcare services, hospitals also harbor a complex interplay of microbial pathogens. Here, we address the hospital microbial landscape as a climax of selective pressure.

To do this, we started with a Web of Science-based search, according to the following query: "(hospital ward bacteria detection) OR (hospital care bacteria detection)". The research was limited to the title and abstract, and it was restricted by publication year (2015/01/01–2024/04/11). We obtained 327 records. Most of the hits (n = 186) are in the intensive care unit, but many records are also focused on surgical contexts (n = 19) and to a lesser extent on general wards (n = 6). Nevertheless, most of the retrieved papers are centered on human-derived samples rather than environmental samples, necessitating further exploration of the literature (ESI S1†).

Tracing their trajectory from intensive care units (ICUs) to general wards, the journey of pathogens across different hospital wards is influenced by selective pressures. The increase in selective pressure, driven by factors such as antimicrobial usage, invasive procedures, and compromised host immunity, fosters the evolution and dissemination of multidrug-resistant organisms (MDROs). As MDROs traverse across hospital settings, they encounter diverse microbial communities and environmental conditions, further shaping their evolutionary trajectories.

2.1 Neonatal intensive care unit (NICU)

There are approximately 6500 newborn deaths every day, amounting to 47% of all child deaths under the age of 5 years (WHO). The neonatal intensive care unit (NICU) plays a crucial role in hospitals by providing intensive care to newborns who face significant health challenges right from birth. National point prevalence surveys conducted in NICUs in the United States in 1999 and in Europe in 2011 revealed that HAIs affected 11.2% of neonates in the US and 10.7% in Europe, respectively.[1,2] The most common infections in the NICU fall into two categories: infections that are acquired during the labor and birth process, and hospital-acquired infections that babies contract when they are patients in the NICU. In fact, bacterial contamination was identified on various surfaces, including neonatal incubators, suction tips, ventilators, stethoscopes, door handles, weighing scales, mother beds, laryngoscopes, telephones, and ultrafiltrate bags. These pathogens can survive for varying durations, further complicating the efforts to maintain a sterile environment.[3] An additional challenge in preventing infections in the NICU comes from colonized healthcare workers and patients, who can act as sources of pathogens. Contributory factors to the spread of infections include poor hand hygiene, overcrowding, understaffing, inadequate training of staff, and insufficient disinfection or fumigation practices.[4] In NICUs, contamination can result from various significant bacterial and fungal pathogens, presenting substantial health risks. Notably, Gram-negative bacteria have been identified as the leading cause of outbreaks, representing 54% with 21 out of 39 reported incidents.[5] Key pathogens include *Staphylococcus aureus*, species of *Klebsiella*, *Escherichia coli*, *Pseudomonas*, *Acinetobacter*, and *Enterococcus* species. A systematic study about pathogen concentrations in NICU is still lacking.

2.2 Intensive care unit (ICU)

Intensive care units treat patients with severe or life-threatening illnesses and injuries. Immunocompromised patients account for an increasing proportion of the typical ICU cases,[6] making them more vulnerable to HAIs from pathogens. The frequent use of antimicrobials and the high cleaning practices, added to the clinical conditions of patients, create a unique microbiota characterized by common bacteria, opportunistic pathogens,[7] and MDROs.[8] This makes treatment more difficult[9] and contributes to substantial mortality and morbidity. ICUs are the hospital wards with the highest number of nosocomial infections, regarding lower respiratory tract infections, urinary tract infections and bloodstream infections in order of decreasing incidence rates.[8,10,11] Infections can be caused by the use of invasive devices[12] and by the transmission of pathogens from contaminated surfaces and the gloves of healthcare personnel.[13] In the ICUs, there is nearly the same likelihood for sanitary workers to have contamination on their hands or gloves after touching the environment in a room where a patient harboring pathogens resides because there is after directly touching the colonized patient and their surroundings.[13] This situation starts a circular process: the patients, the gloves or the environment could be the starting point of the infectious transmission.[14] Different surfaces are contaminated by several microorganisms, such as *S. aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Corynebacterium* spp., and *Bacillus* spp.

2.3 General surgical ward

Patients admitted to general surgical wards are those who have undergone surgical procedures or require postoperative care for their surgical conditions. In addition to the general medical ward, it caters to different patient populations and medical needs. However, patients in general surgical wards

typically have shorter lengths of stay following surgical procedures although this may vary depending on the complexity of the surgery and postoperative recovery. This means a high heterogeneity in the composition of patients undergoing different treatments. Bacterial pathogens in these wards can contribute to surgical site infections (SSIs). Thus, prior to surgical procedures, patients in general surgical wards may receive prophylactic antibiotics to reduce the risk of surgical site infections. These antibiotics are typically administered shortly before surgery and discontinued within 24 hours postoperatively, adhering to antimicrobial stewardship principles. The most threatening infections are due to multidrug-resistant *E. coli*.[15] *E. coli* is a ubiquitous bacterium implicated in SSIs, urinary tract infections, and bloodstream infections in general surgical wards. These bacteria can contaminate surgical sites through fecal–oral transmission or cross-contamination from the gastrointestinal tract.[16] Notably, Cassini *et al.*[15] demonstrated that a large proportion of the burden of antibiotic resistance bacteria was due to health-care-associated bloodstream, respiratory tract, or surgical site infections, and that more than half of health-care-associated infections are considered preventable. The mean bacterial colony count on surfaces in surgical wards was reported at 48.8 CFU cm^{-2}, indicating a potential source of contamination.[17]

2.4 General medical ward

The general medical ward provides acute medical services for adults of all ages across a wide variety of specialties (such as gastroenterology, endocrinology, respiratory medicine, rheumatology, and cardiology) but does not necessitate specialized treatment or monitoring found in ICUs or specialty wards.

Due to the diverse range of medical conditions and patients admitted, and to the dynamicity of this environment, a high heterogeneity of pathogens can be encountered: *S. aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *E. coli*, and *P. aeruginosa* are some examples.[18] Common areas show shared bacteria according to exposure (*e.g.*, high touch and high foot traffic sites, such as patient bed rails, medical equipment, and floor). Moreover, more than one study[18,19] demonstrated that the detection rate of bacteria in general wards is higher than that in intensive care units, supporting the evidence that different cleaning procedures can affect bacteria proliferation. Noteworthy, methicillin-resistant *S. aureus* (MRSA), but not vanB-positive vancomycin-resistant enterococci (VRE), was also detected at a high rate in a newly opened hospital. Bacterial load plateaued at a significantly higher level in common areas than in inpatient rooms ($p < 0.001$, common area median: 2.44×10^5 CFU per swab [2.42×10^5]; inpatient area median [IQR]: 1.10×10^5 CFU per swab [2.17×10^5]).[20]

Understanding the microbial landscape of general medical wards is essential for patient outcomes and healthcare-associated infection rates.

2.5 Main entrance

The main entrances of healthcare facilities are environments characterized by the largest flux of people, including patients, visitors and staff. Hospital lobby areas and bathrooms are characterized by the presence of viruses,[21] fungi,[22] and bacteria, such as *Citrobacter freundii*, *Stenotrophomonas maltophilia*,[23] and *S. aureus*,[24] as reported in Table 1. Because *S. aureus* is a skin commensal,[25] it is found in the most crowded hospital areas and surfaces.[26] The main sources of bacteria in this environment are people and the exchange between indoor and outdoor air. Moreover, floor cleaning and maintenance activities contribute to increased bacteria and fungi levels (the average levels of bacteria and fungi were 7.2 × 102 CFU m^{-3} and 7.7 × 10 CFU m^{-3}, respectively).[27] Despite the number of people, the major transmission of HAIs occurs in areas frequented by the patients and the medical staff, so areas with visitors, such as the main entrances, are less contaminated by pathogens or opportunistic bacteria.[26]

2.6 Environmental sampling challenges in hospital wards

There is a growing body of evidence indicating that hospital surface environments contribute significantly to the dissemination of pathogens. However, the optimal methods for sampling these surfaces remain unclear, and there is a lack of standardized guidelines or legislation to direct these practices. Currently, there is no legal mandate requiring hospitals to conduct routine environmental monitoring of surface contamination. Hospitals that opt to conduct sampling often rely on in-house protocols or guidelines adapted from the food and pharmaceutical industries. Comprehensive, evidence-based guidelines specific to hospital surface sampling are notably absent, and there are limited studies on the efficacy of various sampling methods under the diverse conditions present in hospital environments. Recent reviews[58,59] have detailed the methodologies for sample collection, highlighting the advantages and disadvantages of various approaches. Specifically, it has been noted that both elution-dependent methods (such as pre-moistened swabs, sponges, and wipes) and elution-independent methods (such as replicate organism detection and counting plates, 3M Petrifilm™ plates, and dipslides) require the presence of moisture and neutralizers during sampling to enhance recovery rates.

Most of the studies reviewed were conducted in laboratory settings rather than in actual hospital environments, where numerous variables can influence sampling recoveries. Previous studies do not provide a comprehensive understanding of the hospital surface microbiome mainly due to the scarcity of studies examining the general environment outside of outbreak situations, the tendency to focus on specific organisms or pathogens, and the variability in sampling methods, result analyses, and units of measurement (*e.g.*, few studies report results in colony-forming units per square centimeter). This variability complicates cross-study comparisons.

Table 1 Bacterial contamination of hospital wards and surfaces. Starting from the table by Russotto *et al.*,[28] we present an updated version of bacterial contamination of hospital wards and surfaces commonly found in scientific literature. ICU = intensive care unit; NICU = neonatal intensive care

Hospital wards	Surfaces	Microorganisms	Ref.
ICU	Electrocardiography leads	*Enterococcus* (VRE)	29
		Coagulase-negative staphylococci	30
		P. aeruginosa	
		A. baumannii (CRAB)	31
		Enterobacteriaceae spp.	32
	Cardiac monitor	*A. baumannii*	33
	Blood pressure cuffs	*S. aureus* (MRSA)	34
		A. baumannii (CRAB)	31
		C. difficile	35
		Enterobacteriaceae spp	32
	Ventilator (*e.g.*, buttons, circuits)	*S. aureus*	36
		P. aeruginosa	
	Suction system	*S. aureus*	36
		P. aeruginosa	
		S. maltophilia	37
	Medical charts	Coagulase-negative staphylococci	8
		A. baumannii	
		K. pneumoniae	
	Portable radiography equipment	*S. aureus* (MRSA)	38
		Enterococcus (VRE)	
		A. baumannii	
		K. pneumoniae	
		P. aeruginosa	
		S. maltophilia	
	Ultrasound machine	*S. aureus* (MRSA, MSSA)	39
		Coagulase-negative staphylococci	40
		P. aeruginosa	
		A. baumannii	
		Corynebacterium spp.	
		Bacillus spp.	
	Bed	*A. baumannii*	41
		S. aureus (MRSA)	42
		E. faecium (VRE)	9
		Enterobacteriaceae spp.	32
	Stethoscopes	*S. aureus*	43
		A. baumannii	
	Personnel's uniforms and hands	*A. baumannii*	44
		A. baumannii (CRAB)	31
		Enterococcus (VRE)	13
	Telephone/cell phones	*A. baumannii*	45
		A. baumannii (CRAB)	31
		Coagulase-negative staphylococci	46
		S. aureus	
		Non-fermenting Gram-negative bacteria	
	Computer (keyboards and/or mouse)	Coagulase-negative staphylococci	47
		Non-fermenting Gram-negative bacteria	
		S. aureus (MRSA)	33
	Television	*A. baumannii*	33
		S. aureus (MRSA)	
	Sink	*Klebsiella* spp.	48
		A. baumannii	33
		S. aureus (MRSA)	14
		K. oxytoca	48
		K. pneumoniae	
		E. cloacae	
		E. asburiae	
		C. freundii	
		E. coli	
		Pantoea spp.	
		S. marcescens	
		P. aeruginosa	49
	Ultrafiltrate bag	*P. aeruginosa*	49
	Floor	*S. aureus* (MRSA)	33

Table 1 *(continued)*

Hospital wards	Surfaces	Microorganisms	Ref.
	Sanitary equipment and toilet	*E. coli* *K. pneumoniae* *P. aeruginosa* *S. aureus*	50
	Chair	*A. baumannii*	33
	Patient's table	*A. baumannii* *S. aureus* (MRSA)	33 14
	Door handle or push plate	*S. aureus* (MRSA) *A. baumannii* (CRAB)	14 31
NICU	Neonatal incubator	*S. marcescens* *P. aeruginosa* *Klebsiella* spp. *E. coli* *Enterococcus* spp. *E. faecium* *S. aureus*	51 52 3 53
	Weighing machine	*Klebsiella* spp. *E. coli* *Enterococcus* spp.	3
	Laryngoscope	*E. coli* *S. aureus*	3
	Ventilator (*e.g.*, buttons, circuits)	*S. marcescens* *E. coli*	51 3
	Suction system	*E. coli* *P. aeruginosa* *Klebsiella* spp. *A. baumannii* *Enterococcus* spp.	3
	Bed	*E. coli* *P. aeruginosa* *Klebsiella* spp. *A. baumannii*	3
	Stethoscopes	*S. aureus* *Enterococcus* spp.	3
	Personnel's uniforms and hands	*S. marcescens*	51
	Telephone/cell phones	*A. baumannii* *Enterococcus* spp.	3
	Sink	*S. marcescens* *P. aeruginosa*	51 54
	Ultrafiltrate bag	*Klebsiella* spp.	3
	Door handle or push plate	*S. aureus* *Enterococcus* spp.	3
General surgical ward	Bed rails	*Bacillus cereus* group, *Enterococcus* (*faecalis* and *faecium*), *M. luteus*, *Staphylococcus* spp., *Streptococcus* spp.	55
	Keyboard	*Bacillus cereus* group, *M. luteus*, *Staphylococcus* spp.	55
	Simulation manikin	*Bacillus cereus* group, *M. luteus*, *Staphylococcus* spp.	55
	Table	*Bacillus cereus* group, *E. faecalis*, *M. luteus*, *Staphylococcus* spp., *Streptococcus* spp.	55
	Workstations-on-wheels	*Bacillus cereus* group, *E. faecalis*, *M. luteus*, *Staphylococcus* spp., *Streptococcus* spp.	55
	Faucet, basin, and drain of sinks	*E. coli* *Aeromonas* spp. *S. aureus* *S. epidermidis*	56
	Bed, taps, door handles	*S. aureus* *Pseudomonas* spp. *Enterobacteria* *E. faecalis*	57
	Floor, walls, equipment, instruments, operation tables, sink, light switch, chairs, beds, patient cloths, door/locker handlers, trolley, stretchers, sinks/faucets, intravenous stands, and oxygen cylinder	*S. aureus* *Klebsiella* spp.	17

Table 1 *(continued)*

Hospital wards	Surfaces	Microorganisms	Ref.
General medical ward	Door handles/knobs	*S. aureus* *E. coli* *Citrobacter* spp. *K. pneumoniae* *P. aeruginosa* *S. pneumoniae* *Proteus vulgaris* *Bacillus* spp. *Acinetobacter* spp. Coagulase negative *Staphylococcus* *Enterobacter* spp. *Enterococcus* spp. *Micrococcus* spp. *Diphtheroids*	18
	Bedrail, bedroom floor and toilet flush	*S. aureus* *Enterococcus* spp.	20
	Hospital bed units	*P. aeruginosa* *E. cloacae*	19
	Medical instruments	*K. pneumoniae*	19
	Water taps, thermos bottles, treatment carts, and dishcloths	*P. aeruginosa* *E. cloacae* *K. pneumoniae*	19
Main entrance	Sink	*C. freundii* *S. maltophilia*	24 23
	Door handle or push plate	*S. aureus* (MSSA)	24

Thus, integrating surface sampling methodologies into the workflow of hospital environments presents several challenges. One significant hurdle is the lack of standardized protocols, as mentioned before, which necessitates the development of customized guidelines that can be consistently applied across various hospital settings. This customization is time-consuming and requires substantial training of staff to ensure accurate and reliable sampling.

Moreover, the hospital environment is characterized by a high degree of variability, including differences in surface materials, cleaning practices, and the presence of diverse microbial communities. These factors can influence the effectiveness of sampling methods, making it difficult to develop a one-size-fits-all approach. Additionally, the integration of routine sampling into the daily operations of a hospital requires coordination across multiple departments and disciplines, potentially disrupting clinical workflows and patient care activities.

Finally, the implementation of routine environmental monitoring must be supported by robust data management and reporting systems to track and analyze trends over time. This requires the integration of new technologies and software, which can be a complex and resource-intensive process. Overcoming these challenges is essential to ensure that environmental monitoring effectively contributes to infection control and patient safety in hospital environments. Finally, the awareness that hospitals are fully fledged ecosystems, representing one of the most peculiar built environments with its microbiome: beyond pathogens, a large community of microorganisms, many harmless and some even potentially beneficial, lives in hospitals. These microbial communities could form a kind of "immune system", decreasing opportunistic pathogen accumulation and persistence in hospitals.[60]

3. The most concerning pathogens in the hospital environment

Six species of pathogens are recognized as particularly threatening due to their potential MDR mechanisms and pathogenicity. These are called ESKAPEE pathogens due to their ability to "escape" from commonly used antibiotics due to their increasing multi-drug resistance. They include *Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Enterobacter* spp., and *E. coli*;[61,62] along with *Clostridioides difficile*, these are the most common bacteria causing nosocomial infections.[63] A short description and pathogenicity of these bacteria are presented below.

3.1 *Enterococcus faecium*

Enterococci, particularly *Enterococcus faecium*, have emerged as significant causative agents of infections in humans.[60] They are known to be associated with hospital-acquired infections and are linked to a high rate of mortality.[61] In fact, studies in population genetics and genomics have revealed the existence of two separate subpopulations within the species *E. faecium*. The first group is primarily found as harmless inhabitants of the gastrointestinal tract, rarely causing clinical infections. However, the second group

consists of hospital-associated *E. faecium* strains.[62] In addition, *E. faecium* can resist antibiotics and environmental stressors.[63] Therefore, the continual use of antibiotics in the hospital environment has significantly contributed to the evolution of *E. faecium* into a highly adept pathogen within hospital environments. It is important to highlight that a significant majority of modern *E. faecium* isolates exhibit strong resistance to ampicillin, and a considerable number of these isolates, varying by geographic location, show resistance to glycopeptides.[63] The rapid rise in hospital-acquired infections from *E. faecium*, coupled with limited treatment options, is due to the bacterium's rising resistance to antibiotics and the prevalent challenge of biofilm-associated infections.[64–68]

3.2 *Staphylococcus aureus*

S. aureus is a cocci-shaped Gram-positive bacterium that tends to cluster in "grape-like" bunches. It takes the name "aureus" from the Latin word "gold" due to the golden colonies observed in culture medium.[69] *S. aureus* inhabits the environment, and it is part of the human microbiota; however, when entered into the bloodstream or internal tissue, it is responsible for a wide variety of clinical manifestations[25,70] such as ocular and skin infections, endocarditis, central nervous system infections, and pneumonia.[71] *S. aureus* pathogenic strains include vancomycin-resistant *S. aureus* (VRSA) group and MRSA group[63] that survive on surfaces from 6 h on stainless steel to 3 years on polyethylene.[35] In high-income countries, such as those in the European Union and European Economic Area (EU/EEA), MRSA is one of the three most impactful antibiotic-resistant microorganisms, together with *E. coli* and carbapenem-resistant *P. aeruginosa*. They are commonly acquired in healthcare settings and determine 70% of the burden of AMR in terms of disability and premature mortality, such as disability-adjusted life-years[72] (Global Report on Infection Prevention and Control, 2022). As presented in Table 1, *S. aureus* has a wide distribution in different sites and wards of hospitals, and this has brought the draft of the guidelines for MRSA in 52.5% of countries in 2021. The globally medium proportion of MRSA was 24.9% (interquartile range (IQR) 11.4–42.7) in 2020, and it was 15.5% in EU/EEA countries in 2019.[72,73] The percentage of MRSA isolates with resistance found in North America, Europe and Northeast Asian countries varies from less than 5% in the Scandinavian region to 60% in the U.S.A and China in 2022.[74] Moreover, patients infected with MRSA infections have an essential increase in post-infection length of stay, septic shock and mortality compared with those with methicillin-susceptible *S. aureus* (MSSA) infections, where the risk for discharge to long-term care is more than doubled.[72]

3.3 *Klebsiella pneumoniae*

K. pneumoniae is a Gram-negative bacterium of the *Enterobacteriaceae* family that can be symbiotic with its host by colonizing intestinal mucosa, skin and nasopharynx. However, it can also be a pathogen in humans, often causing nosocomial infections, such as urinary tract infections, blood infections and pneumonia. For other bacteria, the excessive use and misuse of antibacterial agents has led to an increase in resistance to antibiotics and the emergence of carbapenem-resistant (CRKP) and MDR *K. pneumoniae* strains.[75] Antibiotic resistance of this bacterium is further enhanced by biofilm formation, which can protect the pathogen from the host immune responses and can decrease the antibiotic effects, thus making clinical management of *K. pneumoniae* infection more complicated.[76]

3.4 *Acinetobacter baumannii*

A. baumannii is a Gram-negative bacterium that causes HAIs, especially affecting patients in intensive care units. It is responsible for hospital-acquired bloodstream infections and pneumonia, and it is particularly prone to cause outbreaks owing to its ability to survive prolonged periods on dry surfaces under unfavorable environmental conditions and to acquire antibiotic resistance.[77] During an outbreak, *A. baumannii* can be found on linen, furniture, and sinks, as well as on medical equipment, such as ventilator tubing. It can also form biofilms on both non-living and biological surfaces, increasing its resistance to antibiotic agents and yielding medical device-related infections. It can be transmitted through air droplets or the skin of infected patients; however, the most common way of transmission is through the hands of healthcare workers.[78] Consequently, *A. baumannii* carbapenem-resistant (CRAB) bacterium is considered one of the priority pathogens for WHO.[79]

3.5 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative bacterium of the family *Pseudomonadaceae* present in multiple ecological niches, such as soil and aquatic environments, and plant and animal tissues, due to its metabolic versatility.[80] It is also an opportunistic pathogen that causes acute or chronic infections in immunocompromised individuals, such as patients with cystic fibrosis and cancer, as well as in patients in the intensive care unit. It can colonize medical equipment and the hospital environment, leading to HAIs, such as pneumonia, urinary tract infections and bloodstream infections.[81] Strikingly, *P. aeruginosa* can exist in both planktonic form and biofilm, which are especially dangerous because they can infect medical devices but are also particularly harmful for patients with cystic fibrosis who frequently succumb to a chronic infection of the lungs.[82] In fact, *P. aeruginosa* infections are extremely difficult to treat due to antibiotic resistance.[83] For these reasons, *P. aeruginosa* is listed in the critical category of the WHO's priority list of bacterial pathogens.[79]

3.6 *Enterobacter* spp.

Enterobacter comprises a group of common Gram-negative bacteria characterized by rod-shaped, facultative anaerobic

properties. Flagella in *Enterobacter* species serve multiple functions, including adhesion, biofilm formation, protein export, and motility. Each species within the genus produces unique endotoxins, and as Gram-negative bacteria, they possess a lipopolysaccharide capsule that aids in evading phagocytosis and triggering inflammatory responses. Among these bacteria, certain strains can cause opportunistic infections in individuals with compromised immune systems, particularly those in hospital settings or those undergoing mechanical ventilation. Infections most commonly affect the urinary and respiratory tracts. Over the past three and a half decades, *Enterobacter aerogenes* (now known as *Klebsiella aerogenes*) and *Enterobacter cloacae* have emerged as significant threats in neonatal wards and intensive care units, especially among mechanically ventilated patients.[84] Around 2010, *E. cloacae* became more prevalent than *E. aerogenes* as the most frequently isolated species. It is important to note that within the *E. cloacae* complex, other clinically relevant members exist, often posing challenges for accurate species identification using standard tests.[85] MDR *Enterobacter* species are increasingly causing infections acquired in hospital settings. Prior to 2005, almost all *Enterobacter* strains were susceptible to carbapenems, but carbapenem resistance has now been reported in all WHO health regions.[86]

3.7 *Escherichia coli*

E. coli is a common Gram-negative bacterium of the family *Enterobacteriaceae* naturally present as a commensal of the intestinal tract of humans and other animals, with an important role in digestion. However, several pathotypes also exist, which cause infections, such as enteric/diarrhoeal disease, urinary tract infections and sepsis/meningitis, leading to two million deaths annually.[87,88] The intestinal *E. coli* strains can be divided into six well-described categories: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC).[88] Human infection can be acquired through contaminated food/water or *via* direct contact with an infected person, while in neonates, *E. coli* infections often occur through the maternal genital tract.[89] Clinically, *E. coli* infections are commonly treated with ciprofloxacin, levofloxacin, fosfomycin and fluoroquinolones; however, resistance to multiple antibiotics has been reported, making *E. coli* one of the more dangerous pathogens. In 2019, *E. coli* infections were responsible for more than 150 000 deaths associated with antimicrobial resistance in Europe.[90]

3.8 *Clostridioides difficile* (previously known as *Clostridium difficile*)

C. difficile is a rod-shaped Gram-positive anaerobic spore-forming bacterium.[91] Due to its difficulty in isolation and slow growth, it was given the name "*Bacillus difficilis*" from the Latin of difficult, changed to *C. difficile* in the 1970s.[92] It is part of animal and human gut microbiota[93] but becomes pathogenic in *C. difficile* infections (CDI). CDIs are caused primarily by clostridial toxin A (TcdA) and/or toxin B (TcdB) and are the leading cause of hospital-acquired diarrhea and colitis.[94] These diseases are often related to antibiotic treatments because they unbalance the gut microbiota composition; in this situation, *C. difficile* has the opportunity to multiply and produce its toxins.[92] Moreover, it can survive on various materials, from 15 minutes on dry surfaces to 6 hours on wet items, while its spores exhibit high resistance and can be found on floors and equipment for up to 5 months.[35] Due to its pathogenicity, the involvement with antibiotic treatments and the multidrug-resistance of some strains,[95] 23.7% of countries in the world have guidelines for *C. difficile*.[96]

As clearly appears, different surfaces can harbor different bacterial species, and different wards constitute peculiar environments. This is due to various factors; for instance, cleaning procedures, surface material, temperature, relative humidity, and initial titer can affect the range of survival times of bacteria. Moreover, survival is species specific and can vary largely among different microorganisms. However, it is worth mentioning that extensive studies have been conducted on selected species, such as *E. coli* and *A. baumannii*, but the survivability of many species remains widely unexplored. One reason for the lack of specific data is the unavailability of cellular model systems to study the respective pathogens. Many clinically relevant bacteria remain infectious on inanimate surfaces and can survive for months on dry surfaces. *In vitro* studies can provide initial indications to assess the risk of transmission of a particular pathogen by fomites; however, the conditions presented in various experimental studies often do not resemble real-life scenarios (*e.g.*, large inoculums and small surface areas) and therefore require careful interpretation. Moreover, the fraction of pathogens transferred depends on multiple factors, including species and surface material. The efficiency of the transfer of a pathogen between fomite and skin is a critical parameter for modeling its potential for transmission and implementing effective hygiene measures while avoiding unnecessary ones.

Thus, there is a significant gap in knowledge regarding this specific issue, as research is often fragmented into individual, non-standardized studies.[97]

Fig. 1 and Table 1 present the most common areas (wards and surfaces) where the most concerning pathogens have been detected.

4. Innovative methods for pathogen detection exploiting nanotechnology

In conventional laboratories, colony counting culture, polymerase chain reaction (PCR), Gram staining, and analytical profile index (API) systems are some of the most widely used techniques for identifying the presence of pathogens in clinical samples.[98–100] These strategies are often analytically concrete in terms of selectivity and reliability. However, these methods are expensive, time-consuming, and

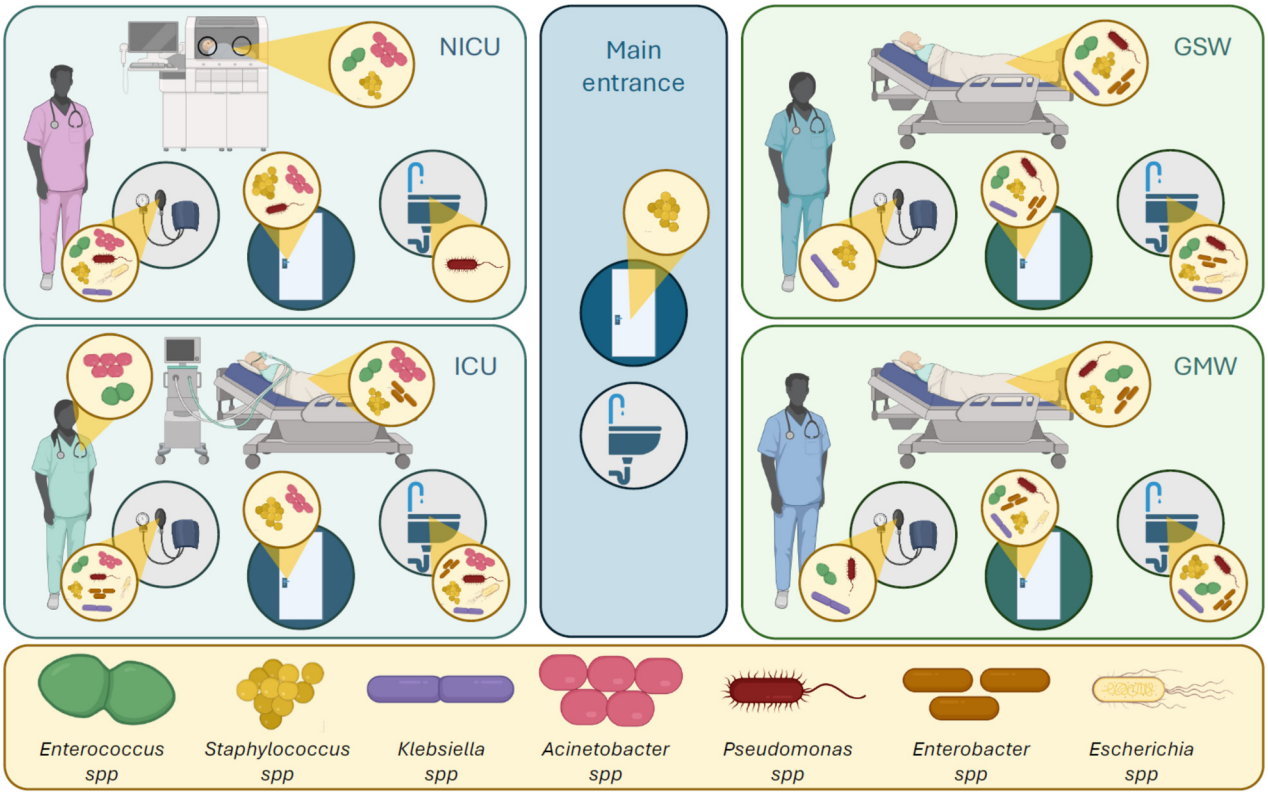

Fig. 1 Most concerning pathogens in different hospital wards and surfaces. Starting from Table 1, the image shows various surfaces in different hospital departments contaminated with ESKAPEE pathogens and other congeneric pathogen species. The surfaces are divided into personnel uniforms and/or hands, represented by the nurse image; beds and/or bed rails and sheets, represented by the hospital bed and the neonatal incubator in NICU; medical equipment, represented by the sphygmomanometer; room furniture and/or door handles, represented by the door; and bathroom, represented by the sink. The hospital wards are ICU – intensive care unit; NICU – neonatal intensive care unit; main entrance; GSW – general surgical ward; GMW – general medical ward. At the bottom is the bacterial genera legend the image is made using Biorender.

require qualified staff to interpret the results when the experiment is completed.[101–103]

With the increasing attention to point-of-care (POC) testing, there is a need for rapid and reliable diagnostic tools that can be used directly at the patient's bedside or in community settings. Rapid detection platforms fulfill this need by providing quick results for on-the-spot decision-making.[104] Biosensors, with their unique features, play an important role in meeting the demand for POC tests. They provide rapid and real-time results, are portable and cost-effective, and generally exhibit high sensitivity and selectivity, providing quick, accurate, and user-friendly diagnostic solutions that are ideal for decentralized healthcare settings.[105,106]

A biosensor is a device with a biological sensing component integrated into or closely connected to a transducer. The ability to miniaturize the transduction element and the absence of an economical production method are frequently the primary obstacles to developing POC and sensing devices.[107]

Nanotechnologies have become highly valuable in the field of biosensing due to their versatility and exceptional properties.[108,109] Specifically, characteristics of nanomaterials, such as their high reactive capacity, high adsorption, quantum size effects, and surface-to-volume ratio compared to their bulk form, are essential for developing biosensing methods.[110] Furthermore, as nanomaterials can be easily tailored in terms of size and shape, it is possible to modify or immobilize their surfaces with various biological species through covalent or non-covalent bonding, improving the biosensing characteristics in terms of high sensitivity, selectivity, and quick response to the analytes in the sample.[111–113]

POC biosensors typically leverage one or more of the following six approaches to signal transduction: I) optical, II) electrochemical, III) mechanical, IV) magnetic, V) thermometric, and VI) microgravimetric. Electrochemical and optical techniques are the most frequently used and sensitive ones for chemo- and biosensors.[114] Among the optical techniques, the most appealing for the production of point-of-care devices is colorimetric because it does not require sophisticated instruments and qualified personnel.[115,116]

4.1 Two fundamental parameters: sensitivity and selectivity towards pathogenic subtypes

To enable efficient risk assessment critical for healthcare, food safety analysis, and environmental monitoring, effective

pathogen diagnostic methods have to be rapid, ultrasensitive, specific and affordable to be applied in low-resource settings.[117] When designing biosensors, sensitivity is a crucial parameter to consider because it is the ability to quantify the analyte within a wide range of concentrations. Another equally important aspect is the sensitivity of the device, expressed as the limit of detection (LoD) of the colony forming units (CFU) per mL or the ng mL^{-1} of the analyte to be detected. Ideally, the higher the sensitivity, the lower the minimum concentration of the analyte that can be detected.

In this respect, the use of nanotechnologies plays a significant role in improving the sensing capability of the detection of biosensors. Various nanomaterials, such as gold nanoparticles (AuNPs), quantum dots (QDs), carbon nanotubes (CNT), metal nanoclusters (MNCs), and up-conversion nanoparticles (UCNPs), are integrated into biosensors to enhance the sensitivity and stability of these devices by amplifying their signal and expanding detection limits.[118] Some examples of nanotechnology-based biosensors are provided in the next paragraph. These nanomaterials can be manipulated to contain functionality for specific molecular recognition. Several recognition elements have been explored so far, including enzymes, antibodies, nucleic acids, aptamers, and cells. Their selective interaction with a particular analyte determines the effectiveness of the biosensor. Thus, the selectivity of the biosensor towards a certain target or strain changes depending on the recognition element used on the biosensor. To obtain excellent detection results, the development of bacterial recognition elements with higher efficiency and specificity is significantly needed.[119]

Antibodies used as recognition elements provide high specificity due to their unique antigen–antibody interactions but are often associated with high costs and lack of reproducibility. Moreover, the instability of protein-based recognition elements presents challenges in maintaining long-term sensor performance.[120] A less expensive alternative is represented by aptamers, short, single-stranded DNA or RNA molecules (20–100 nucleotides in length) with defined structures that can specifically bind to a wide range of targets *via* three-dimensional structures. However, aptamers are prone to degradation, and assays using a single aptamer as a recognition element are less specific.[121] Combinations of antibiotics and aptamers as dual recognition elements can be used to increase selectivity. For example, Shen *et al.* developed the broad-spectrum glycopeptide antibiotic vancomycin (Van) and aptamer-based dual-recognition CD nanoprobe combined with quantum dots to detect *S. aureus* *via* ratiometric fluorescence.[122] The detection time of the method is 30 min with a LoD of 1.0 CFU mL^{-1}.[119] Other examples are presented in Table 2. Overall, by carefully choosing the optimal combination of nanoparticles and recognition elements, one can design highly selective and sensitive nanotechnology-based biosensors. Nevertheless, further advancements are required to reduce their limitations and enhance their efficiency.

Some recent nanotechnology-based biosensors for the detection of the ESKAPEE pathogen are presented in Table 2 with a focus on the sensitivity of the device, the recognition element used to increase the selectivity and the reaction time. Particularly, in the context of pathogen diagnostics critical for healthcare, food safety analysis, and environmental monitoring, the development of a biosensor with rapidity and high accuracy is essential. Compared to traditional diagnostic methods, which are characterized by long reaction times, nanotechnology-based biosensors can be tailored to achieve faster reaction times.

4.2 Nanosensing methods for pathogen detection

This subsection briefly discusses the fundamental principles of signal transduction that exploit nanomaterials, with a focus on electrochemical, optical, and colorimetric methods for the detection of the pathogens presented above (Fig. 2).

Electrochemical nanobiosensors. Electrochemical sensors rely on the conductive interface of an electrode to generate measurable signals in response to binding events close to the surface.[151–153] The signal transduction interface between the electrode surface and the biological sample plays a key role in capturing target ions or biomolecules. It then transduces the electrical properties of ions or biomolecules and electrochemical reactions in output signals.[154] Electrodes are thus fabricated from conducting and semiconducting materials, including metals, such as gold (Au), and nonmetals, such as carbon.[109] Sohouli *et al.* developed an electrochemical aptasensor for detecting *S. aureus* using gold nanoparticles (AuNPs) and nitrogen-doped carbon nano-onions (NCNOs). The combination of AuNPs and NCNOs improved the electrode's conductivity and the active surface area, leading to a high-performing aptasensor with a linear range of 10–10^8 CFU mL^{-1} and a low detection limit of 3 CFU mL^{-1}. This biosensor also demonstrated excellent repeatability, reproducibility, and long-term stability, allowing for the detection of small amounts of *S. aureus* in human serum samples.[155]

C. difficile is the leading cause of hospital-acquired diarrhea (see Subsection 3.8). The two major toxins, TcdA and TcdB, have been studied intensively since their initial recognition as major *C. difficile* virulence factors,[156] and they are related to the microorganism infection.[157] Thus, Zhu *et al.* developed a sandwich-type electrochemical impedance immunosensor based on single-domain antibody-conjugated AuNPs applied to amplify the detection signal. In these biosensors, the primary antibody is immobilized on a gold electrode; then, a solution of monodispersed AuNPs conjugated with the secondary antibody is added. When proteins are adsorbed to the electrode surface, they form an inert electron transfer blocking layer and hence increase electron transfer resistance.[128]

Silica nanoparticles (SNPs) have recently emerged as one of the most up-to-date biocompatible materials because they have strong surface properties, high stability, chemical

Table 2 Some of the latest nanotechnology-based biosensors for the detection of ESKAPEE pathogens

Pathogen	Transduction method	Nanotechnology	Recognition element	Assay time	Linear range	LOD	Sample	Ref.
Acinetobacter baumannii	Electrochemical	Au-electrode with beta cyclodextrin	DNA probe	105 min	0.3 nM to 0.24 μM	0.14 nM	Food	123
		Cit-Ag NPs	DNA probe	2 min after DNA hybridization	1 μM to 1 ZM	1 ZM (LLOQ)	Bacteria DNA	124
	Optical	Au nanoprisms with Fe(III) siderophore	LSPR-based whole-cell sensing with aptamer-based molecular recognition motifs	3h	4×10^2 to 4×10^6 CFU mL^{-1}	80 cell per mL	Bacterial culture	125
		NCND/GO	Aptamer ssDNA	20 min	2×10^3 to 4.5×10^7 CFU mL^{-1}	3×10^2 CFU mL^{-1}	Bacterial culture, urine sample	126
		MNPs	Tail fiber proteins	10 min	1×10^4 to 1×10^5 cells per mL	4.48×10^4 cell per mL	FBS	127
Clostridioides difficile	Electrochemical	AuNPs	Antibody	—	1 pg mL^{-1} to 100 pg mL^{-1} of toxin	0.61 pg mL^{-1}; 0.60 pg mL^{-1} of toxin	Stool sample	128
Enterococcus faecalis	Electrochemical	Au crystals	DNA aptamer, toluidine blue (TB) as DNA hybridization indicator	6 h (immobilization time); 5 min (TB binding time)	10^{-17} to 10^{-10} M	4.7×10^{-20} M	Urine and stool sample	129
		Cysteine-modified AuNPs	Clavanin A peptide	—	10^1 to 10^4 CFU mL^{-1}	10 CFU mL^{-1}	Bacterial culture	130
	Colorimetric	Quercetin-mediated AgNPs	LAMP amplification DNA	—	10 to 10^5 CFU mL^{-1}	10 CFU mL^{-1}	Food	131
Escherichia coli	Optical	AuNCs	Cu reduction	<30.0 min	10^3 to 10^6 CFU mL^{-1}	89 CFU mL^{-1}	Bacterial culture	132
		AuNPs plus CeO_2NPs	Aptamer and azithromycin	<30.0 min	10 to 1.5×10^5 CFU mL^{-1}	1.04 CFU mL^{-1}	Food	133
		AgNCs plus MNPs	RNA-cleaving DNAzyme probe	—	10^2 to 10^7 CFU mL^{-1}	60 CFU mL^{-1}	Food, tap water	134
		MNPs	Fluorescent proteins	<30.0 min	—	10^8 CFU mL^{-1}	Bacterial culture	135
		CDs	Receptors (boronic acid, polymixin and Van)	>60.0 min	—	OD600 = 1.0	Tap water	136
		BCDs	Cu quenching and reduction	—	10^3 to 10^7 CFU mL^{-1}	1.5×10^2 CFU mL^{-1}	Food	137
		Tb-MOF	Antibody	5.0 min	1.3×10^2 to 1.3×10^8 CFU mL^{-1}	3 CFU mL^{-1}	Food	138
		COF-BA Au@Ag nanoparticles	Magnetic IgG@Fe_3O_4 nanoparticles	30 min	10 to 10^3 CFU mL^{-1}	10 CFU mL^{-1}	Bacterial culture	139
	Colorimetric	Fe_3O_4/Au-PEI NPs	Antibody	60 min	10 to 10^7 CFU mL^{-1}	0.52 CFU mL^{-1}	Clinical sample	140
	Electrochemical	SiO_2-NPs	Polyclonal antibodies	30 min	8×10^4 to 8×10^6 CFU mL^{-1}	2×10^3 CFU mL^{-1}	Bacterial culture	141
		CO NPs	Cu reduction	60 min	10^3 to 10^7 CFU mL^{-1}	2 CFU mL^{-1}	Food and water	83
Klebsiella pneumoniae	Colorimetric	AuNPs	Aptamer	1 min	10^2–10^8 CFU mL^{-1}	3.4×10^3 CFU mL^{-1}	Bacterial culture, clinical sample, urine	142
	Optical	COF-BA Au@Ag nanoparticles	Magnetic IgG@Fe_3O_4 nanoparticles	30 min	10 to 10^3 CFU mL^{-1}	10 CFU mL^{-1}	Bacterial culture	139
	Electrochemical	GO–ICA hybrid film	ssDNA aptamer	—	10^{-6} to 10^{-10} M	3×10^{-11} M	—	143

Table 2 *(continued)*

Pathogen	Transduction method	Nanotechnology	Recognition element	Assay time	Linear range	LOD	Sample	Ref.
Pseudomonas aeruginosa	Electrochemical	AuNPs	ssDNA aptamers	10 min	60 to 6 × 10^7 CFU mL^{-1}	60 CFU mL^{-1}	Bacterial culture	144
		CO NPs	Cu reduction	60 min	10^3 to 10^7 CFU mL^{-1}	1.6 × 10^4 CFU mL^{-1}	Food and water	83
	Colorimetric	MNPs and gold	Specific protease substrate peptide	1 min	45 to 4.5 × 10^7 CFU mL^{-1}	10^2 CFU mL^{-1}	Clinical sample	145
	Optical	COF-BA Au@Ag NPs	Magnetic IgG@Fe_3O_4 nanoparticles	30 min	10 to 10^3 CFU mL^{-1}	10 CFU mL^{-1}	Bacterial culture	139
Staphylococcus aureus	Electrochemical	MPDA/MnO_2	SA31 aptamer	—	5 to 10^7 CFU mL^{-1}	3 CFU mL^{-1}	Food	146
		PtNPs@Van	Aptamer-coated magnetic $CuFe_2O_4$ nanoprobes	—	5 to 10^4 CFU mL^{-1}	1 CFU mL^{-1}	Bacterial culture, clinical sample, food	147
		Fe_3O_4@Au NPs	Van and aptamer	Within 50 min	10 to 10^7 cells per mL	3 CFU mL^{-1}	Bacterial culture	148
		AuNP	ssDNA aptamer	—	6.2 × 10^2 to 6.2 × 10^5 CFU mL^{-1}	3 CFU mL^{-1}; 2.51 fg μL^{-1} for genomic DNA	Bacterial culture	149
	Optical	UCNPs	Aptamer	—	50 to 10^6 CFU mL^{-1}	25 CFU mL^{-1}	Food	150
		COF-BA Au@Ag NPs	Magnetic IgG@Fe_3O_4 nanoparticles	30 min	10 to 10^3 CFU mL^{-1}	10 CFU mL^{-1}	Bacterial culture	139
		MNPs	Fluorescent proteins	<30.0 min	—	10^8 CFU mL^{-1}	Bacterial culture	135
		CDs	Antibody	—	1 to 2 × 10^2 CFU mL^{-1}	1 CFU mL^{-1}	Food	151
		MOF	Bacteriophages	—	40 to 4 × 10^8 CFU mL^{-1}	31 CFU mL^{-1}	Food	152
		CQDs plus NFs	Aptamer		10 to 10^8 CFU mL^{-1}	10 CFU mL^{-1}	Food	190
		CNPs plus QDs	Van and aptamer-based dual-recognition CD nanoprobe	<30.0 min	10 to 10^6 CFU mL^{-1}	1 CFU mL^{-1}	Food	122
		Au nanodisk	ssDNA aptamer	120 s	10^3–10^8 CFU mL^{-1}	10^3 CFU mL^{-1}	Bacterial culture, food	163
	Colorimetric	ALP-labeled Fe_3O_4 and Au NPs	Aptamer	Within 60 min	10 to 10^6 CFU mL^{-1}	2.4 CFU mL^{-1}; 50 CFU mL^{-1} (naked eye)	Bacterial culture	191
		Au NPs	*S. aureus* protein A gene	10–15 min	5 to 40 ng μL^{-1}	8.73 ng μL^{-1}	Stool and urine samples	192

Abbreviations: Au: gold; Cit-Ag NPs: citrate capped silver nanoparticles; NCND: nitrogen-doped carbon nanodots; GO: graphene oxide; MNPs: magnetic nanoparticles; AuNPs: gold nanoparticles; Ag: silver; AgNPs: silver nanoparticles; AuNCs: gold nanoclusters; CDs: carbon dots; Van: vancomycin; BCDs: blue carbon dots; Tb: terbium; MOF: metal–organic framework; COF-BA: boronic acid-functionalized covalent-organic framework; @: conjugation; Fe_3O_4/Au-PEI NPs: polyethyleneimine coated magnetic gold nanoparticles; CO NPs: cupric oxide nanoparticles; ICA: indole-5-carboxylic acid; MPDA: mesoporous polydopamine; PtNPs: platinum nanoparticles; UCNPs: up-conversion nanoparticles; CQDs: carbon quantum dots; NFs: nanofibers; CNPs: carbon nanoparticles; QDs: quantum dots; ALP: alkaline phosphatase.

inertness, and facile functionalization.[158] To specifically identify and bind *E. coli*, Mathelié-Guinlet *et al.* created an electrochemical biosensor that employs SNPs modified with specific polyclonal antibodies. The gold electrode is initially coated with a polyelectrolyte multilayer to enable the electrostatic immobilization of a layer of NPs functionalized with specific polyclonal antibodies. The transducer without nanoparticles recognizes bacteria although quantification is

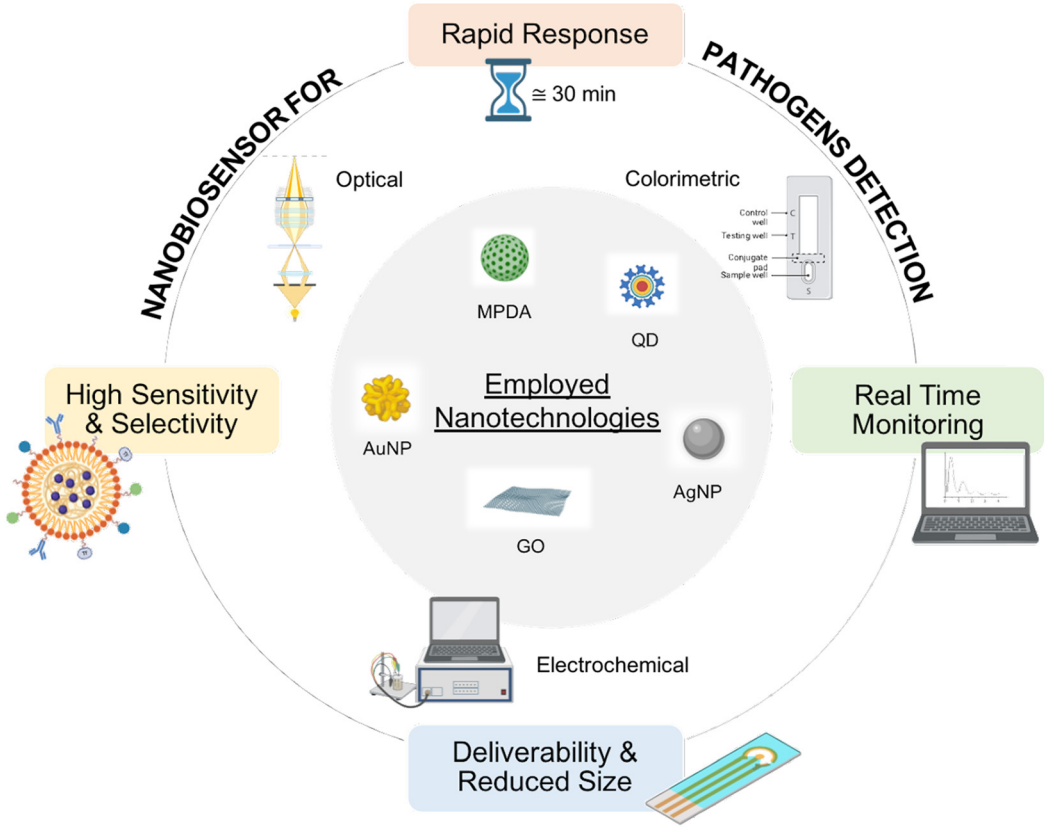

Fig. 2 Schematic of the described nanotechnologies, methods of detection and their main properties.

difficult due to random oscillations in many parameters detected by cyclic voltammetry. However, with the presence of nanoparticles in the biosensor, bacteria are consistently and reliably detected over the measured range.[141]

Gram-negative bacteria, such as *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *A. baumannii*, can tolerate an environment with a large amount of Cu. After internalization, the enzyme cupric reductase starts reducing Cu^{2+} to Cu^{+}.[159]

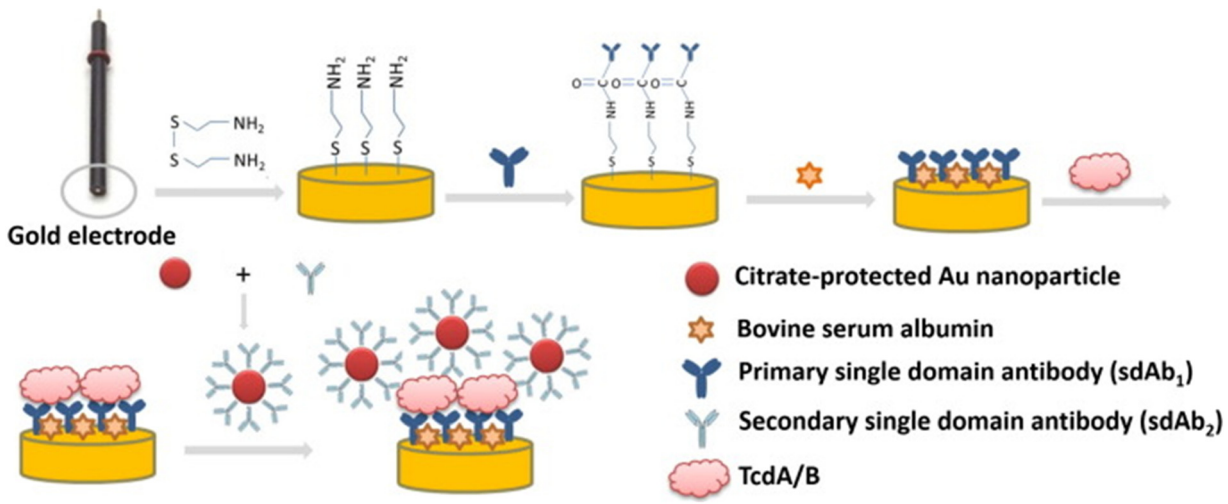

Fig. 3 Sandwich-type electrochemical immunosensor for the detection of TcdA and TcdB. Toxins are bonded onto the electrode through antigen–antibody interaction; then, secondary antibody-coated AuNPs are introduced onto the electrode surface as an amplifying probe to optimize the immunosensing performance. Reprinted from Bioelectrochemistry, Zanzan Zhu, Lianfa Shi, Hanping Feng, H. Susan Zhou, "Single domain antibody-coated gold nanoparticles as enhancers for *Clostridioides difficile* toxin detection by applying electrochemical impedance immunosensors",[128] copyright 2015, with permission from Elsevier.

Recently, a biosensor has been developed that exploits the mechanism of copper homeostasis of Gram-negative bacteria associated with the intrinsic oxidase-like activity of cupric oxide nanoparticle (CuONP). In the presence of gram-negative bacteria, reduced Cu^{+} catalyzes the oxidation of *o*-phenylenediamine (OPD) to form 2,3-diaminophenazine (oxOPD), which has a fluorescence emission at 573 nm under excitation at 423 nm.[54]

Nazari-Vanani *et al.* developed an innovative electrochemical biosensor designed *via* the electrodeposition of a new gold nanostructure of ice crystals-like as the sensing substrate combined with toluidine blue as the DNA hybridization indicator. Here, a particular thiolated ssDNA was stabilized on the transducer superficies, and the hybridization of the DNA was assessed by differential pulse voltammetry[129] (Fig. 3).

Optical nanobiosensors. In optical sensors, the measuring element produces, directly or through a recognition process (*e.g.*, the formation of an antibody–antigen complex), an optical signal (*e.g.*, color, fluorescence, or chemiluminescence), or causes a change in the optical properties of the environment.[114] The optical signal produced may be observed by the naked eye or measured by applying a photodetector (a device that converts optical signals into measurable electrical signals).[160] Optical biosensors are mostly subcategorized into localized surface plasmon resonance (LSPR), colorimetric sensors, surface-enhanced Raman spectroscopy (SERS), fluorescence resonance energy transfer (FRET), fluorescence, and luminescence chemistry groups according to their properties, including absorption, reflection, and scattering.[161]

Localized surface plasmon resonance is an optical phenomenon that occurs when a dielectric surrounds a group of electrons in a metal. The extremely intense and highly confined electromagnetic fields of the LSPR provide a very sensitive probe for detecting small changes in the environment around the nanostructures, which is particularly attractive for sensor applications.[162] LSPR-based aptasensors have become important biological and chemical sensing tools for detecting target analytes in real time without labeling agents. Khateb *et al.* used hole-mask colloidal lithography to fabricate arrays of gold nanodisks on a glass substrate. These nanodisks are then functionalized with DNA aptamers to capture *S. aureus* within the local optical field of the metal nanostructure, providing a signal readout with a total analysis time of 120 seconds.[163]

Peptides can act as reducing and stabilizing agents to synthesize AuNPs, thereby regulating the size, morphology, hydrophobicity, and surface charge of the nanoparticles.[164] Yu *et al.* used a one-pot method to synthesize peptide-functionalized gold nanoparticles (P_AuNPs). These P_AuNPs are made with different positive/negative charges and hydrophilic/hydrophobic characteristics tailored for bacterial recognition. The interaction between P_AuNPs and bacteria produces a response in the LSPR spectrum that acts as a bacterial fingerprint. The antibiotic-resistant and antibiotic-susceptible strains of ESKAPEE pathogens are then identified using machine-learning algorithms based on the bacterial fingerprint supplied by the plasmonic nanosensor. The study shows that the surface chemistry of AuNPs changes their plasmonic capabilities, allowing for the fabrication of highly sensitive biosensors for bacterial identification.[121]

FRET is a non-radiative phenomenon with the energy transferred from an excited donor fluorophore to an acceptor fluorophore through intermolecular dipole–dipole coupling.[165] Nanoparticles, such as semiconductor quantum dots (QDs), graphene quantum dots (GQDs), and upconversion nanoparticles (UCNPs), have gained significant attention as photo-stable fluorescence probes and potential donors in FRET. Furthermore, nanoparticles with relatively large sizes have unique electrical properties that account for their quenching capacity. AuNPs and graphene oxide (GO) are two examples of effective fluorescence quenchers in FRET assays.[166] Nevertheless, Bahari *et al.* employed carbon dots (CD) as the donor species and graphene oxide as the acceptor in the FRET process. When the modified *ortho*-phenylenediamines carbon dot (*o*-CD) with aptamer (*o*-CD-ssDNA) binds to the GO surface, the fluorescence of *o*-CD is efficiently quenched. The aptamer (ssDNA) acts as a biorecognition element and specifically binds with *A. baumannii*. This binding permits the release of the *o*-CD-ssDNA from GO, leading to the recovery of the fluorescence signal of *o*-CD.[126]

Wang *et al.* defined a SERS-based sensor for the simultaneous detection of *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. SERS spectra exhibit fingerprint-like patterns that are highly specific to the molecular composition of the analyte, allowing the detection of different bacteria even in complex samples. In particular, the biosensor proposed exploits Au@Ag nanoparticles coated with polydopamine and functionalized with boronic acid as a SERS tag. The nanoprobe forms a sandwich structure by combining the magnetic separation element ($IgG@Fe_3O_4$) with the Raman amplification element (SERS tag). The use of IgG-functionalized magnetic nanoparticles enables more efficient capture and isolation of bacteria.[139,167,168]

Zheng *et al.* established a method for producing highly photoluminescent AuAg nanoclusters by employing silver ions (Ag^{+}) as linkers to connect the Au-thiol motifs, resulting in Au/Ag–thiol motifs on thiolated Au nanoclusters. This approach significantly increased the photoluminescence in the AuAg bimetallic nanoclusters. When photoluminescent AuAg nanoclusters are exposed to the bacterium *A. baumannii*, their fluorescence is selectively quenched[169] (Fig. 4).

Colorimetric nanobiosensors. A successful technique for lowering the expenses associated with the use of electronic readers is to build sensors whose signals can be detected with the naked eye.[116] Typically, this requires either an increase/decrease in coloration or a change in the tone of a colored solution.[170] After establishing a clear distinction between the color produced by the analyte-containing

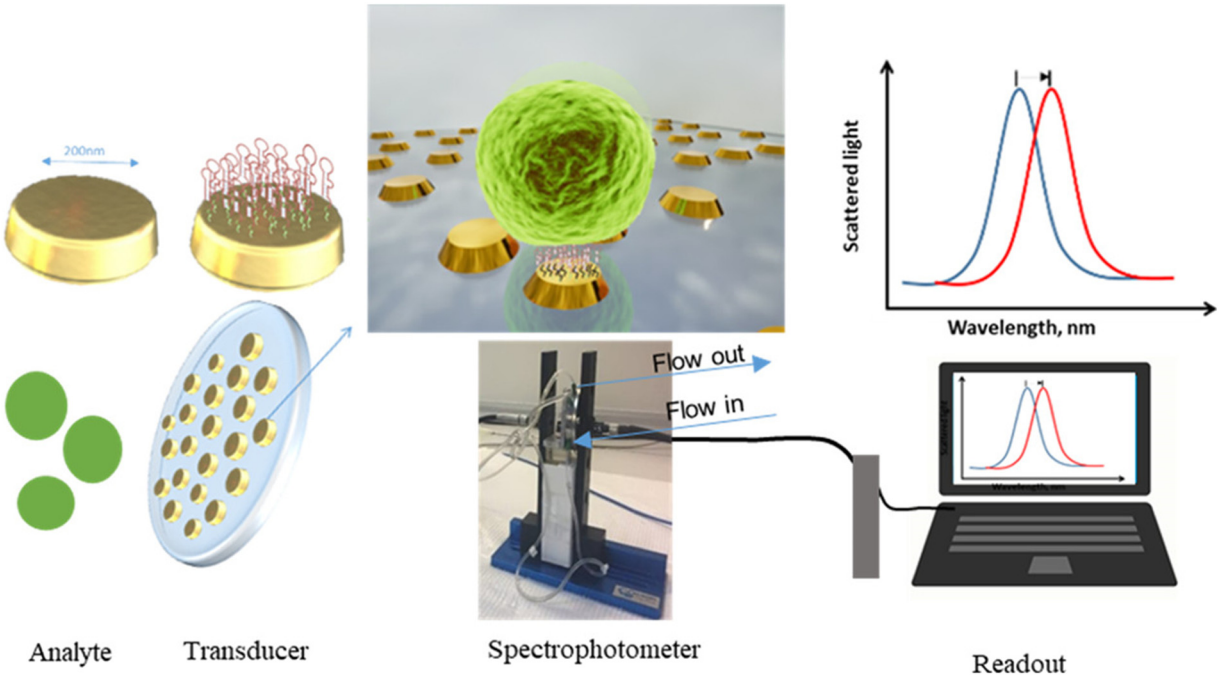

Fig. 4 Schematic representation of a label-free optical nanobiosensor *via* aptamer recognition of *Staphylococcus aureus*. Arrays of plasmonic gold nanodisks with disk diameters of either 100 or 200 nm are employed to enable refractometric detection of the bacteria. The LSPR spectra shift is measured using a compact custom-built fiber spectrometer and showed a maximum absorption peak at 650 nm for the 100 nm disks and 990 nm for the 200 nm disks. Reprinted with permission from Khateb, Heba *et al.*[163] Development of a label-free LSPR-Apta sensor for *Staphylococcus aureus* detection, *ACS Applied Bio Materials*, vol. 3 and 5, 2020, 3066–3077. Copyright 2020, American Chemical Society.

solution and the color produced by the blank solution, this technique permits the identification of a specific molecule without the need for additional equipment. Frequently, the results are qualitative in the form of a positive/negative outcome.[171]

Gold nanoparticles have outstanding optical features because of their distinct size and shape-dependent interactions with light. The color of AuNPs can be controlled by modulating their size, shape, or the surrounding media. Smaller nanoparticles are red, while larger ones are blue.[172] Madkour *et al.* developed a sensor based on the optical properties of gold nanoparticles by synthesizing AuNPs of 10 nm and functionalizing them with a thiolated oligonucleotide probe. The detecting element is designed using protein A sequence data obtained from the gene bank. This protein is specifically tailored to bind to the target DNA sequence of the *S. aureus*–SPA gene, an important virulence factor of *S. aureus*. Gene detection is based on a color change that can be observed with the naked eye and quantitatively measured using a UV-vis spectrophotometer. The nanosensor exhibits a stable red color in the presence of the target DNA, while the color changes to blue in the absence of the target. This colorimetric assay allows for the rapid detection of DNA samples without DNA amplification.[173] The same process is exploited by Tondro *et al.*, who conjugated gold nanoparticles with a thiolated oligonucleotide probe from a partial sequence of the 16S ribosomal RNA gene of *Enterococcus faecalis*. In this case, the aggregation is induced by the addition of an acid solution, but when the target sequence is present, it hybridizes with the sequence conjugated on the surface of AuNPs, preventing aggregation.[174]

Sivakumar *et al.* exploited the characteristic properties of silver for the selective detection of *E. faecium* and *A. baumannii*. In this sensor, nanoparticles are not directly synthesized: the formation of nanoparticles occurs instead under specific conditions to produce a signal that could be detected by the naked eye. The recognition element is the loop-mediated isothermal amplification (LAMP) amplicons of the target DNA. The interaction between the LAMP amplicons, Ag^{+} ions, and quercetin, a polyphenolic flavonoid with high reducing potential, leads to the formation of silver nanoparticles (AgNPs), which display an intense brown color.[131]

A lateral flow assay (LFA) is a diagnostic technique used to detect target analytes rapidly and easily in various samples. In an LFA, the sample containing the target analyte is applied to a test strip made up of several components, such as a sample application pad, conjugate pad, nitrocellulose membrane, and an adsorption pad. The nitrocellulose membrane usually contains test and control lines that immobilize specific biorecognition molecules, such as antibodies. When the sample flows through the strip *via* capillary action, the target analyte interacts with the labeled molecules and moves along the strip. If the target analyte is present, it binds to the capture molecules on the test line, producing a visible signal that indicates a positive result.[175]

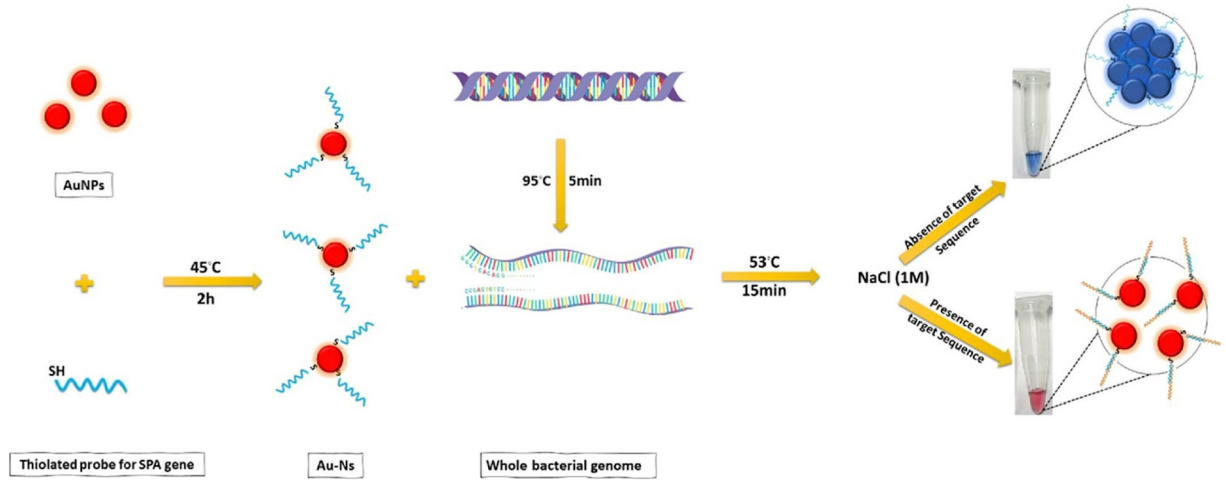

Fig. 5 Colorimetric detection strategy for the *Staphylococcal* protein A (SPA) gene based on gold nanosensors (Au-Ns). AuNP surface is functionalized with a specific thiolated probe. *S. aureus* genome is denatured by heating at 95 °C for 5 min, then mixed with 50 μL Au-Ns and incubated for 15 minutes. Finally, 5 μl of NaCl solution was added to the mixture, and the result was evaluated by the naked eye.[167]

A way to detect *P. aeruginosa* was developed by Alhogail *et al.* They created a biosensor on paper by applying a special protease substrate. This substrate was designed to allow the LasA protease from *P. aeruginosa* to access and break it down easily. The substrate is a peptide linked to magnetic nanoparticles (MNPs) at one end. At the other end, a cysteine allowed the peptide–MNP complex to form a self-assembled monolayer on a gold sensor surface. When *P. aeruginosa* protease cleaves the peptide, the MNP portion is detached, revealing the golden color of the sensor[145] (Fig. 5).

5. Future perspectives suggested by promising approaches based on nanosensors

The recent COVID-19 global pandemic has further confirmed the need to develop miniaturized diagnostic devices that can be run at home by a non-technical operator, thereby supporting the healthcare system in containing infections and allowing for immediate clinical decision making. Even the World Health Organization has stressed the importance of creating such devices by summarizing the required characteristics for an ideal POC with the acronym ASSURED: affordable, sensitive, specific, user-friendly, robust and rapid, equipment-free, and deliverable.[176–178] Although traditional methods for detecting pathogens exhibit adequate sensitivity, their speed and flexibility fall short of effectively addressing a significant outbreak. POC analyses emerge as the sole practical approach to handle such situations rapidly, with minimal operator demands on a large scale.[179]

In this review, we focused our attention on the integration of nanotechnology and pathogen detection devices considering that the unique physicochemical properties of nanoparticles have led to the development of all the nanobiosensors listed above. The application of nanotechnologies to produce POC has several advantages, such as increased sensitivity, miniaturization, multiplexing capabilities, improved specificity, enhanced imaging and visualization, and paves the way to personalized medicine.

Electrochemical nanobiosensors are considered the most widespread class of sensors for tracking bacteria. They can offer high sensitivity because they can detect target molecules even at very low concentrations. Moreover, they can be designed to be very selective towards specific biomolecules such as the nanosensor developed by Sohouli *et al.*, which can detect even small amounts of *S. aureus* in human serum samples. These nanosensors can also be miniaturized, allowing for portable and on-site detection systems for risk evaluation. Even if significant progress has been made in the development of such devices, there are still some issues to solve, such as improving their biocompatibility through surface modification and coating. Furthermore, most electrochemical biochips are currently engineered for single use; thus, future research should focus on making detectors reusable in sensor design.[117,155]

Optical biosensors have attracted considerable interest as substitutes for conventional methods due to their rapid, straightforward, and accurate outcomes. The integration of nanotechnology has introduced numerous strategies to upgrade conventional optical biosensors into smart, advanced, and efficient optical biosensing platforms.[180] Compared with traditional strategies, nanomaterials used in optical biosensors have excellent chemical, physical, and optical properties, which make them suitable for obtaining low detection limits and high sensitivity for identifying viruses and bacteria, depending on their color change or fluorescence conversion.[181] Although the use of biosensors employing nanomaterials has shown promising results in laboratory settings, several challenges must still be overcome to implement them effectively in practical applications. At

present, optical and photoelectrochemical biosensors primarily rely on precious metals, such as gold, platinum, and silver, for construction. However, their limited availability and high expenses hinder the commercial availability of these sensors. Hence, it has become crucial to explore alternative nanomaterials sourced abundantly and at lower costs to promote the widespread use of these sensors.[182]

The colorimetric detection technique is one of those interesting optical methods that provides a straightforward and easily interpretable output by producing visible color changes in response to the presence of the target analyte. These nanosensors can also be designed and fabricated to be portable, enabling on-site testing without sophisticated instrumentation, and they can detect pathogenic bacteria in a very short period.[161] Compared with fluorescence sensors, colorimetric ones have the advantages of simplicity, fast response, and visualization detection. Although they have been applied to differentiate many chemicals and bioanalytes, there are few reports on colorimetric sensors for identifying bacteria.[183] This could be because the interpretation of the color changes associated with the results may introduce variability, impacting reproducibility. Moreover, this kind of sensor is usually solely qualitative and cannot indicate the amount of the pathogen present in the tested sample.[176]

Focusing on nanobiosensors applied to healthcare-associated infections, the examples reported above (Table 2) were mostly tested on food and biological samples, such as serum, stool, and urine samples. To the best of our knowledge, only a few nanobiosensors have been applied to detect pathogens in personnel, equipment, devices, hospital surfaces and environments, and patient support accessories, which are the main mediated factors responsible for the spread of nosocomial infection pathogens. One strategy to minimize the risk of patient-to-patient transmission of pathogens from other contaminated items is to perform regular sanitary controls of all inanimate surfaces and to implement quick methods for determining cleanliness and hygiene.[184,185] Nanotechnologies have the potential to contribute to the implementation of these new rapid, reliable, and sensitive sensors that can improve the traditional detection systems applied in hospitals. Now, this family of nanosensors remains a goal to reach. We can speculate that to develop such a sensor, it is crucial to address challenges related to standardization, regulatory approval, and ethical considerations. Moreover, further studies are required to ensure the accuracy and reliability of the nanobiosensors, which must be validated in real hospital settings.

Other challenges that can hinder the implementation of nanobiosensors in monitoring pathogens on hospital surfaces include diverse microbe-rich background matrices found in environmental samples and low pathogen concentrations. To overcome the latter issue, different strategies have been developed to enrich pathogen concentration besides being applied so far mainly to agriculture, food and bioaerosol monitoring.[186,187] These methods can be divided into material-based enrichment methods, electric-based enrichment methods and bio-organism-based enrichment methods. Among the material-based enrichment strategies, noble metal ion characteristics have been exploited to build up nanoparticle aggregation-based enrichment methods where the aggregation of the nanoparticles on the target analytes leads to a change in the color of the solution in a proportional manner.[188,189] Recently, to improve the stability, sensitivity and specificity of these nanoparticle-based enrichment assays, research has focused on the development of systems based on the cooperation between DNA/RNA chains and nanoparticles. These strategies are yet to be tested in hospital settings with real environmental samples.

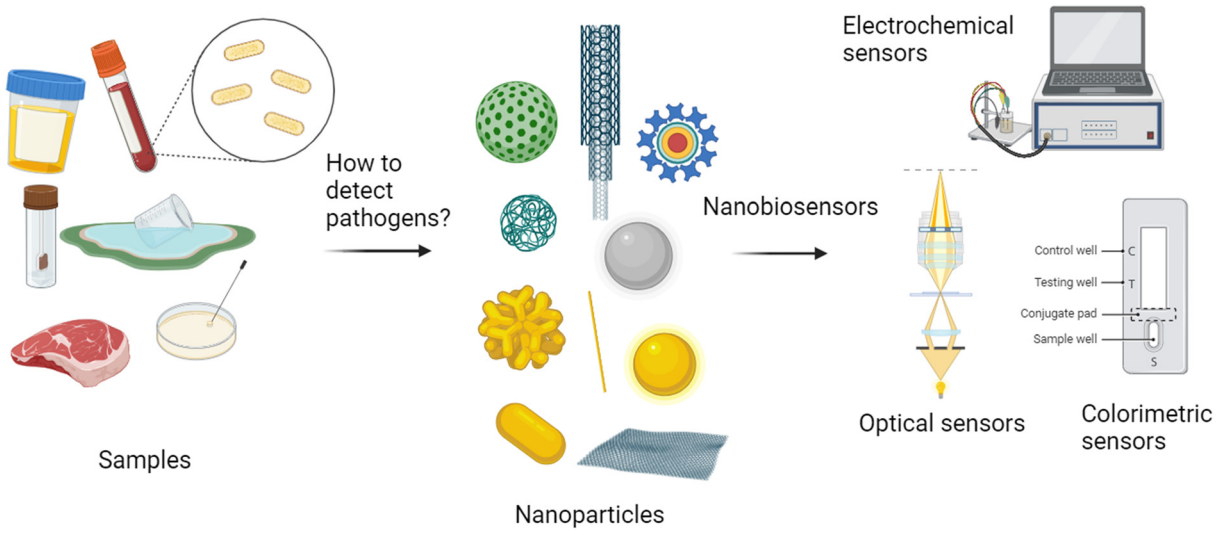

Fig. 6 Some of the typical pathogens containing samples, such as blood, saliva, and environmental samples, are depicted alongside electrochemical, optical, and colorimetric sensors utilizing nanotechnologies (*e.g.* gold, silver, or metal nanoparticles).

Nevertheless, with the increasing demand for POC devices, nanotechnologies and their properties can be a real resource for designing a new generation of efficient yet easy-to-use pathogen detection and identification assays for clinical environment surveillance. To move toward this new concept, a change in mentality is likely to come with the advent of new technologies not only in the diagnosis and treatment of human diseases but also in the more general management of the healthcare system (Fig. 6).

Abbreviations

AgNPs	Silver nanoparticles
AuNPs	Gold nanoparticles
CDI	*Clostridioides difficile* infections
CFU	Colony forming unit
CNT	Carbon nanotube
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
CRKP	Carbapenem-resistant *Klebsiella pneumoniae*
CuONPs	Cupric oxide nanoparticles
DAEC	Diffusely adherent *Escherichia coli*
EU/EEA	European Union and European Economic Area
EPEC	Enteropathogenic *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ESKAPEE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Escherichia coli*
FRET	Fluorescence resonance energy transfer
GO	Graphene oxide
GQDs	Graphene quantum dots
HAI	Healthcare-associated infection
ICA	Indole-5-carboxylic acid
ICU	Intensive care unit
LAMP	Loop-mediated isothermal amplification
LFA	Lateral flow assay
LoD	Limit of detection
LSPR	Localized surface plasmon resonance
MDR	Multiple drug resistance
MDRO	Multidrug-resistant organism
MNCs	Metal nanoclusters
MNPs	Magnetic nanoparticles
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NCNOs	Nitrogen-doped carbon nano-onions
NICU	Neonatal intensive care unit
o-CD	*Ortho*-phenylenediamines carbon dot
P_AuNPs	Peptide-functionalized gold nanoparticles
POC	Point of care
PCR	Polymerase chain reaction
QDs	Quantum dots
SERS	Surface-enhanced Raman spectroscopy
SNPs	Silica nanoparticles
SPA	*Staphylococcal* protein A
SSI	Surgical site infection
TcdA	Toxin *Clostridioides difficile* A
TcdB	Toxin *Clostridioides difficile* B
UCNPs	Upconversion nanoparticles
Van	Vancomycin
VRE	Vancomycin-resistant *Enterococcus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WHO	World Health Organization

Data availability

No primary research results, software or code has been included and no new data were generated or analysed as part of this review.

Author contributions

Miriam Colombo: conceptualization, methodology, resources, writing – review & editing, supervision, project administration, funding acquisition. Antonia Bruno: conceptualization, methodology, resources, writing – original draft, supervision. Farida Tripodi: conceptualization, methodology, resources, writing – original draft, project administration, funding acquisition. Alice Armanni, Linda Barbieri, Alessandro Colombo, Giulia Tomaino: methodology, resources, writing – original draft, visualization. Sara Fumagalli, Hind Moukham: methodology, resources, writing – original draft. Ekaterina Kukushkina, Roberto Lorenzi, Letizia Marchesi, Alberto Paleari, Alessandra Ronchi, Valeria Secchi: writing – review & editing. Laura Sironi, Angelo Monguzzi: conceptualization, supervision, project administration, funding acquisition. All authors read and approved the submitted version.

Conflicts of interest

There are no conflicts to declare.

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