New Microbes and New Infections 65 (2025) 101589

Contents lists available at ScienceDirect

New Microbes and New Infections

journal homepage: www.journals.elsevier.com/new-microbes-and-new-infections

Targeted next-generation sequencing enhances precision and rapid detection in healthcare-associated infection Surveillance: Unveiling multidrug-resistant colonization in ICUs

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ARTICLE INFO

Keywords:
tNGS
Microorganisms
Healthcare-associated infections

ABSTRACT

Objectives: This study aims to evaluate the potential advantages of targeted next-generation sequencing (tNGS) over conventional bacterial culture methods for pathogen detection in hospital-associated infections (HAIs).
Methods: All EICU medical staff and all medical staff from the Physical Examination Centre completed a questionnaire. Nasopharyngeal specimens were collected from medical staff who met all of the inclusion criteria and none of the exclusion criteria. EICU medical staff provided 2 samples each, while Physical Examination Centre staff provided 1 sample each. For EICU medical staff, one of their two nasopharyngeal swabs was subjected to tNGS testing, and the other to bacterial culture testing. For the PEC staff, their nasopharyngeal swabs were subjected to tNGS testing. Additionally, six pairs of spectacles and six keyboards used by EICU medical staff were randomly selected, and the surfaces were swabbed with sterile swabs for tNGS testing.
Results: In 23 nasal swab samples from EICU group, tNGS detected 14 species of microorganism in 29 instances within 19 h. Bacterial culture detected 2 species of microorganism in 4 instances, 2 positive samples within 19 h and confirmed another 2 positive samples within 69 h. A total of 42 samples with 14 different microorganism species were collected from the nasopharyngeal swabs of 23 EICU members and 15 PEC members. Among them, 29 cases (69 %) of 14 different microorganisms were detected in EICU staff, with an average of 1.3 microorganism species detected per person, while 13 cases (28 %) of 6 different microorganisms were detected in PEC staff, with an average of 0.9 microorganism species detected per person. The most common colonizing bacteria included *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella* spp. Compared to bacterial culture, tNGS offers advantages in monitoring HAIs, including a broad range of detectable microorganisms, high sensitivity of results, and shorter reporting time for positive results. Bacteria colonizing the EICU carry more antibiotic resistance genes.
Conclusions: tNGS outperforms conventional culture in healthcare-associated infection surveillance, with higher sensitivity and accelerated pathogen identification. Simultaneously, tNGS revealed extensive colonization of multidrug-resistant (MDR) pathogens (e.g., *Acinetobacter baumannii*, MRSA) in EICU environments, highlighting its utility in monitoring complex antimicrobial resistance patterns.

1. Introduction

Hospital-associated infections (HAIs) are a major challenge in the global healthcare sector [1]. Intensive Care Unit (ICU) patients are a high-incidence population for hospital infections, due to the patients' critical condition, multiple underlying diseases, weakened immunity, frequent invasive procedures, prolonged hospital stays, and the widespread use of broad-spectrum antibiotics [2,3]. According to data from the International Nosocomial Infection Control Consortium (INCCC) in 2024, the overall incidence rate of HAIs in ICUs is as high as 7.28 % [4], accounting for 30 % of in-hospital infections [5]. Their occurrence implies additional medical costs, extended hospital stays, and increased mortality rates [6,7].

The occurrence of HAIs is closely related to the hospital environment and staff. The nasopharynx, hands, and spectacles of hospital staff, and the surfaces within the hospital environment can serve as carriers and

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https://doi.org/10.1016/j.nmni.2025.101589
Received 30 January 2025; Received in revised form 5 April 2025; Accepted 23 April 2025
Available online 24 April 2025

transmission media for microorganisms. The microorganisms may survive long-term in those locations and spread through medical activities, thereby promoting the incidence of HAIs [8–10]. Reducing surface microbial contamination plays a crucial role in controlling the risk of HAIs. Effective environmental surface cleaning and disinfection, hand hygiene, and nasopharyngeal cleaning have all been proven to be associated with a reduction in the transmission of pathogens to patients [11, 12]. However, practical implementation is far more complex than the theory, as it is challenging to achieve rapid and accurate monitoring of microorganisms in clinical settings, making it difficult to prove that these preventative measures are truly effective.

To date, the monitoring of microorganisms has been primarily based on culturing methods, which often require several days and necessitate the selection of appropriate culture media and environments according to different microbial habits. Thus there are limitations in terms of detection time and sensitivity [13,14]. There is an urgent need for a rapid and accurate microbial detection technology for the prevention and control of HAIs. In recent years, next-generation sequencing (NGS) has demonstrated great potential for microbial detection due to its rapidity and sensitivity [15]. Some studies have attempted to use NGS technology for microbial detection on hospital environmental surfaces, which not only provides real-time and accurate microbial information but also provides a detailed description of the entire microbial community [16,17]. However, due to its high cost, large-scale clinical implementation remains challenging. The advent of targeted next-generation sequencing (tNGS) has addressed this critical issue. By narrowing the gene sequencing scope and targeting the enrichment of antibiotic resistance genes and virulence genes, tNGS achieves faster, more precise, and cost-effective results. tNGS has been widely applied in disease diagnosis [18,19]. But its application in the prevention and control of HAIs requires further research.

Multidrug resistance has been increased all over the world that is considered a public health threat. Several recent investigations reported the emergence of multidrug-resistant (MDR) bacterial pathogens from different origins, including food supplies, community environments, and animal reservoirs [20–26]. These pathogens facilitate horizontal gene transfer of resistance determinants across species via mobile genetic elements such as plasmids and transposons [22,23]. Core mechanisms driving antimicrobial resistance development encompass the production of antibiotic-inactivating enzymes, modification of drug target sites, and overexpression of efflux pumps [22]. The escalation of resistance underscores the critical need for rational antibiotic stewardship. Besides, the routine application of the antimicrobial susceptibility testing to detect the antibiotic of choice as well as the screening of the emerging MDR strains is imperative [24,25].

The complex interplay between antimicrobial resistance mechanisms and pathogen dissemination underscores the necessity of establishing context-specific surveillance systems, particularly in high-risk hospital units where microbial ecology differs markedly from general care settings. Understanding the distribution and resistance of pathogens is of great importance for the prevention and treatment of HAIs. However, the distribution of pathogens varies across different countries, regions, and hospitals, and even different departments within the same hospital or different monitoring periods within the same department, posing considerable challenges.

In terms of the distribution of HAI pathogens, the main pathogens causing HAIs include meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter baumannii* [27]. There are typical differences between the ICU and general wards, which are not only manifest in the different distribution of infectious strains but also in the higher incidence of MDR bacteria infections in the ICU [28–30]. The major HAIs pathogens exhibit robust pathogenic and drug-resistant mechanisms: *Staphylococcus aureus* produces toxic shock syndrome toxin-1 (TSST-1) that induces toxic shock syndrome (TSS) [31], while *Pseudomonas aeruginosa* disrupts host cells through its type III secretion system [32]. *Acinetobacter baumannii* survives under high antibiotic pressure in ICUs via biofilm-mediated drug resistance [33], whereas extended-spectrum β-lactamases (ESBLs) produced by *Klebsiella pneumonia*e and *Escherichia coli* can hydrolyze antibiotics [34], making these pathogens particularly dangerous in healthcare settings.

This study aims to address two critical challenges in HAIs control: (1) the limitations of conventional microbial monitoring methods in tracking MDR pathogens, and (2) the understudied relationship between hospital environmental ecology and pathogen transmission dynamics. We propose a dual-strategy approach combining targeted next-generation sequencing tNGS technology with comparative environmental surveillance. First, we will systematically validate tNGS against standard culture methods for detecting bacterial colonization on healthcare workers' nasopharyngeal mucosa and high-touch surfaces, quantifying its advantages in sensitivity, turnaround time, and resistance gene profiling capability. Second, through parallel monitoring of EICU and PEC, we seek to establish how variations in antimicrobial pressure, patient vulnerability, and infection control protocols shape pathogen evolution and transmission risks. The integrated findings are expected to provide actionable insights for developing precision surveillance frameworks in heterogeneous healthcare settings.

2. Methods

2.1. Ethics statement

This study has been approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (Ethics Approval Number SYSKY-2024-817-01). All participants in the study have signed informed consent forms.

2.2. Study population

The study population comprises medical staff from the EICU and the PEC of Sun Yat-sen Memorial Hospital, Sun Yat-sen University.

2.3. Inclusion criteria

There were four inclusion criteria for the EICU and PEC population, as below: (1) Medical staff who have been working in the relevant unit for 12 months or more.

(2) Adults aged 18 years and above. (3) Willing to participate in the study and able to understand the study content and requirements.(4) Have read and voluntarily signed the informed consent form, indicating understanding of the study's purpose, procedures, potential risks, and measures for rights protection.

2.4. Exclusion criteria

There were several exclusion criteria, as below. (1) Use of antibiotics, antiviral drugs, immunosuppressants, or other medications within the preceding 4 weeks that could affect the study results. (2) Presence of severe chronic diseases such as heart disease, diabetes, kidney disease, *etc*., which could affect the study results or cause adverse reactions. (3) Pregnant or breastfeeding women, as the study may have adverse effects on the foetus or infant. (4) History of mental illness or cognitive disorders, which could affect compliance with the study and the reliability of data. (5) Having unhealthy habits such as alcoholism or drug addiction, which could affect the accuracy of the study results. (6) Currently participating in other clinical studies, which may have interactions with the interventions of this study.

2.5. Study procedure

All EICU medical staff and all medical staff from the Physical Examination Centre completed a questionnaire assessing their respiratory

status, confirming their respiratory conditions and medication use for the past four weeks. Nasopharyngeal specimens were collected in July 2024 from medical staff who met all of the inclusion criteria and none of the exclusion criteria. EICU medical staff provided 2 samples each, while Physical Examination Centre staff provided 1 sample each. A polypropylene fibre swab was used to swab both the tonsils and the posterior pharyngeal wall for 15 s. Those nasopharyngeal swabs were then preserved in sterile specimen tubes.

For EICU medical staff, one of their two nasopharyngeal swabs was subjected to tNGS testing, and the other to bacterial culture testing. For the PEC staff, their nasopharyngeal swabs were subjected to tNGS testing. Additionally, six pairs of spectacles and six keyboards used by EICU medical staff were randomly selected, and the surfaces were swabbed with sterile swabs for tNGS testing. The study flowchart is shown in Fig. 1.

2.6. tNGS analysis

Samples were sent to Guangzhou KingMed Diagnostics Group Co. for tNGS sequencing. Nucleotide extraction was automated using a MagPure Viral DNA/RNA Kit (IVD5412, Magen Biotechnology, Guangzhou, China) on a KingFisher Flex Purification System (Thermo Fisher Scientific, Waltham, MA, USA). Nuclease-free water (catalogue item number 10977023, Invitrogen, Waltham, MA, USA) was used as the non-template-control (NTC) to detect contamination.

The reverse transcription, multiplex PCR pre-amplification, and library preparation for tNGS were performed using a Respiratory Pathogen Microorganisms Multiplex Testing Kit (Guangzhou KingMed Diagnostics Group Co. Guangzhou, Guangdong, China). Generated libraries were quantified using an Equalbit DNA HS Assay Kit (Vazyme Biotech Co., Nanjing, China) with an Invitrogen Qubit 3.0/4.0 fluorometer (EQ121-02, Thermo Fisher Scientific, Waltham, MA, USA) to ensure all samples had a library density $\geq$0.5 ng/μL or else were subjected to library reconstruction. DNA fragment analysis was performed using a Standard Cartridge Kit (C105201, BiOptic Inc., Jiangsu, China) and the compatible Qsep100 capillary electrophoresis system. After library qualification, sequencing was performed on the KM MiniSeq Dx-CN Platform (KM MiniSeqDx-CN, KingCreate, Guangzhou, Guangdong, China).

The sequencing data generated was analysed using a customized bioinformatic workflow, and base-calling was performed using bcl2fastq software; fastp was then used for adaptor trimming and low-quality filtering, after which Bowtie2 was used to map the reads of each sample against our special authoritatively classified tNGS database in very-sensitive mode. If the best alignment of reads was dropped into one target amplicon region, then the read was counted. The number of counts of each target amplicon was summed and normalized to 100,000, then indicated as this target amplicon pathogen result. Results were interpreted by a clinical microbiologist [35].

2.7. Statistical methods

In this study, statistical analyses were performed using R software (version 4.3.0). Normally distributed continuous data are expressed as mean ± standard deviation (SD). Intergroup comparisons between two independent samples were analysed using the independent two-sample *t*-test, with a P value < 0.05 considered statistically significant.

3. Results

To identify more effective tools for monitoring HAIs, we conducted a comparative analysis between tNGS and traditional culture-based methods to validate whether tNGS demonstrates superior accuracy and faster processing speed compared to conventional bacterial culture. Furthermore, this study investigated the impact of distinct environments on HAIs by analyzing differences in infection rates and pathogen profiles between ICUs and emergency departments.

3.1. Demographic characteristics

A total of 28 EICU medical staff and 19 PEC medical staff completed the questionnaire. Five EICU medical staff and four PEC staff were excluded due to the use of antibiotics within the preceding four weeks. Nasopharyngeal swabs were ultimately collected from 23 EICU medical staff and 15 PEC medical staff. Characteristics of the study group are shown in Table 1.

3.2. Comparative analysis of tNGS and bacterial culture

Among the 38 medical staff participating in the study, a total of 42 instances of microorganisms were detected, comprising 14 different species. EICU medical staff had 29 instances of 14 species of microorganisms detected (69 %), with an average of 1.3 microorganism species per person. In the PEC, 13 instances of 6 species of microorganism were detected (31 %), with an average of 0.9 microorganism species per person.

Among the 23 EICU medical staff members tested, tNGS detected a total of 14 species and 29 instances of microorganisms, while bacterial culture detected 2 species and 4 instances of microorganisms (Fig. 2).

tNGS results were released within 19 h for all samples. For bacterial

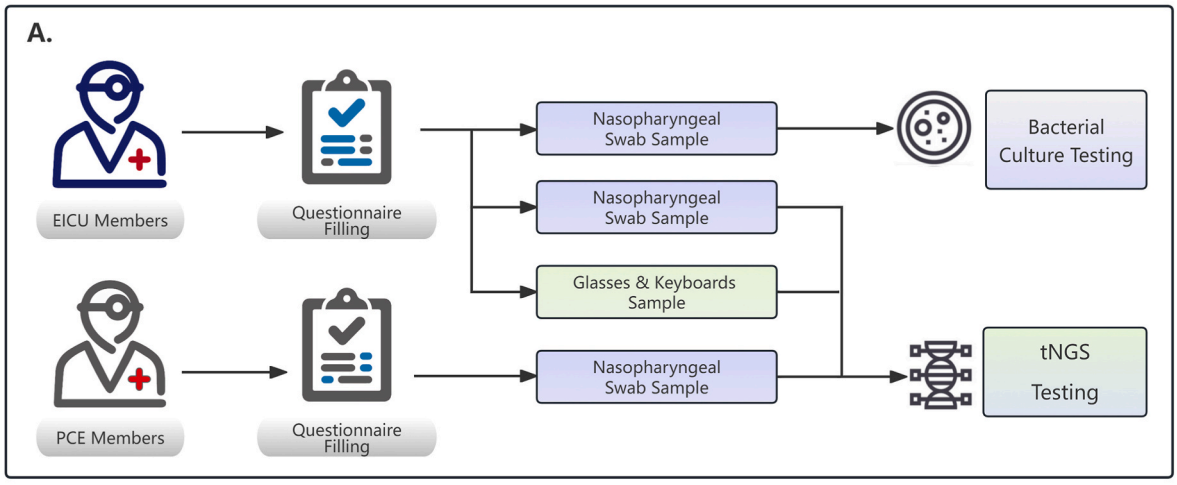

Fig. 1. Research flowchart.

Table 1
Characteristics of the study group.

Department	Count (*n*)	Age (y)	Sex		Upper respiratory symptoms		Smoking history	
			Male	Female	Yes	No	Yes	No
EICU	23	37.1 ± 6.1	12 (52 %)	11 (48 %)	5 (21 %)	18 (79 %)	1 (4 %)	22 (96 %)
PEC	15	39.1 ± 7.2	6 (40 %)	9 (60 %)	2 (13 %)	13 (87 %)	0 (0 %)	15 (100 %)
Total	38	37.9 ± 6.6	18 (47 %)	20 (53 %)	7 (18 %)	31 (82 %)	1 (3 %)	37 (97 %)

NOTE. Data are expressed as counts (percentages), mean ± SD, or as otherwise indicated.

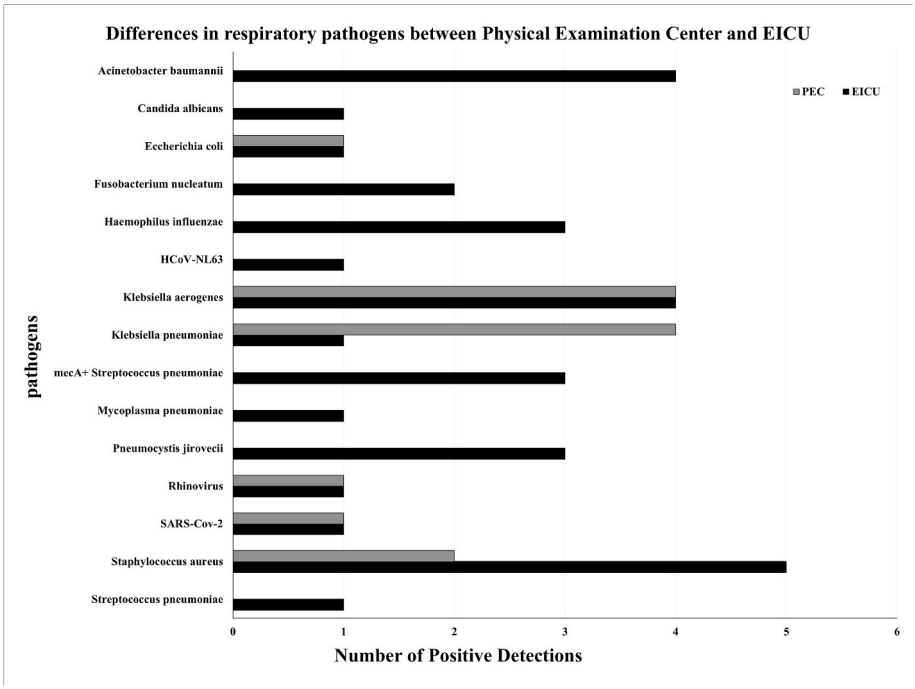

Fig. 2. Bacterial culture and tNGS results of nasopharyngeal swabs from EICU healthcare workers. tNGS detected a total of 14 species and 29 instances of microorganisms, while bacterial culture detected 2 species and 4 instances of microorganisms.

culture, 2 samples had positive results released within 19 h, 2 samples had positive results released within 69 h, and the remaining 19 samples had negative results released within 168 h (Fig. 3).

3.3. Comparative analysis of ICU and Non-ICU settings

Among the 23 EICU staff, 5 (21.7 %) had no microorganisms detected, 9 (39.1 %) had 1 species of microorganism detected, 8 (34.8 %) had 2 species of microorganism detected, and 1 (4.3 %) had 4 species of microorganism detected. Among the 15 PEC staff, 6 (40.0 %) had no microorganisms detected, 6 (40.0 %) had 1 species of microorganism detected, 2 (13.3 %) had 2 species of microorganism detected, and 1 (6.7 %) had 3 species of microorganism detected (Fig. 4).

The microorganisms detected by tNGS and their frequencies are as follows. In EICU staff *Staphylococcus aureus* (with or without mecA resistance gene): 5 instances (17.2 %), *Staphylococcus aureus* (with *mecA* gene): 3 instances (10.3 %), *Acinetobacter baumannii* and *Klebsiella aerogenes*: 4 instances (13.8 %), *Haemophilus influenzae* and *Pneumocystis jirovecii*: 3 instances (10.3 %), *Fusobacterium nucleatum*: 2 instances (6.9 %), *Candida albicans*, *Escherichia coli*, Human coronavirus NL63, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Rhinovirus* sp., SARS-CoV-2 and *Streptococcus pneumoniae*: 1 instance (3.4 %). In PEC staff: *Klebsiella aerogenes*: 4 instances (30.8 %), *Staphylococcus aureus* (without *mecA* gene): 2 instances (15.4 %), *Escherichia coli*, *Klebsiella pneumoniae*, *Rhinovirus* sp. and SARS-CoV-2: 1 instance (7.7 %) (Fig. 5).

In the EICU, 6 surface swab samples from keyboards and 6 from spectacles were analysed, which resulted in a total of 32 instances of microorganisms being detected using tNGS, comprising 12 different species. On keyboards 24 instances of 11 species of microorganism were detected: *Acinetobacter baumannii*: 5 instances (20.8 %), *Candida tropicalis*: 4 instances (16.7 %), *Klebsiella pneumoniae*: 3 instances (12.5 %), *Candida parapsilosi*, *Enterobacter cloacae* complex, *Mycobacterium abscessus and* Nontuberculous mycobacteria: 2 instances (8.3 %), *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Rhizopus* sp. and *Stenotrophomonas maltophilia*: 1 instance (4.2 %). On spectacles 8 instances of 5 species of microorganism were detected: *Acinetobacter baumannii*: 5 instances (62.5 %), *Candida parapsilosis*, *Klebsiella pneumoniae*, Nontuberculous mycobacteria, and *Staphylococcus aureus* (with *mecA* gene): 1 instance (12.5 %) (Fig. 6).

4. Discussion

We compared tNGS with traditional bacterial culture methods and found that tNGS detected significantly more types and instances of microorganisms than bacterial culture. tNGS can simultaneously detect more than 100 types of microorganism, including bacteria, fungi, and viruses, whereas bacterial culture testing can only detect bacteria and has difficulty obtaining positive results for bacteria that require special culture conditions, such as anaerobes and mycobacteria. Numerous studies have demonstrated that tNGS has higher sensitivity and a broader pathogen detection range than bacterial culture. The high sensitivity and extensive pathogen spectrum analysis of tNGS highlight its potential as a leading diagnostic tool in clinical microbiology [28,29,36]. Additionally, tNGS can significantly improve the ability to detect

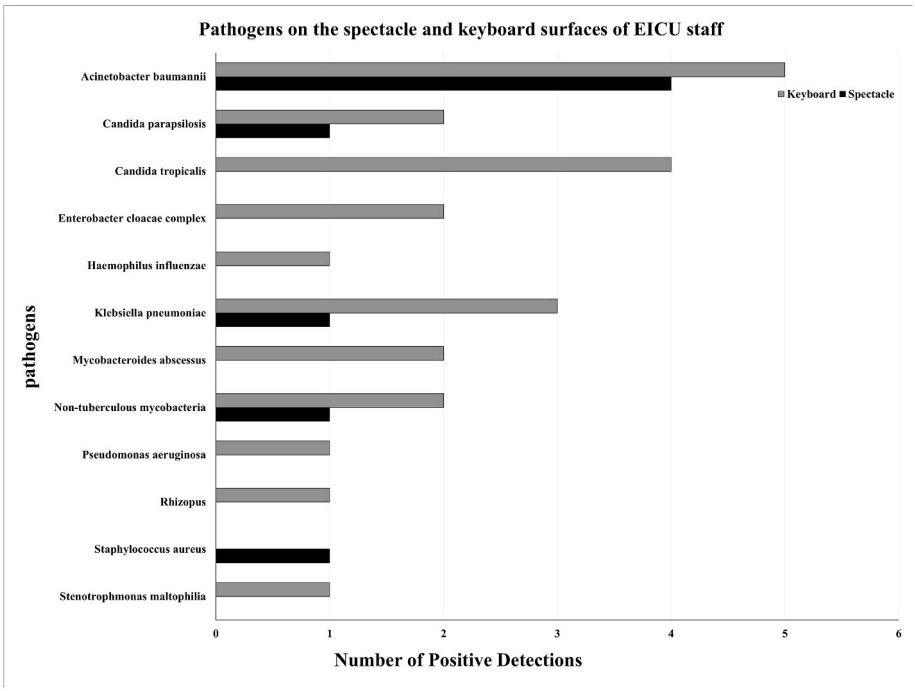

Fig. 3. Detection times for tNGS and bacterial culture. tNGS results were released within 19 h for all samples. For bacterial culture, 2 samples had positive results released within 19 h, 2 samples had positive results released within 69 h, and the remaining 19 samples had negative results released within 168 h.

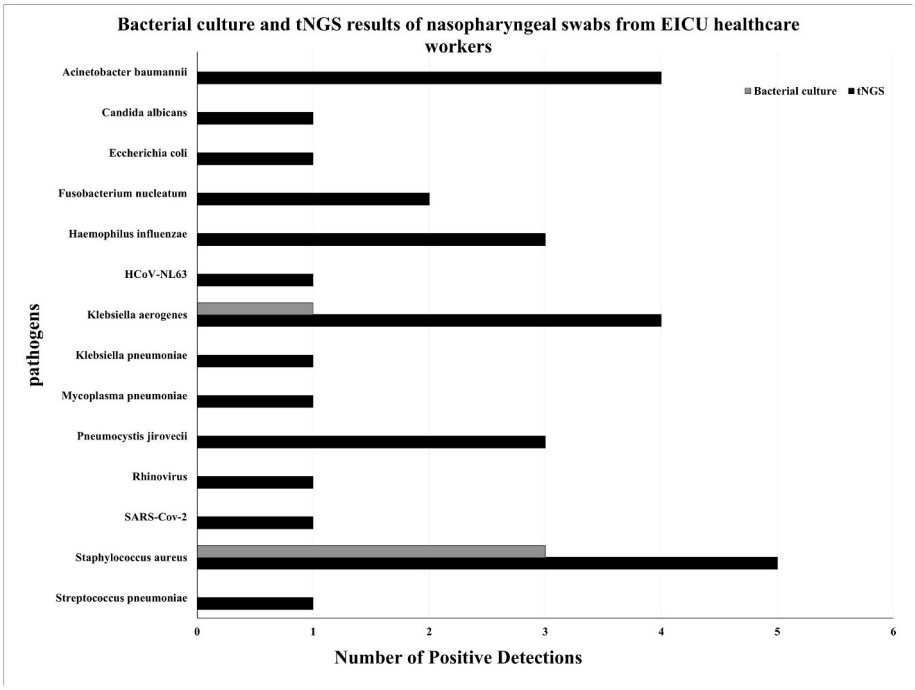

Fig. 4. The number of microorganism species detected in medical staff working in the EICU and PEC from nasopharyngeal swabs analysed using tNGS.Among the 23 EICU staff, 5had no microorganisms detected, 9 had 1 species of microorganism detected, 8 had 2 species of microorganism detected, and 1 had 4 species of microorganism detected. Among the 15 PEC staff, 6 had no microorganisms detected, 6 had 1 species of microorganism detected, 2 had 2 species of microorganism detected, and 1 had 3 species of microorganism detected.

multiple microorganisms, while bacterial culture testing usually reports only dominant bacteria and cannot fully reflect the microbial diversity in the sample [36]. Theoretically, tNGS can obtain results within 8–16 h, whereas bacterial culture testing usually takes 1–2 days to yield positive results and releases negative results on the 7th day of culturing. tNGS has a substantial advantage over traditional bacterial culture methods in hospital infection surveillance.

EICU medical staff had a higher average number of microorganisms detected in their nasopharyngeal swabs compared to the medical staff from the PEC. More individuals from the EICU had multiple microorganisms detected — particularly *Staphylococcus aureus* carrying the *mecA* gene and *Acinetobacter baumannii*, which were not detected in the

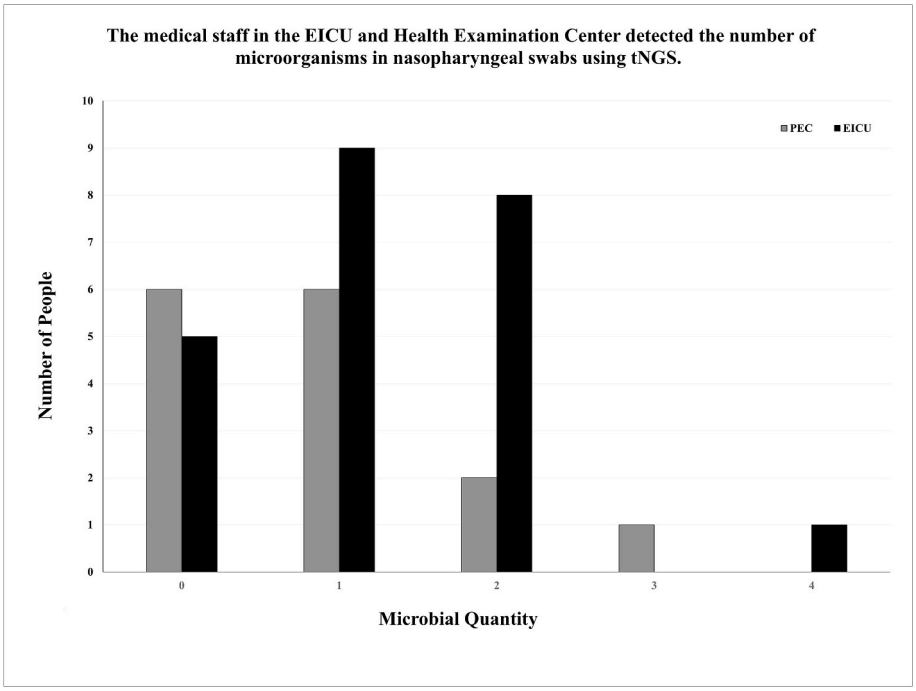

Fig. 5. The species and quantities of microorganisms detected in the EICU and PEC. A total of 42 instances of microorganisms were detected, comprising 14 different species. EICU medical staff had 29 instances of 14 species of microorganism detected. In the PEC, 13 instances of 6 species of microorganism were detected.

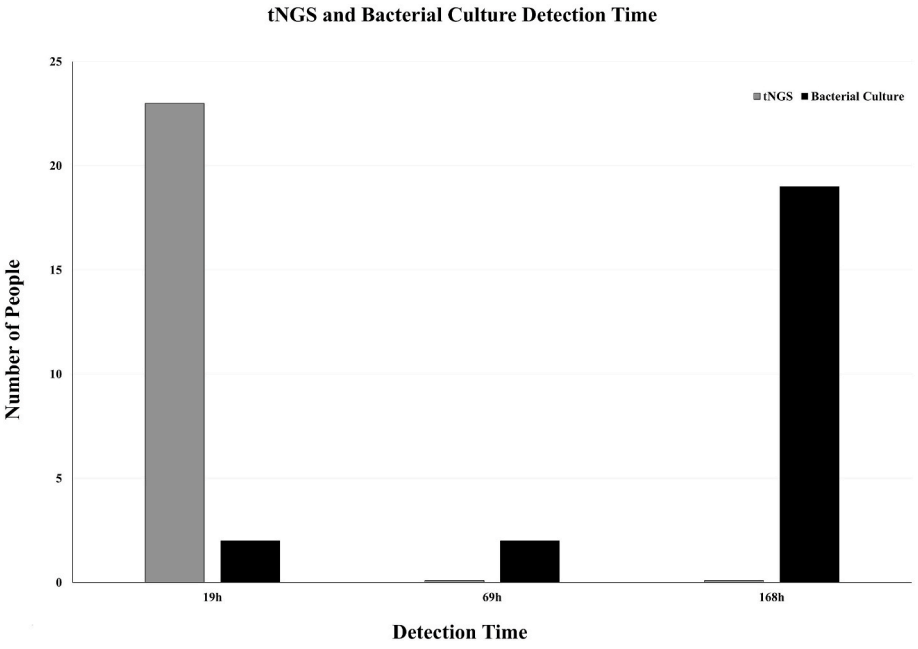

Fig. 6. Pathogens on the surfaces of spectacles and keyboards used by EICU staff. In the EICU, 6 surface swab samples from keyboards and 6 from spectacles were analysed, which resulted in a total of 32 instances of microorganisms being detected using tNGS, comprising 12 different species.

PEC staff but had detection rates of 10.3 % and 13.8 % respectively among EICU staff. This finding suggests that ICU staff are more susceptible to microbial contamination compared to their non-ICU counterparts. This disparity may be attributed to factors such as the higher prevalence of infected patients, elevated antibiotic resistance rates, and more frequent staff-patient interactions in ICUs. Notably, procedures performed post-treatment may further facilitate bacterial transmission in these high-risk environments [37,38].

The *mecA* gene encodes an altered penicillin-binding protein (PBP2a or PBP2'), which has very low affinity for β-lactam antibiotics, thereby mediating meticillin resistance [39]. *Staphylococcus aureus* that is positive for the *mecA* gene is referred to as MRSA. Colonization by MRSA within hospitals has been widely reported. A systematic review covering 16 studies indicated that the carriage rate of MRSA among ICU medical staff is 9.5 % [40]. Another systematic review reported the MRSA carriage rate among healthcare workers to be 1.8 %, with higher rates noted in emergency departments [41]. Furthermore, an Iranian study demonstrated a nasal MRSA carriage rate of 5.3 % among healthcare workers, with higher rates in the emergency and surgical departments [42]. Those reports are consistent with the findings of the present study,

highlighting the high carriage rates of MRSA among healthcare workers in emergency and intensive care units. This underscores the critical need for stringent infection control measures in these high-risk areas to mitigate the spread of resistant and potentially harmful microorganisms.

Acinetobacter baumannii also exhibited a high detection rate among EICU medical staff, yet it was not detected among the PEC staff. This could be because *Acinetobacter baumannii* is one of the most common pathogens of HAIs in intensive care units [43,44]. *Acinetobacter baumannii* not only has a high detection rate in the nasopharynx of emergency intensive care unit medical staff, but even more alarmingly is detected on the surfaces of items. Out of the 12 items, *Acinetobacter baumannii* was detected on 9 items, with a detection rate of 75 %. Previous studies have not researched microbial colonization on the surfaces of keyboards and eyeglasses. A European study on the microbial colonization of surfaces indicated that 100 % of ICU staff's mobile telephones were colonized by bacteria, with the most common being coagulase-negative staphylococci, *Bacillus* sp., and MRSA (97 %, 56 %, and 17 %, respectively) [45]. Another study isolated bacteria from 77.3 % of mobile telephones, with the most frequently isolated microorganisms being coagulase-negative staphylococci and *Staphylococcus aureus* [46]. Regarding stethoscopes, a study showed that out of 99 stethoscopes, bacteria could be cultured from 36 of them, 34 of which were Gram-positive bacteria, and the rest were Gram-negative bacteria. Among the 34 Gram-positive bacteria, 29 were identified as *Staphylococcus aureus*, and 5 as coagulase-negative *Staphylococci* [47]. *Acinetobacter baumannii* exhibits remarkable adaptability, continuously mutating to meet the demands of specific environments. Its biofilm formation mediates antibiotic resistance, thereby enabling its survival in ICU settings under high antibiotic pressure [48].

We also observed a high detection rate of *Klebsiella* species among healthcare workers in both the EICU and the PEC, with 13 individuals testing positive, resulting in a detection rate of 28.9 %. *Klebsiella* species are typically transmitted during hospital care procedures. Research has indicated that interaction with patients carrying *Klebsiella* species results in the contamination of gloves or protective clothing in 14 % of healthcare workers [49]. Another study has shown that assisting patients with toileting, handling wet secretions, and providing in-bed baths are high-risk factors for the transmission of *Klebsiella* species [50]. This indicates that our medical staff were likely contaminated with *Klebsiella* species from patients during healthcare procedures.

Three healthcare workers in the EICU were found to have *Pneumocystis jirovecii*. There is less research on colonization by *Pneumocystis jirovecii*, in comparison to bacteria, primarily because it cannot be cultured *in vitro*. Studies have used serum 1,3-β-D-glucan combined with metagenomic next-generation sequencing (mNGS) to detect colonization by *Pneumocystis jirovecii* in patients with unexplained pulmonary infiltrates, showing a sensitivity of up to 95.5 % [51]. Other studies have investigated colonization in patients with underlying pulmonary conditions, including a PCR test on the bronchoalveolar lavage fluid (BALF) of 80 patients with interstitial pneumonia, finding a colonization rate of 33.8 % for *Pneumocystis jirovecii* [52]. In healthy adults, some studies have not detected *Pneumocystis jirovecii* colonization, while others have found about 20 % of adults to be colonized [53,54]. Among healthcare workers, there are studies indicating colonization of *Pneumocystis jirovecii* in two medical doctors who performed bronchoalveolar lavage on patients with *Pneumocystis jirovecii* pneumonia [55]. Herein three EICU healthcare workers tested positive for *Pneumocystis jirovecii*, all of whom were nurses and had provided care for patients with *Pneumocystis jirovecii*, suggesting possible transmission from patients to nurses during their work.

Our study found that the microorganisms colonizing surfaces were more often Gram-negative bacteria, fungi, and non-tuberculous mycobacteria, which are more likely to be HAI pathogens rather than normal environmental colonizing bacteria, compared to Gram-positive bacteria like coagulase-negative *Staphylococci* and *Staphylococcus aureus*. This may be related to the following factors. First, our use of the tNGS method is more sensitive than culture methods for detecting microorganisms that have special or stringent culture conditions, in particular fungi and *Mycobacteria*. Second, the items we sampled differ from those in other studies. Our sampled keyboards were placed in the EICU for a long time, and the keyboards have many crevices that are difficult to clean thoroughly. Spectacles are items that need to be worn for a long time and have many opportunities to be contaminated by droplets and sputum during daily medical and nursing work. Compared to hand hygiene, cleaning the surface of eyeglasses is often overlooked.

We also compared tNGS with bacterial culture testing and found that tNGS detected a considerably higher variety and number of species of microorganisms compared to bacterial culture testing, and it provided results more quickly. tNGS can simultaneously detect over 100 types of microorganism, including bacteria, fungi, and viruses, and can deliver results in as little as 8 h. By contrast, bacterial culture can only detect bacteria — and it struggles to yield positive results for anaerobic bacteria, *Mycobacteria*, and other bacteria requiring special culture conditions. Typically, positive results from bacterial culture are available within 1–2 days, while negative results are not reported until the 7th day of culturing. tNGS offers a definite advantage over traditional bacterial culture methods in hospital infection monitoring.

In conclusion, tNGS outperforms conventional culture in healthcare-associated infection surveillance, with higher sensitivity (69 % vs. 28 %) and accelerated pathogen identification (19 h vs. 69 h). Simultaneously, tNGS revealed extensive colonization of multidrug-resistant pathogens (e.g., Acinetobacter baumannii, MRSA) in EICU environments, highlighting its utility in monitoring complex antimicrobial resistance patterns.

CRediT authorship contribution statement

Tangchun Liu: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Shuyan Deng:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Wandi Liu:** Software, Investigation, Formal analysis. **Jinzhao Zhang:** Software, Investigation, Formal analysis. **Pengfei Wang:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Zhengfei Yang:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Data availability

Data will be made available on request.

Ethical approval

This study has been approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (Ethics Approval Number SYSKY-2024-817-01).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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