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ORIGINAL ARTICLE

Prevalence of *Burkholderia cepacia* recovered from clinical specimens in the Zainoel Abidin general hospital, Banda Aceh, Indonesia

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ABSTRACT

Background and Objectives: *Burkholderia cepacia* is one of the multiple intrinsic resistant bacteria causing opportunistic infections. The study aimed to determine the distribution of *B. cepacia* isolates based on types of clinical specimen, hospital wards, and the patient's gender-age and to evaluate their antibiotic susceptibility.
Materials and Methods: This study involved isolating, identifying, and testing antibiotic susceptibility of *B. cepacia* isolates recovered from clinical specimens of Dr. Zainoel Abidin general hospital (RSUDZA) Banda Aceh Indonesia during March 2019-March 2022.
Results: In total, there were 3,622 Gram-negative bacterial isolates of 10,192 clinical specimens obtained during the study period and *B. cepacia* was positively detected in 127 isolates (1.24%). Most of the 127 isolates of *B. cepacia* were found in blood and sterile body fluid samples (55.11%) followed by urine and pus samples accounting for 23.62% and 13.37%, respectively. The internal medicine wards had the highest number of detected *B. cepacia* isolates at 28.3%. *B. cepacia* infections were more common in men (59.05%) and people over 45 years old (41.73%). The bacteria were highly sensitive to the antibiotic ceftazidime (92.7%).
Conclusion: Culture examination of clinical specimens is not required for confirmed infections, despite being essential for appropriate antibiotic treatment. Implementing surveillance programs and judicious use of antibiotics can prevent bacterial transmission.

Keywords: Antimicrobial resistance; *Burkholderia cepacia*; Prevalence

INTRODUCTION

Bacterial infections remain the major concern generating significant ramifications in communal and healthcare settings. *Burkholderia cepacia* often referred to as the *Burkholderia cepacia* complex (Bcc) is one of the pathogenic bacteria causing opportunistic infections. *Burkholderia cepacia* complex are free-living, motile, bacilli-shaped organisms that do not form spores and belong to the group of aerobic Gram-negative bacteria that do not ferment glucose and comprise of nine genomovars, namely *B. cepacia* (genomovar I), *B. multivorans* (genomovar II), *B. cenocepacia* (genomovar III), *B. stabilis* (genomovar IV), *B. vietnamiensis* (genomovar V), *B. dolosa* genomovar VI, *B. ambifaria* (genomovar VII), *B. an-*

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thina (genomovar VIII), and *B. pyrrocinia* (genomovar IX) (1, 2).

Blood agar and MacConkey agar are commonly used to grow pathogenic Gram-negative bacteria, including *B. cepacia*. These bacteria typically produce colonies with rounded edges and a convex shape on these media. However, the colony color can vary depending on the medium, with *B. cepacia* colonies appearing white on blood agar and colorless to opaque on MacConkey agar. While these media are not specific for *Burkholderia cepacia*, other selective media such as *Burkholderia cepacia* selective agar, BD Cepacia media, and MAST Cepacia media can be used to prevent the growth of other Gram-negative bacteria and fungi (3).

In hospital, *Burkholderia cepacia* have been linked to major contaminant for solution, medication, and equipment in healthcare facilities. It was reported that, the pathogens contaminated alcohol-free mouthwash solution leading to healthcare-associated respiratory tract infection and colonization (4). Moreover, *B. cepacia* was also detected as contaminant in hemodialysis machines leading to bacteremia in patients in Brazil (5). Additionally, the pathogens also contaminated 0.5% chlorhexidine gluconate (CHG) solution which is widely used as antiseptic for skin disinfection, hand washing, and oral hygiene (6).

In addition to bacterial contaminants, *B. cepacia* are also involved in healthcare-associated infections. As any other opportunistic pathogens in hospital settings, *B. cepacia* are causative agents for infections in pre-infected individuals or individuals with weakened immune systems, such as in cystic fibrosis (CF) patients (7). *B. cepacia* infection in patients with cystic fibrosis (CF) cause a significant decrease in lung function that can progress into a life-threatening systemic infection and necrotizing pneumonia called cepacia syndrome (8). Cepacia syndrome patients would exhibit fever, respiratory distress, leukocytosis, pulmonary infiltration, bronchopneumonia, and confluent septicemia (9). In addition to CF patients, *B. cepacia* also cause serious infections in non-CF patients. It was reported that *B. cepacia* affects patients with co-morbidities such as chronic granulomatous, hematological malignancies, chronic renal failure, and uncontrolled diabetes mellitus (10).

B. cepacia are highly problematic to manage the dissemination and persistence of the pathogens. *B. cepacia* harbor resistance to multiple antimicrobial resistance so that these bacterial pathogens fail to respond to treatment leading to prolonged hospitalization and the increasing mortality risk (11). Another reason for persistence of *B. cepacia* is its ability of forming biofilm structures allowing the pathogen to attach and colonize on both biotic and abiotic surfaces leading to persistent infections (12).

In Indonesia, *B. cepacia* infections have not been widely studied and antibiotics to treat the infections are limited. Therefore, the present study was conducted to evaluate the prevalence and determine the antibiotic susceptibility of *Burkholderia cepacia* isolates recovered from clinical specimens of patients at RSUDZA Banda Aceh, Indonesia.

MATERIALS AND METHODS

Clinical sample collections. Blood, urine, sputum, body fluids, pus, and swabs were collected from inpatients and outpatients at the Zainoel Abdin general hospital in Banda Aceh, Indonesia, between March 2019 and March 2022. All clinical samples were evaluated for quality and the types of clinical samples. The research has been approved for ethics from the Ethics Committee for Health Research, Faculty of Medicine, Universitas Syiah Kuala (No. 399/EA/FK-RSUDZA/2021).

Bacterial isolation, observation, and identification. Except for blood samples, which were pre-incubated using BacT/ALERT® 3D (Biomeriux, Lyon, France), all clinical samples were inoculated on blood agar and MacConkey agar (Biomeriux, France) plates according to the manufacturer's instructions. The plates were then incubated for 24 hours at 35 ± 2 degrees Celsius before being Gram-stained and inspected for their morphology under a microscope with 1,000x magnification.

VITEK® 2 Compact (Biomeriux, Lyon, France) was used for further identification as well as antibiotic susceptibility assay. A pure bacterial colony obtained from clinical samples was suspended in 0.45% NaCl solution, which was equal to 0.50 - 0.63 McFarland Standard solution, and then inoculated onto Gram-negative (GN) cassettes for identification and antibiotic susceptibility testing. Susceptibility testing was determined for the following antibiotics: amikacin, cefotaxime, cefoperazone, ceftazidime, ceftriaxone, doxycillin, gentamicin, imipenem, levofloxacin, piperacillin/tazobactam, and tobramycin.

Statistical analysis. The distribution of *B. cepacia* isolates was descriptively evaluated based on clinical species, hospital wards, age, and gender. All data were tallied using Microsoft Excel to offer descriptive information in tables or charts. When applicable, a statistical analysis was conducted using the Chi-square test or Fisher's exact test. At a two-tailed significance level of $p \leq 0.05$, all XLStat Cloud (Addinsoft, New York, USA) tests were considered statistically significant.

RESULTS

Bacterial isolation, observation, and identification. Bacteria growing on blood agar media showed colony with white, rounded shape, smooth edges, and convex elevation (Fig. 1a), whereas on MacConkey Agar the bacteria exhibited opaque colonies with rounded shape, smooth edges, and convex elevation (Fig. 1b). Based on the results of the Gram stain that has been carried out, bacterial cells of *B. cepacia* showed Gram negative bacilli (rod) shapes with pink cell color (Fig. 1c).

Distribution of *Burkholderia cepacia* isolates. Fig. 2 depicts the distribution of *B. cepacia* isolates based on the clinical specimen submitted to the lab for testing. During the period of present study (March 2019 to March 2022), a total of 10,192 clinical samples showing microbial growth were submitted for testing, and *Burkholderia cepacia* was positively detected in 127 (1.24%) of these clinical samples.

The majority of the 127 *B. cepacia* isolates were found on blood and sterile body fluids samples, followed by urine and pus samples, which accounted for 70 (55.11%), 30 (23.62%), and 12 (13.37%) isolates, respectively (Fig. 3). Based on the units, *B. cepacia* isolates were predominantly detected on clinical specimens from the internal medicine wards accounting for 36 (28.3%) isolates. The distibution of *B. cepacia* based on the hospital wards and types of clinical specimens in the present study was highly significant linked ($p < 0.0001$; $\chi 2 = 105.18$), i.e., there was association affecting the distribution of *B. cepacia* isolates on the hospital wards and types of clinical specimens.

In terms of patients' gender and age, men are riskier to be infected rather than women (59.05% vs 40.94%) (Fig. 4). Moreover, the majority of *B. cepacia* isolates (41.73%) were identified in individuals aged 46 to 55 years. The distibution of *B. cepacia* based on the patients' gender and age groups in the present study was statistically significant ($p = 0.003$; $\chi 2 = 21.51$), i.e., the distribution of *B. cepacia* isolates was associated with patients' gender and age groups.

Antibiotic susceptibility of *Burkholderia cepacia* isolates. *B. cepacia* isolates (n=127) exhibited high sensitivity to ceftazidime (92.7%) and intermediate sensitivity to cefoperazone (76.9%), ceftriaxone (66.6%), and cefotaxime (65.9%), levofloxacin (63.4%), and doxycycline (53.2%). However, *Burkholderia cepacia* isolates demonstrated low sensitivity to imipenem (40.4%), amikacin (38.7%), tobramycin (36.5%), gentamicin (34.6%), and piperacillin/tazobactam (30.2%) (Fig. 5).

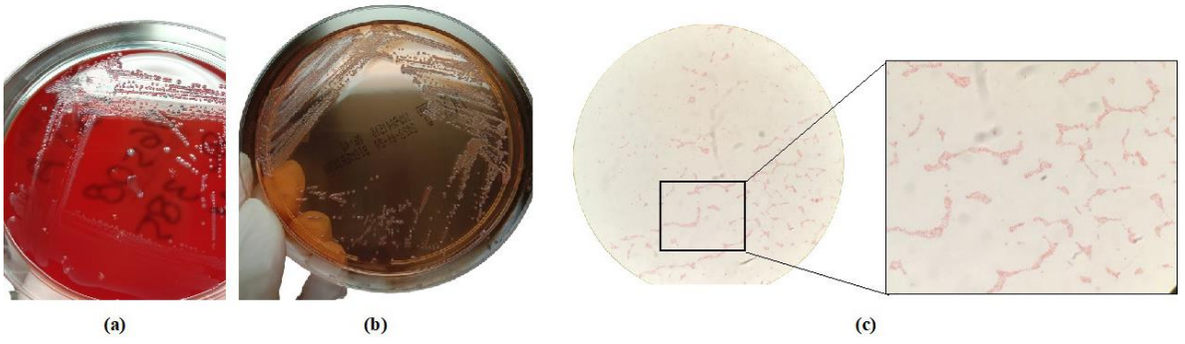

Fig. 1. Growth of *Burkholderia cepacia* after 24 hours showing (a) white, rounded shape, smooth edges, and convex elevation colonies on blood Agar media and (b) opaque, rounded shape, smooth edges, and convex elevation colonies on MacConkey media; (c) Cells of *Burkholderia cepacia* Gram staining results showing Gram-negative bacilli (rod) shapes observed under a microscope at a magnification of 1000x.

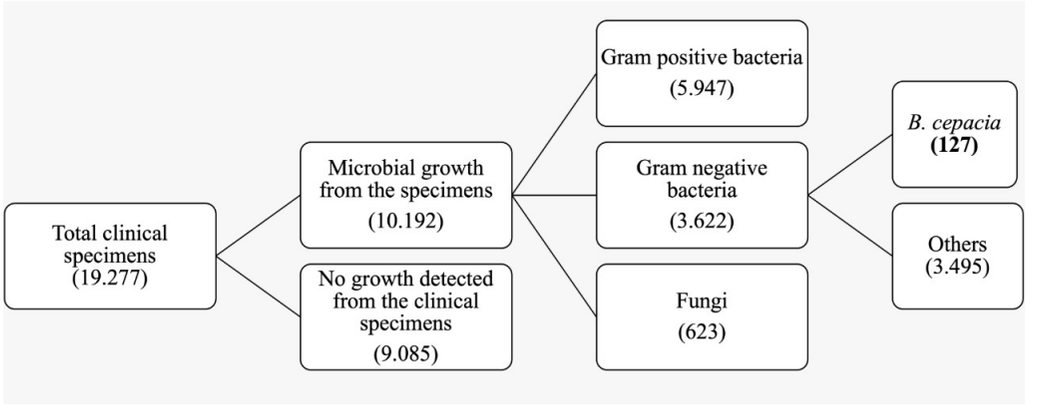

Fig. 2. Distribution of *Burkholderia cepacia* isolates (n = 127) relative to Gram-positive and Gram-negative bacterial as well as fungal pathogens isolated from clinical specimens during a study period of March 2019 – March 2022 at the Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia. Numbers in brackets represent total clinical specimens or isolates.

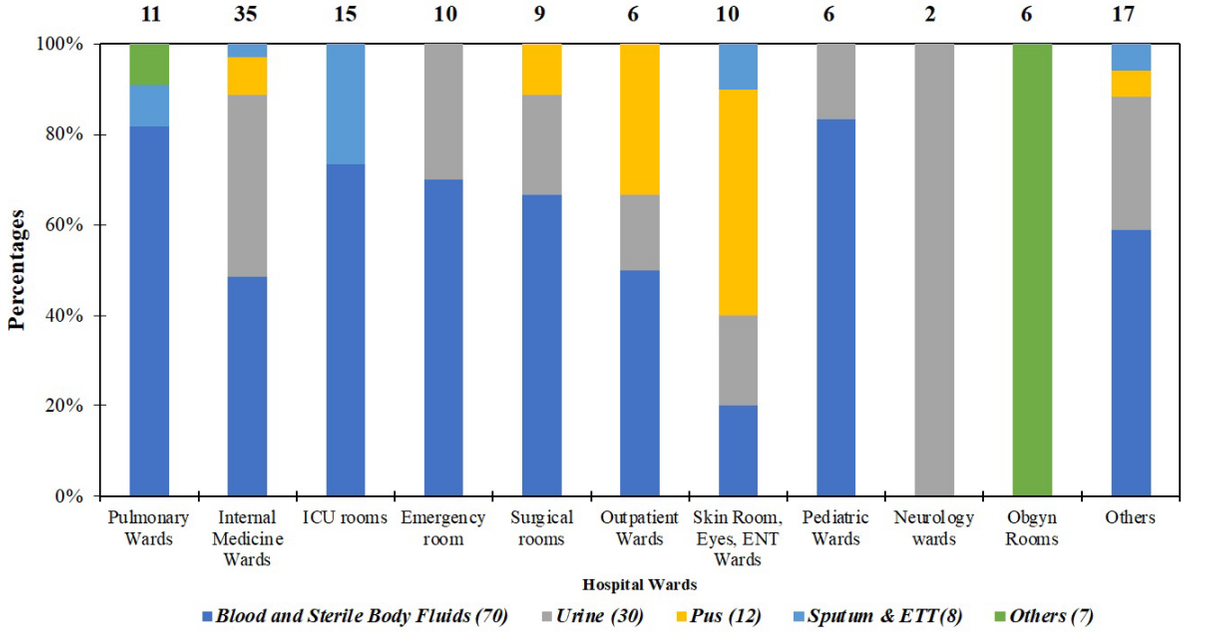

Fig. 3. Frequency of occurrence (%) of *Burkholderia cepacia* isolates (n = 127) distributed based on types of specimens collected in a variety of hospital wards during a study period of March 2019 – March 2022 at the dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia. Numbers above each column are the total number of clinical isolates positive for *Burkholderia cepacia*. Numbers on the right of the chart legend in brackets represent total clinical isolates for each clinical specimen. Based on the Chi-square test for independence test, the types of clinical specimens and hospital wards were highly linked ($P < 0.0001$; $\chi 2 = 105.18$).

DISCUSSION

Due to their genomic plasticity and metabolic adaptability, *Burkholderia cepacia* has been recognized as one of the concerning bacteria causing infections in hospital and environmental settings. During the study period (March 2019 to March 2022), 10,192 clinical samples were tested, and *B. cepacia* was found in roughly 127 isolates (1.24%) of these clinical samples. The results of this study were lower than the results of a previous study that found 150 isolates of *B. cepacia* from 112 patients in a hospital in Shanxi, China (13). However, the results of the present study were higher than those of another study that detected 29 isolates at a hospital in Bali, Indonesia (14).

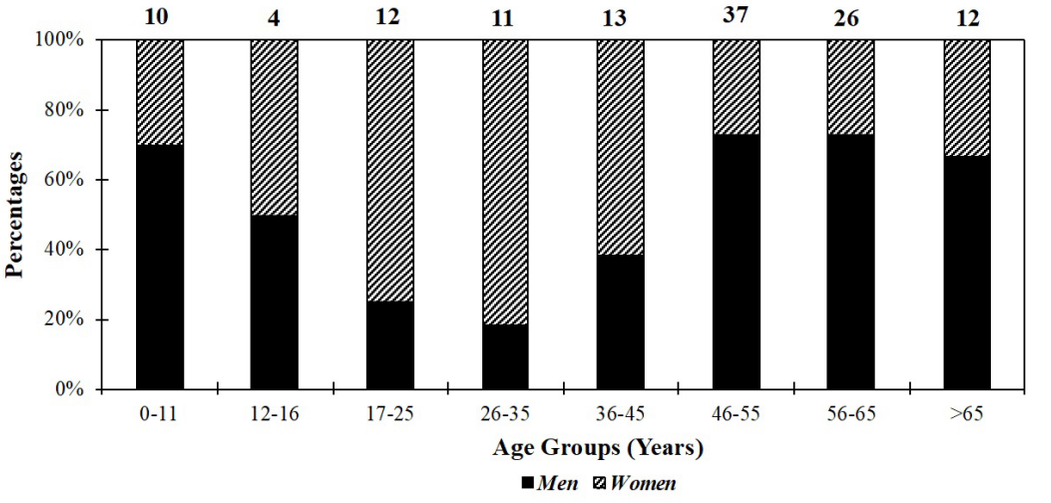

Fig. 4. Frequency of occurrence (%) of *Burkholderia cepacia* isolates (n =127) based on patients' gender and age groups in the Zainoel Abidin general hospital in Banda Aceh, Indonesia during a period of March 2019 – March 2022. Numbers above each column are the total number of *Burkholderia cepacia* isolates.

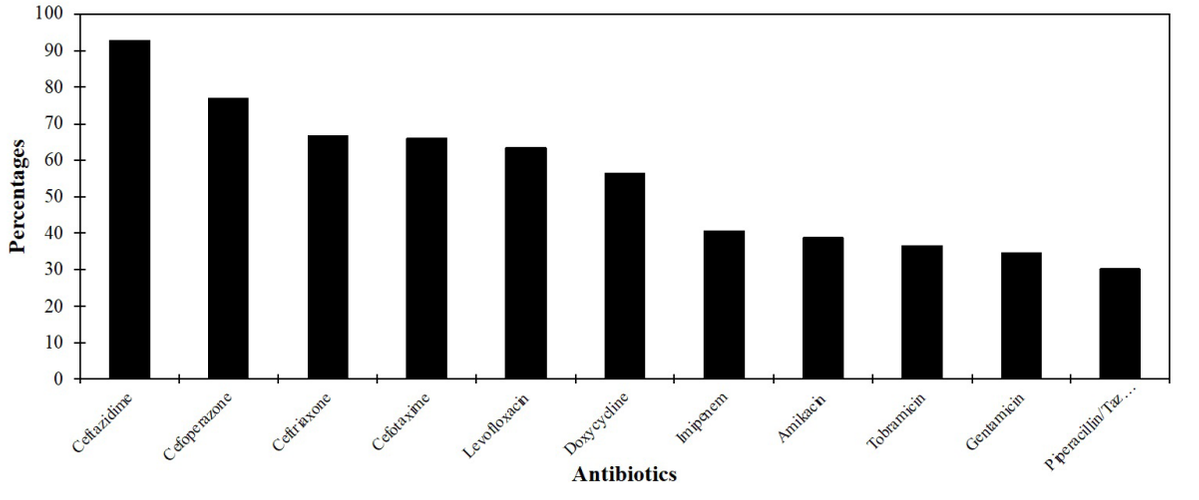

Fig. 5. Antibiotic susceptibility of *Burkholderia cepacia* (n =127) isolates of clinical specimens collected from patients in the Zainoel Abidin general hospital in Banda Aceh, Indonesia during a period of March 2019 – March 2022.

In the present study, *B. cepacia* isolates were more predominantly detected in blood samples indicating the higher prevalence of bloodstream infections (BSI) or bacteremia. Most of these bacteria isolated from blood cultures come from patients who have a background of end stage renal disease. This is in accordance with previous study demonstrated that *B. cepacia* were the most commonly found in blood specimens accounting for which was associated with bloodstream infections (15, 16). Moreover, other investigators highlighted the prevalence of *B. cepacia* detected in 30 patients with bloodstream infections during two-year period (17) indicating *B. cepacia* as emerging pathogens in blood stream infections. Furthermore, *B. cepacia* as BSI pathogens are also prevalently detected in patients with central venous catheters. It was previously reported that *B. cepacia* infections occurred in 42.1% of 216 non-CF patients with bacteremia using central venous catheter (CVC) (18). The organic matter present in the implanted catheter for a certain period of time creates a favorable environment for bacterial colonization and the formation of microbial biofilms. Biofilm formation begins when microbes stick to surfaces by creating

extracellular polymers that mediate adhesion and structural matrix formation; in the patient's body, *B. cepacia* biofilm formation occur if adhesion to the organic environment already exists (12). In addition to blood samples in the present study, *Burkholderia cepacia* isolates were also detected in urine (23.62%) indicating the occurrence of the pathogens as ethiological agents for urinary tract infections. *Burkholderia cepacia* is one of the Gram negative uropathogenic bacteria causing urinary tract infections (UTI) after *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (19).

The present study found that patients infected with *Burkholderia cepacia* were predominantly detected in the Internal Medicine wards. *B. cepacia* is known to cause various diseases and is often found in patients with a history of conditions that affect the function of internal organs, such as cystic fibrosis (7, 8), infectious endocarditis (20), and chronic granulomatous disease (10). In addition to the internal medicine wards, patients treated in ICU rooms also had a higher number of *B. cepacia* isolates during the study, similar to previous research showing a higher occurrence of *B. cepacia* in ICU rooms compared to non-ICU rooms (14).

In this investigation, men were more likely to be infected with *B. cepacia* than women. This is consistent with previous research showing that men are more susceptible to these infections than women (14, 15, 21). It is believed that this may be due to the role of genetic and hormonal factors, i.e. the X chromosome and the hormone estrogen, in the immune response to infectious agents. In men, sex hormones may regulate the immune response in a way that leads to more severe and prolonged responses to infection, resulting in more systemic damage compared to women (22). In addition, the majority of cases of *B. cepacia* infection were found in individuals aged 46 to 55 years which was in line with previous findings (21, 23). This may be due to the effects of aging on the immune system, as well as other risk factors such as previous infections and invasive medical procedures (24).

B. cepacia isolates in the present study remain high sensitivite to ceftazidime (92.7%) and cefoperazone (76.9%). These antibiotic susceptibility results showed similarity with others signifying the high sensitivity *Burkholderia cepacia* to ceftazidime and/or cefoperazone (13, 17, 25). The use of surveillance programs and careful administration of antibiotics can help prevent the spread of *B. cepacia* infections, even though some medications are still effective against it. This is because other groups of *Burkholderia* bacteria, such as *Burkholderia pseudomallei*, which causes meliodosis and has been detected in human and environmental samples, can develop resistance to pan-drug resistance in certain regions (26), including Indonesia (27).

CONCLUSION

During the three-year period from March 2019 to March 2022, the prevalence of *Burkholderia cepacia* infections was around 1.2%. The bacteria appear to be linked to bloodstream infections or bacteremia, based on the prevalence of *Burkholderia cepacia* isolates found in blood and sterile body fluids. Furthermore, the infection was more common in men and individuals over the age of 45. While ceftazidime and cefoperazone are still effective in treating *Burkholderia cepacia* infections, establishing regular surveillance programs and using antibiotics wisely may help to reduce the spread of the infection, particularly in hospital settings.

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