

## Epidemiology of Nosocomial Infections in the Intensive Care Unit (ICU) at Beni-Suef Hospital and their Control Strategies

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### ABSTRACT

Nosocomial infections are more prevalent in the Intensive Care Unit (ICU) than in other hospital departments. This study aimed to elucidate the epidemiology and characteristics of ICU-acquired infections, with a focus on the impact of various risk factors. This study retrospectively analyzed data from 174 ICU patients admitted between December 2022 and November 2023 in Beni Suef Hospital, Beni Suef, Egypt. The primary focus was on the epidemiology of bacterial infections, including their incidence, causative organisms, and infection sites. Additionally, the study aimed to identify risk factors associated with ICU-acquired infections. Out of 174 patients, 98 (56.3%) acquired nosocomial infections in the ICU. *Acinetobacter baumannii* was the primary causative agent (19.0%), with urinary tract infections being the most common type (29.0%). Patients with nosocomial infections experienced longer mechanical ventilation and ICU stays. However, mortality rates did not significantly differ between infected and non-infected patients. Independent risk factors identified through multivariate analyses included intubation, urinary catheterization, and mechanical ventilation each lasting over 7 days, as well as an ICU stay exceeding 7 days. This study highlights a substantial occurrence of nosocomial infections in the ICU and identifies specific risk factors associated with their acquisition in this critical care setting.

**Keywords:** Epidemiology; Hospital surveillance; ICU patients; ICU-acquired infection; Nosocomial infection.

### INTRODUCTION

Nosocomial infections, or hospital-acquired infections, pose a significant risk to patient safety in healthcare facilities, particularly in Intensive Care Units (ICUs) where their occurrence is notably higher than in other hospital departments (Ramaraj, 2014). This increase affects mortality and morbidity patterns in ICUs and imposes considerable financial burdens on individuals and society (Campanella *et al.*, 2023). Nosocomial infections, also known as Healthcare-Associated Infections (HAIs), occur in patients receiving medical care in a hospital or healthcare facility and were not present prior to admission (van Buijtene and Foster, 2019).

Nosocomial infections can manifest during the treatment of another ailment or even after the patient has been discharged from the healthcare facility. Additionally, these infections can occur when healthcare workers contract them while performing their professional duties (Henderson *et al.*, 2010). These infections are closely associated with the use of invasive medical devices common in modern healthcare, such as ventilators and catheters (Dadi *et al.*, 2021). In affluent nations, one of these healthcare-related infections can affect up to seven out of every 100 hospitalized patients, while in developing countries, the figure rises to ten out of every 100 patients (Iscimen *et al.*, 2008). Populations particularly

vulnerable to these infections include patients in burn units, intensive care units (ICUs), organ transplant recipients, and newborns. Nosocomial infections can also encompass those contracted by hospital staff, visitors, or other healthcare professionals (Douglas *et al.*, 2023). Infections excluded from the nosocomial classification comprise: (1) infections existing at the time of admission but deteriorating, with alterations in the pathogens or symptoms leading to a new infection; and (2) infections acquired trans-placentally under specific conditions such as cytomegalovirus, toxoplasmosis, rubella, or syphilis, which become apparent 48 hours after birth (Zinjani, 2023).

Nosocomial infections, commonly caused by bacteria like *Staphylococcus aureus*, *E. coli*, *Enterococci*, and fungi such as *Candida*, often originate from sources like urinary catheters, surgical procedures, and central venous catheters (Joshi *et al.*, 2019). These infections are prevalent in healthcare settings due to factors like inadequate hygiene and antibiotic-resistant strains like Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Garoy *et al.*, 2019). Urinary catheters, used to assist patients with bladder control issues, can introduce pathogens leading to urinary tract infections characterized by symptoms like painful urination and fever (Warren, 2001). Surgical site infections, occurring post-surgery, result from pathogens on the skin or the operating room environment, manifesting in symptoms such as skin

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redness and tenderness (Iyengar *et al.*, 2021). Central venous catheters, inserted into large veins for therapies like total parenteral nutrition (TPN), can cause bloodstream infections due to pathogens entering the skin during insertion. These infections have a high mortality rate and symptoms may include skin redness and drainage at insertion sites (Hammes *et al.*, 2015).

Transmission pathways of nosocomial infections within healthcare settings encompass various modes, each presenting unique challenges for infection control. Direct contact, a common route, involves physical interaction with infected individuals or contaminated surfaces, facilitating the transfer of pathogens (Zhang *et al.*, 2020). Indirect contact occurs when susceptible individuals come into contact with objects or surfaces contaminated by infected individuals, amplifying the risk of transmission. Airborne transmission poses a significant threat, with pathogens spreading through respiratory droplets or particles expelled by infected individuals, remaining suspended in the air, and inhaled by others (Sze-To *et al.*, 2014). Nosocomial infections can spread through various transmission pathways: droplet transmission, which requires close proximity; fomite transmission, involving inanimate objects that carry infectious agents; and vectors such as insects or animals, as well as waterborne sources and contaminated food or beverages. Understanding these pathways is crucial for effective infection prevention and control in healthcare settings. The National Nosocomial Infections Surveillance System defines nosocomial infections as localized or systemic conditions resulting from an adverse response to infectious agents or their toxins. These conditions were not evident in the incubation phase during the initial entry of the patient into the hospital (Samson, 2020). In essence, nosocomial infections are those acquired during a hospital stay, distinct from infections present or developing before admission (Sharma and Paul, 2023). Such infections can manifest in specific body sites or affect the entire system, posing challenges to patient well-being and necessitating careful monitoring and preventive measures within healthcare settings (Dhole *et al.*, 2023). Nosocomial infections that are a matter of considerable concern are recognized by assessing their frequency and potential severity (Choudhuri *et al.*, 2017). The study aims to thoroughly investigate the factors contributing to these infections, the microorganisms responsible, and their impact on ICU mortality rates and patient length of stay, with the goal of improving outcomes and intervention strategies (Choudhuri *et al.*, 2017). The rise and dissemination of antibiotic resistance across diverse pathogens pose a substantial and increasing global health concern (Castañeda-Barba *et al.*, 2024). Over time, bacteria, viruses, fungi, and other microorganisms have developed the ability to withstand the effects of commonly used antibiotics, rendering once-effective treatments ineffective (Sharma and Yumnam, 2024). The rise of antibiotic-resistant pathogens poses a significant public health challenge, making the treatment of infections more difficult and leading to

increased illness, mortality, and healthcare costs. To address this issue, it is crucial to understand the mechanisms of resistance, the factors contributing to its spread, and the specific pathogens involved. This introduction will explore the landscape of antibiotic resistance, focusing on the factors driving it and the need for innovative solutions to preserve antibiotic effectiveness. By analyzing these dynamics, the aim is to improve our understanding of nosocomial infections in ICU settings and develop more effective prevention and management strategies.

## MATERIALS AND METHODS

### Study design and data collection

A retrospective observational study was conducted using prospectively gathered data from a group of 174 consecutive patients admitted to a seven-bed mixed medical-surgical Intensive Care Unit (ICU) at Beni-Suef Hospital, Beni-Suef, Egypt, between December 2022 and November 2023. Ethical approval for the study was obtained from the Faculty of Medicine Beni-Suef University Research Ethical Committee (approval number: FMBSUREC/02012024/Bani Melhem).

### Data retrieval and criteria definition

Patient data, covering the period from December 2022 to November 2023, were obtained from the legitimate database preserved for clinical and administrative purposes. The inclusion criteria comprised patients with an ICU stay exceeding 2 days. Infections acquired in the ICU were characterized as infections that emerged within the ICU after 2 days of admission, differentiated from preceding infections based on clinical and microbiological criteria.

### Clinical samples and method of collection

The selection of the clinical samples and the collection method may vary based on the suspected infection and the type of organism implicated as illustrated in Figure (1). In the case of samples collected from surgical wounds, a sterile swab was rubbed over the infected area, in the case of a tissue biopsy, a sample was taken from the affected site using a sterile procedure (Ben-Artzi *et al.*, 2023). In the case of isolation from a blood sample, aseptic techniques were used to draw blood from a vein, and the sample was then cultured to isolate any microorganisms (Ko *et al.*, 2023). In urine samples, the initial stream was not collected to avoid contamination, as recommended by Ribay *et al.* (2023). For catheterized samples, a sterile catheter was used, and the initial 15-30 mL of urine was discarded before collecting the sample to minimize contamination risks. Throat samples were collected using a sterile swab, following standard procedures to ensure accuracy and reliability in testing (Otu *et al.*, 2023). This method is commonly employed for the isolation of respiratory pathogens.

### Comprehensive analysis of ICU-acquired bacterial infections, risk factors, and patient outcomes

The primary aim was to assess the epidemiology of bacterial infections acquired in the ICU, with a

focusing on the occurrence of new infections, the organisms responsible, and the locations of infection. Secondary objectives included the evaluation of risk factors related to ICU-acquired infections, the comparison of demographic and clinical factors between groups with and without nosocomial infections, and the examination of outcomes for patients with ICU-acquired infections, including mortality, duration of mechanical ventilation, and ICU length of stay.

#### Isolation and identification of bacterial isolates

Ten strains were cultured in the Laboratory of Beni-Suef Hospital. The selection of these bacterial strains for this study was based on their prevalence and clinical significance in hospital settings, particularly within the ICU. These strains were chosen due to their common occurrence in nosocomial infections and their impact on patient outcomes. Where bacterial strains were introduced to designated culture media, and identification was conducted through an analysis of culture, morpho-tinctorial characteristics, and biochemical properties (Okoye *et al.*, 2023). The pathogens responsible for nosocomial infections in the ICU were cultured using various media: Blood Agar, Chocolate Agar, MacConkey Agar, Middlebrook Agar, and Mannitol Salt Agar, and incubated at 37 °C for 24 hours but in the case of Middlebrook Agar, incubation was extended for up to 6 to 8 weeks to ensure proper growth (Atmanto *et al.*, 2022).

#### Molecular identification of bacterial culture

Molecular identification was performed on the ten chosen bacterial isolates based on their clinical significance and prevalence in nosocomial infections. Polymerase chain reaction (PCR) was performed to amplify a conserved region within the bacterial 16S rRNA gene using specific primers. The PCR conditions included an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was

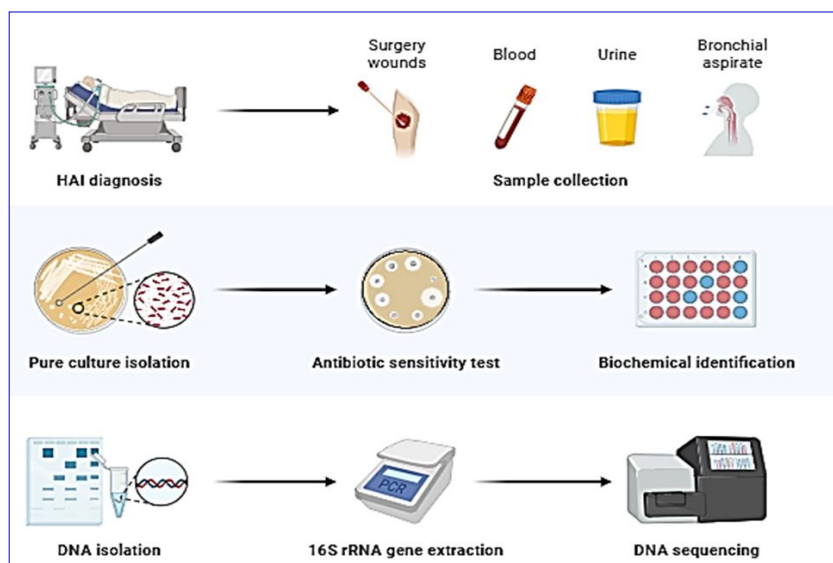
performed at 72°C for 5 minutes. The primers used were: forward primer (27F) 5'-AGAGTTTGA-TCMTGGCTCAG-3' and reverse primer (1492R) 5'-TACGGYTACCTTGTTACGA-CTT-3'. These primers target a conserved region of the 16S rRNA gene. The amplified product was sequenced using the Big TriDye sequencing kit (ABI Applied Biosystems) at Macrogen, Korea. The sequence was submitted to the GenBank NCBI database, and homology was confirmed by conducting a BLASTn search at NCBI BLAST. The bacterial strain's identity was validated through these analyses. Phylogenetic trees were constructed using MEGA 11 software to understand the evolutionary relationships among the bacterial isolates, ensuring precise identification and classification of the bacterial species in the collected samples.

#### Testing the antibiotic susceptibility

Antibiotic resistance profile tests were conducted following the guidelines established by the Clinical Laboratory Standards Institute (CLSI) (Afhami *et al.*, 2020). Screening involved the use of antibiotics targeting extended-spectrum beta-lactamase (ESBL), carbapenem-resistant strains (CRE), multi-drug-resistant TB (MDR-TB), beta-lactams and fluoroquinolones, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant strains (VRSA), and Coagulase-negative staphylococci.

#### Statistical analysis

The incidence of infections acquired in the ICU was expressed as a percentage. Mean  $\pm$  standard deviation was used for presenting continuous variables, while frequency (%) represented categorical variables. Statistical significance for qualitative variables was assessed using the Chi-square test or Fisher exact test, and for quantitative variables, the student's t-test or Wilcoxon test was applied following the method of (Riina *et al.* (2023). Multivariate analysis was conducted to identify independent risk factors associated with mortality and morbidity. The results are presented as *p*- values.



**Figure (1):** Workflow for diagnosing and identifying pathogens in post-operative infections: A Step-by-Step Analysis of sample collection, antibiotic sensitivity testing and genetic sequencing.



## RESULTS

### Isolation and identification of bacterial isolates

In this study, Figure 2 depicts the macroscopic characteristics of the common pathogenic bacteria isolated from ICU patients. In MacConkey agar, *Acinetobacter baumannii* appeared as small, pink colonies with no lactose fermentation. *E. coli* produced large, pink colonies with a mucoid appearance due to lactose fermentation. *K. pneumoniae* typically forms large, mucoid colonies with a pink coloration due to lactose fermentation. On Chocolate agar, *K. variicola* exhibited large, mucoid colonies with irregular edges. *S. haemolyticus* exhibited small, pink colonies with no mannitol fermentation. *S. pneumoniae* typically forms small, grayish colonies. On Blood agar, *S. aureus* typically forms large, round, golden-yellow colonies with a smooth and shiny appearance. These colonies may exhibit a slightly raised elevation and can have a hemolytic zone surrounding them, indicating the hemolysis of red blood cells. On Mannitol Salt agar, *S. lentus* appeared as small, white colonies with a matte surface. *S. epidermidis* forms small, white colonies with no change in the color of the agar. On Middlebrook agar, *M. tuberculosis* is typically slow, taking several weeks for visible colonies to develop. They provide a suitable environment for the growth of slow-growing mycobacteria. These observations provide insights into the distinct colony morphologies and growth characteristics of pathogenic bacteria on various media, facilitating their identification and diagnosis in clinical settings.

### Epidemiological profile of nosocomial infections among patients

The majority of the 174 patients included in the study had conditions like stroke, postoperative, and chronic liver diseases and 98 patients, or 56.3% of the cohort, had an ICU-acquired nosocomial infection (Figure 3).

### Bacterial strain distribution in antibiotic-resistant infections experiment

A study was conducted on a variety of bacterial isol-

ates associated with antibiotic-resistant infections. The identified isolates included *Acinetobacter baumannii* (19.0%), *Escherichia coli* (12.0%), *Klebsiella pneumoniae* (17.0%), *Mycobacterium tuberculosis* (11.0%), *Pseudomonas aeruginosa* (8.0%), *Staphylococcus aureus* (9.0%), *Staphylococcus epidermidis* (4.0%), *Staphylococcus haemolyticus* (6.0%), *Staphylococcus lentus* (3.0%), *Streptococcus pneumoniae* (4.0%), and multiple strains (7.0%) (Figure 4).

In this study, various bacterial species were categorized based on their characteristics into three main groups: Gram-negative bacteria, Gram-positive bacteria and acid-fast bacteria. Among the Gram-negative bacteria examined were *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, all known for their diverse roles in nosocomial infections and antibiotic resistance. The prevalence of these organisms can vary depending on the healthcare facility and patient population, but they remain significant contributors to healthcare-associated morbidity and mortality. Additionally, *M. tuberculosis*, classified as an acid-fast bacterium, was included in the analysis due to its significance in causing tuberculosis infections. For the Gram-positive group, several species were identified, including *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. lentus*, and *S. pneumoniae*, each associated with a range of infections and varying antimicrobial susceptibilities. This comprehensive classification provides a fundamental understanding of the diverse bacterial species prevalent in clinical settings, assisting in the development of targeted diagnostic and therapeutic strategies to combat infectious diseases.

### Clinical outcomes of nosocomial infections: a comparative analysis between patient groups

When comparing Group 1 (those acquiring nosocomial infections) and Group 2 (those without nosocomial infections), no significant differences were observed in terms of age, sex, disease severity, or coexisting conditions. However, Group 1 exhibited longer ICU stays ( $11.2 \pm 5.9$  vs.  $3.6 \pm 1.03$ ) and increased durations of mechanical ventilation ( $9.9 \pm 5.3$  vs.  $3.7 \pm 2.25$ ) compared to Group 2 ( $p \leq 0.001$ ).

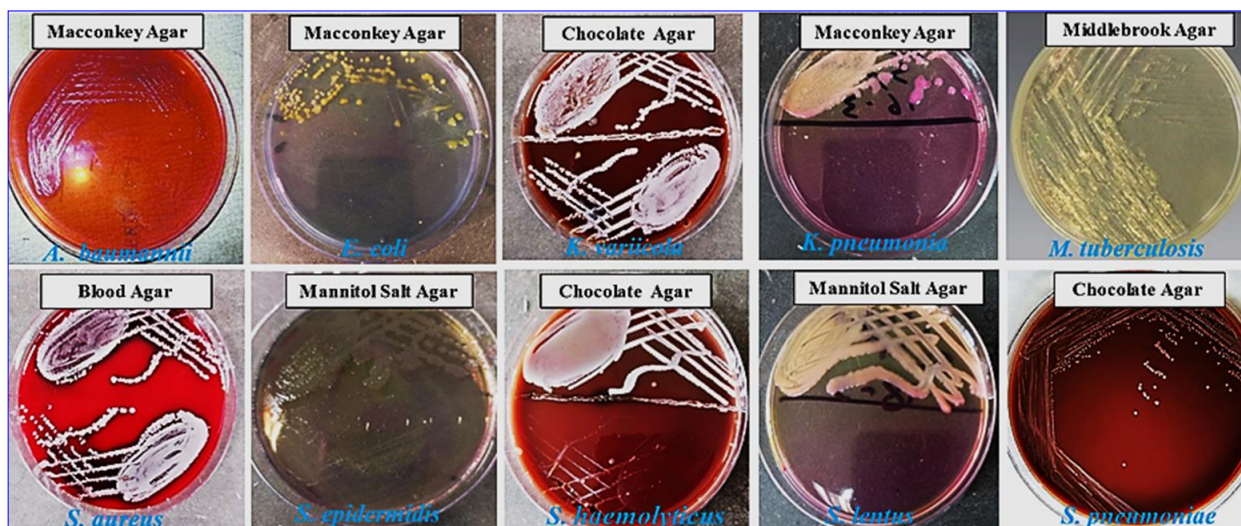


Figure (2): Culture characteristics of the pathogenic bacteria isolated from ICU patients cultured on different media incubated at 37 °C for 24 hrs.

In addition, there was no statistically significant variation in rates of mortality among the two groups (20.5% vs. 33.5%) as represented in Table (1). Multivariate evaluation revealed various independent factors linked to nosocomial infections in the ICU. These factors encompassed the length of ICU stay ( $p \leq 0.001$ ), duration of mechanical ventilation ( $p \leq 0.001$ ), period of tracheal intubation ( $p \leq 0.001$ ), and duration of urinary catheterization ( $p \leq 0.001$ ) (Table 1). The primary causative agents for these infections (were identified as *A. baumannii* followed by *K. pneumoniae*, with urinary tract infections (UTIs) emerging as the predominant infection type (29.0%), followed by, bloodstream infections (19.0%), skin and soft tissue infections (17.7%) (Figure 5).

### Antimicrobial sensitivity

Pathogenic bacteria isolated from patients who were infected during their existence at Beni-Suef Hospital. We found that 10 pathogenic bacterial isolates represent the most common that cause of nosocomial infections. We identified a noteworthy multidrug-resistance pattern. This pattern, of the 21 antibiotics listed in Table (3) belongs to 11 different classes, namely Penicillin, Aminoglycosides, Cephalosporins, Fluoro-quinolones, Tetracyclines, Monobactams, Carbap-enems, Oxazolidinones, Glycopeptides, Nitrofurans, and Polymyxins. This finding underscores the concerning prevalence of organisms demonstrating resistance to a broad spectrum of antimicrobial agents, highlighting the urgent need for effective and targeted interventions in the realm of antimicrobial sensitivity. In the antimicrobial resistance profile analysis, we identified and characterized the resistance patterns of 10 different organisms, each displaying a multidrug-resistance (MDR) profile.

The susceptibility of various pathogens to a range of antibiotics was investigated in this study. The pathogens included *A. baumannii*, *K. variicola*, *K. pneumoniae*, *M. tuberculosis*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. lentus*, and *S. pneumoniae*. The results revealed varying degrees of susceptibility or resistance among these organisms to the antibiotics tested. For Gram-negative bacteria such as *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, resistance was commonly observed across multiple antibiotics including ampicillin, cefazolin, ceftazidime, and ceftriaxone. However, susceptibility was noted to antibiotics like amikacin, imipenem, and colistin, highlighting potential treatment options. *M. tuberculosis*, an acid-fast bacterium, exhibited resistance to ampicillin, amikacin, and cefazolin among others. However, susceptibility was observed to antibiotics like rifampicin and isoniazid, which are commonly used in tuberculosis treatment regimens. Among Gram-positive bacteria such as *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. lentus*, and *S. pneumoniae*, resistance was noted to antibiotics like ampicillin, oxacillin, and ceftoxitin. Nevertheless, susceptibility was observed to antibiotics including vancomycin, linezolid, and minocycline, suggesting potential treatment avenues. These findings underscore the importance of

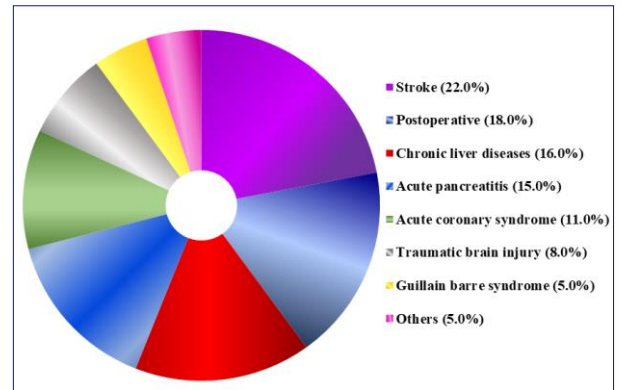


Figure (3): Patient demographics and Enrollment Distribution.

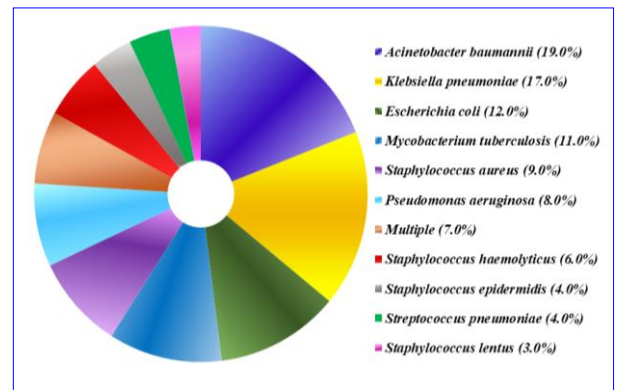


Figure (4): Causative agents of nosocomial infections in the intensive care unit.

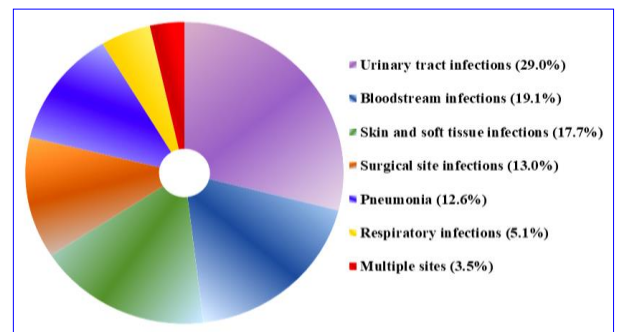


Figure (5): Patterns in nosocomial infection distribution.

understanding the antibiotic susceptibility profiles of various pathogens to guide appropriate treatment strategies and combat antimicrobial resistance effectively.

### Molecular identification of the pathogenic bacteria

In this study, we conducted a comprehensive analysis of bacterial strains associated with nosocomial infections, providing detailed information on their genomic characteristics. Table (3) presents a curated list of bacterial species identified as common causal agents of nosocomial infections, along with their corresponding GenBank accession numbers. Among the notable findings, *A. baumannii*, *E. coli*, and *K. pneumoniae* emerge as prominent pathogens, exhibiting 100% query cover and identity. These strains are known for their virulence and propensity to cause infections in healthcare settings.

Interestingly, *K. variicola* and *M. tuberculosis* also feature prominently, underscoring the diverse array of

**Table (1):** Demographic and clinical characteristics of groups with (Group1) and without nosocomial infections (Group2): comparative analysis.

Demographic criteria	Groups of nosocomial infections		p-value
	Group 1	Group 2	
Age - >60 years (%)	47 (48.0)	33 (43.4)	0.6
<59	60 (61.2)	43 (56.6)	0.6
≥60	47 (48.0)	33 (43.4)	
Sex - male (%)	53 (54.1)	43 (56.6)	0.7
Male	53 (54.1)	43 (56.6)	0.7
Female	42 (42.8)	34 (44.6)	
APACHE II - >24 (%)	39 (39.8)	44 (57.9)	0.2
< 23	25 (25.5)	19 (25)	0.2
≥ 24	39 (39.8)	44 (57.9)	
Comorbidities (hypertension, diabetes, heart, liver, kidney diseases) (%)	83 (84.7)	52 (68.4)	0.6
Mechanical ventilation	9.9 ±5.3	3.7 ± 2.25	≤0.001*
Duration of stay	11.2 ±5.9	3.6 ±1.03	≤0.001*
ICU stay (days)			
<7	37 (37.7)	34 (44.7)	≤0.01*
≥7	65 (66.3)	55 (72.3)	
Ventilator duration (days)			
<7	47 (47.9)	70(92.1)	≤0.01*
≥7	62(63.3)	11 (14.5)	
ETT/TT duration (days)			
<7	41 (41.8)	73 (96.0)	≤0.01*
≥7	66 (67.3)	13 (17.1)	
CVP duration (days)			
<7	39 (39.8)	33(43.4)	0.7
≥7	76 (77.5)	56 (73.7)	
Urinary catheter duration (days)			
<7	36 (36.7)	69 (90.7)	≤0.01*
≥7	74 (75.5)	18 (23.7)	
Mortality (%)	20.5	33.5	0.009

Group1, n=98; Group 2, n=76; \*, statistically significant at level  $p \leq 0.05$ ; APACHE: Acute physiology and chronic health evaluation; ; ICU: Intensive Care Unit.; ETT/TT: Endotracheal tube/tracheostomy tube and . CVP, Central venous pressure.

**Table (2):** Susceptibility of bacterial isolates to tested antibiotics.

Tested Antibiotics	Bacterial isolates																				
	A. baumannii		K. varicola		K. pneumoniae		M. tuberculosis		P. aeruginosa		S. aureus		S. epidermidis		S. haemolyticus		S. lentus		S. pneumoniae		
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	
Ampicillin	.		.		.		.		.		.		.		.		.		.		.
Amikacin	.		.		.		.		.		.		.		.		.		.		.
Oxacillin	.		.		.		.		.		.		.		.		.		.		.
Cefazolin	.		.		.		.		.		.		.		.		.		.		.
Cefoxitin	.		.		.		.		.		.		.		.		.		.		.
Ceftazidime	.		.		.		.		.		.		.		.		.		.		.
Ceftriaxone	.		.		.		.		.		.		.		.		.		.		.
Cefepime	.		.		.		.		.		.		.		.		.		.		.
Marbofloxacin	.		.		.		.		.		.		.		.		.		.		.
Levofloxacin	.		.		.		.		.		.		.		.		.		.		.
Tetracycline	.		.		.		.		.		.		.		.		.		.		.
Aztreonam	.		.		.		.		.		.		.		.		.		.		.
Gentamicin	.		.		.		.		.		.		.		.		.		.		.
Imipenem	.		.		.		.		.		.		.		.		.		.		.
Ticarcillin	.		.		.		.		.		.		.		.		.		.		.
Minocycline	.	.	.		.		.		.		.		.		.		.		.		.
Linezolid	.		.		.		.		.		.		.		.		.		.		.
Vancomycin	.		.		.		.		.		.		.		.		.		.		.
Nitrofurantoin	.		.		.		.		.		.		.		.		.		.		.
Colistin	.	.	.		.		.		.		.		.		.		.		.		.
Tobramycin	.		.		.		.		.		.		.		.		.		.		.

\*R= resistant; S= sensitive

pathogens encountered in hospital environments. Furthermore, our analysis reveals high levels of genomic similarity among species, including *S. aureus*, *S. epidermidis*, *S. haemolyticus*, and *S. lentus*, with identity percentages exceeding 99%. Additionally, these similarity percentages reflect comparisons with reference genomic sequences from established databases, which provide a benchmark for identifying and distinguishing these bacterial species.

This emphasizes the importance of infection control measures to combat the spread of staphylococcal infections within healthcare facilities. Based on the results of phylogenetic tree analysis, the isolated bacterial species were closely related to *A. baumannii*, *E. coli*, *K. variicola*, *K. pneumoniae*, *Staphylococcus*

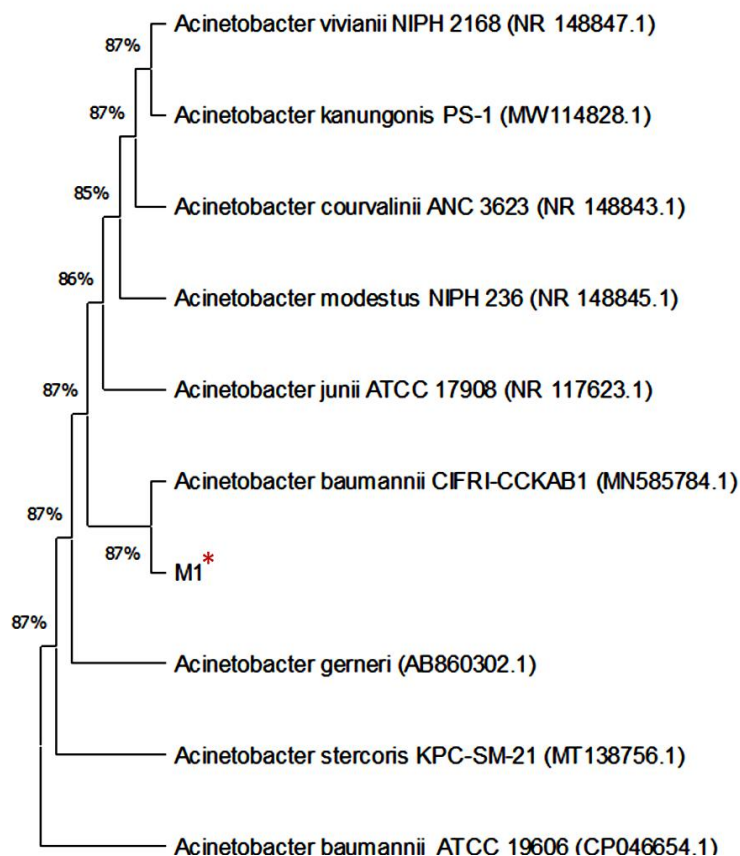
*aureus*, *S. epidermidis*, and *S. haemolyticus*, respectively as shown in (Figures 6-9). Overall, these findings shed light on the genomic diversity and virulence potential of bacterial strains implicated in nosocomial infections, providing valuable insights for targeted surveillance and prevention strategies aimed at reducing the burden of healthcare-associated infections.

## DISCUSSION

Despite constituting a relatively small proportion of hospital beds, ranging from 15% to 20%, ICUs play a disproportionate role in the prevalence of severe nosocomial infections (Groeger *et al.*, 1992, Groeger *et al.*, 1993). Over 50% of severe nosocomial infections

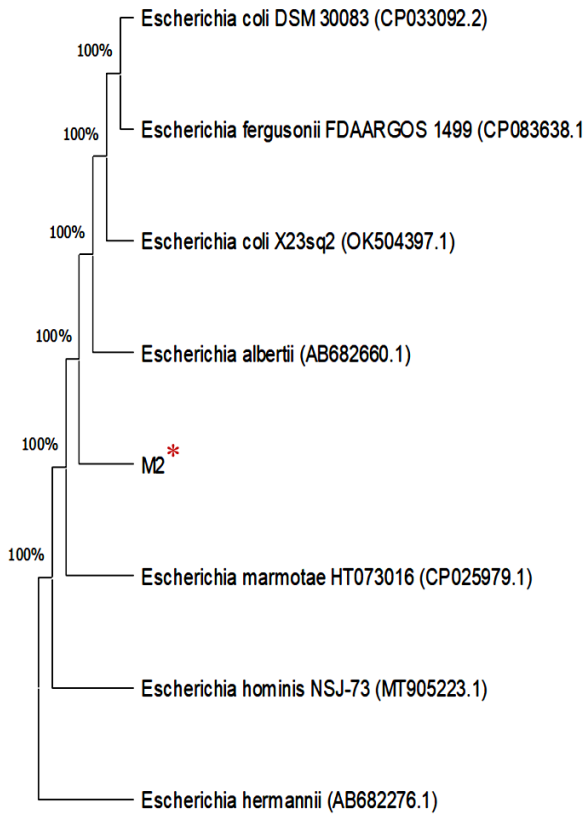
**Table (3):** List of bacterial isolates causing nosocomial infection and their GenBank accession numbers.

Isolate identification	Accession number	Query cover	E-value	Identity %
<i>Acinetobacter baumannii</i>	MN585784.1	100%	0	100%
<i>Escherichia coli</i>	OK504397.1	100%	0	100%
<i>Klebsiella variicola</i>	MN725742.1	100%	0	99.24%
<i>Klebsiella pneumoniae</i>	CP121133.1	100%	0	99.43%
<i>Mycobacterium tuberculosis</i>	UOP13931.1	100%	0	99.70%
<i>Staphylococcus aureus</i>	NCIM 2079	100%	0	99.24%
<i>Staphylococcus epidermidis</i>	MG645279.1	100%	0	99.81%
<i>Staphylococcus haemolyticus</i>	MT622590.1	100%	0	99.70%
<i>Staphylococcus lentus</i>	MT622590.1	100%	0	99.70%
<i>Streptococcus pneumoniae</i>	CP054883.1	100%	0	99.90%

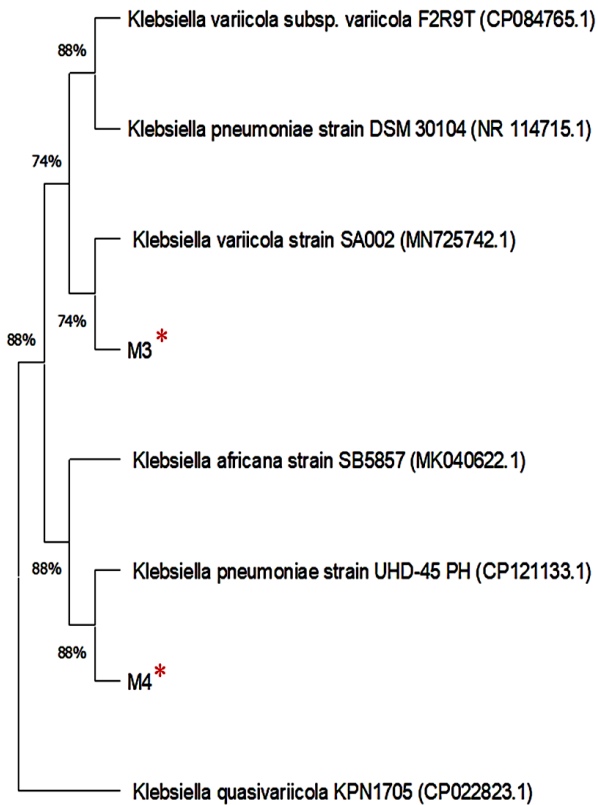


**Figure (6):** Phylogenetic tree of 16S rRNA sequences from the bacterial strain *Acinetobacter baumannii* (M1) constructed using MEGA 11 software.





**Figure (7):** Phylogenetic tree of 16S rRNA sequences from the bacterial strain *Escherichia coli* (M2) constructed using MEGA 11 software.



**Figure (8):** Phylogenetic tree of 16S rRNA sequences from the bacterial strain *Klebsiella variicola* (M3) and *Klebsiella pneumoniae* (M4) constructed using MEGA 11 software.

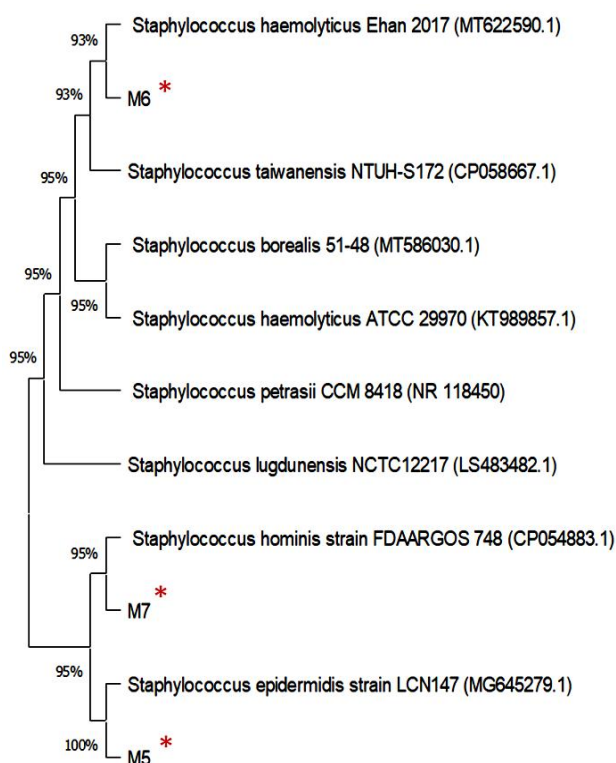
are linked to Intensive Care Units (Li *et al.*, 2023). This alarming statistic underscores the heightened vulnerability of critically ill patients to hospital-acquired infections within these specialized care settings. Comparing our study's results to global trends, the reported incidence of nosocomial infections exceeds rates documented in various studies worldwide. The variability in reported figures emphasizes the complex nature of nosocomial infections and underscores the need for region-specific interventions and surveillance programs. The higher-than-average incidence observed in our study raises concerns about the potential impact on patient outcomes and emphasizes the pressing need for targeted strategies to mitigate and prevent nosocomial infections, particularly in ICU settings. This variability is attributed to the unequal distribution of risk factors across healthcare settings (Pastores *et al.*, 2019).

In our study encompassing 174 participants, a significant proportion presented with underlying health conditions such as stroke, postoperative, and chronic liver diseases. Notably, 56.3% of the cohort, comprising 98 individuals, experienced nosocomial infections acquired in the intensive care unit (ICU). The primary causative agents for these infections were identified as *A. baumannii* followed by *K. pneumoniae*, with urinary tract infections (UTIs) emerging as the predominant infection type, followed by, bloodstream infections, skin and soft tissue infections (Aiesh *et al.*, 2023) Similar studies were reported by (Motbainor *et al.*, 2020, Al Salman *et al.*, 2020), that *A. baumannii* was the main cause of nosocomial infections in hospitals and caused infections of the urinary tract and bloodstream.

Upon comparing two distinct groups within cohort-Group 1, comprising individuals who acquired nosocomial infections, and Group 2, consisting of those without such infections, the results indicated no significant differences in terms of age, sex, disease severity, or comorbid conditions. However, Group 1 exhibited substantially longer ICU stays ( $11.2 \pm 5.9$  days vs.  $3.6 \pm 1.03$  days) and extended durations of mechanical ventilation ( $9.9 \pm 5.3$  days vs.  $3.7 \pm 2.25$  days) compared to Group 2, with these findings showing statistical significance ( $p \leq 0.001$ ). Despite these differences in treatment duration and intensity, the mortality rates between the two groups did not show a statistically significant distinction (20.5% vs. 33.5%), as outlined in Table (1).

The multivariate analysis performed to identify independent factors associated with nosocomial infections in the ICU, revealing several significant contributors. These included the duration of ICU stay, mechanical ventilation, tracheal intubation, and urinary catheterization, all demonstrating statistical significance ( $p \leq 0.001$ ), as shown in Table (1). The extended duration of ICU stay and mechanical ventilation observed in Group 1 highlights the vulnerability of this subgroup to nosocomial infections, underscoring the necessity for targeted preventive measures for these high-risk individuals. Importantly, the lack of a signifi-





**Figure (9):** Phylogenetic tree of 16S rRNA sequences from the bacterial strains *Staphylococcus epidermidis* (M5), *Staphylococcus haemolyticus* (M6) and *Staphylococcus hominis* (M7) constructed using MEGA 11 software.

cant difference in mortality rates between the groups suggests that, despite the increased morbidity associated with nosocomial infections, these infections may not directly correlate with a higher overall mortality rate in our study population. Notably, *A. baumannii* emerged as the predominant cause of nosocomial infections in our study, consistent with its established role in hospital-acquired infections (Hamidian *et al.*, 2022). *A. baumannii*, particularly the strains producing carbapenem-resistant *A. baumannii*, (CRAB), poses a considerable challenge due to increased resistance to antibiotics (Jiang *et al.*, 2022).

Our study exhibited an elevated occurrence of *K. pneumoniae* involvement in urinary tract infections (UTIs), consistent with findings from other regions (Al Yousef *et al.*, 2016). Proper differentiation between asymptomatic bacteriuria and symptomatic UTI is crucial to prevent antibiotic-associated complications and reduce misuse. *Pseudomonas* and *E. coli* identification at an early stage facilitates timely treatment for UTIs, especially in critically ill patients. Our study did not explore the associated mortality, existing literature suggests higher mortality rates in cases of multiple-organism bacteremia compared to single-organism bacteremia. In contrast to certain studies, our investigation did not demonstrate a substantial disparity in overall mortality between patients who contracted hospital-acquired infections and those who did not (Rodriguez-Acelas *et al.*, 2017). Nevertheless, distinctions in the length of ICU stay and the duration of mechanical ventilation were apparent, consistent with observations from another study. This study also

pinpointed autonomous factors that predict hospital-acquired infections in the ICU, such as the length of intubation, urinary catheterization, mechanical ventilation, and ICU stay. These factors contribute to the complexity of heterogeneous cases in the ICU setting. Other studies have identified various predictors such as age, disease severity scores, colistin resistance, aminoglycoside use, and specific medical conditions. Despite its contributions, our study has limitations. The simplicity of the observed variables may not fully capture the complexity of the outcomes. Factors dealt with before admission to the ICU in the emergency department, along with physiological determinants, were not taken into account. Further-more, the inherent limitations of retrospective studies, including potential biases, should be acknowledged, impacting the interpretation of our findings. Table (2) provides information on the resistance patterns of various pathogenic organisms to commonly used antibiotics. The numbers in the column indicate how many different resistance patterns are mentioned for each organism. The presence of multidrug-resistant patterns, such as CRAB, ESBL, CRE, and MDR-TB, underscores the challenge of treating infections caused by these organisms. The emergence of resistant strains, particularly MRSA and VRSA, is a significant concern in healthcare settings. The information highlights the importance of ongoing surveillance, proper antibiotic stewardship, and the development of new therapeutic strategies to address antibiotic resistance.

Our findings highlight several key insights regarding the bacterial species identified in nosocomial infections. Notably, *A. baumannii*, *E. coli*, and *K. pneumoniae* emerged as predominant pathogens, consistent with previous epidemiological studies. Also, previous research has established that three major antimicrobial-resistant (AMR) pathogens, namely *E. coli*, *K. pneumoniae*, and *A. baumannii*, were carried out in the ICU in Vietnam (Roberts *et al.*, 2020). These organisms are well-known for their ability to cause a wide range of infections, including urinary tract infections, bloodstream infections, and pneumonia, particularly among immunocompromised and critically ill patients. The high genomic similarity observed among these strains underscores the importance of targeted interventions to prevent their transmission within healthcare facilities.

Interestingly, our analysis also identified *K. variicola* and *M. tuberculosis* among the prevalent bacterial species. While *K. variicola* has been less extensively studied compared to its closely related counterpart, *K. pneumoniae*, emerging evidence suggests its potential role in nosocomial infections. Similarly, the detection of *M. tuberculosis* highlights the ongoing challenges posed by tuberculosis transmission in healthcare settings, particularly in regions with a high prevalence of the disease. Furthermore, our study elucidated the genetic relatedness among various *Staphylococcus* species implicated in nosocomial infections. *S. aureus*, *S. epidermidis*, *S. haemolyticus*, and *S. lentus* exhibited high levels of genomic similarity, emphasizing the

need for comprehensive strategies to prevent staphylococcal infections, including methicillin-resistant *S. aureus* (MRSA), which can be particularly challenging to control.

Generally, our findings underscore the complex nature of nosocomial infections and highlight the importance of ongoing surveillance, infection control practices, and antimicrobial stewardship efforts to mitigate their impact. By elucidating the genomic characteristics of common bacterial pathogens associated with HAIs, our study provides valuable insights that can inform targeted interventions aimed at reducing the burden of nosocomial infections and improving patient outcomes in healthcare settings.

In the intensive care unit (ICU), preventing and managing nosocomial infections is of paramount importance to ensure patient safety and well-being. Therefore, implementing healthy guidelines for preventing and managing hospital-acquired infections is essential. These guidelines encompass a multifaceted approach, including stringent adherence to infection control protocols, such as proper hand hygiene, appropriate use of personal protective equipment, and meticulous environmental cleaning and disinfection. Additionally, active surveillance for early detection of infections, timely implementation of isolation precautions, and judicious use of antibiotics through antimicrobial stewardship programs are crucial components of effective infection prevention and control strategies in the ICU. By rigorously adhering to these guidelines, healthcare facilities can significantly reduce the incidence of nosocomial infections, minimize patient morbidity and mortality, and improve overall healthcare quality and safety in the ICU setting. In the Intensive Care Unit (ICU), ensuring the safety of both patients and healthcare workers is paramount, particularly in the prevention of nosocomial infections.

Effective personal protection standards and supplies are also essential components of infection control strategies in this critical healthcare setting. From critical care nurses to environmental services staff, each role is outlined with specific recommendations tailored to their responsibilities. These recommendations include the use of N95 respirators or equivalent masks, surgical masks, disposable gloves, impermeable gowns or aprons, eye protection, and hand hygiene facilities with hand sanitizer. Regular training on proper donning and doffing procedures further ensures adherence to these essential infection control measures. By implementing these guidelines, healthcare facilities can effectively mitigate the risk of nosocomial infections and safeguard the well-being of both patients and healthcare workers in the ICU.

## CONCLUSION

Our research revealed a high prevalence of hospital-acquired infections in the intensive care unit (ICU); however, the overall mortality rate in this context was not significantly impacted by these infections. *A. baumannii*-caused pneumonia was found to be the

main cause of infections in intensive care units (ICU). Remarkably, the time of exposure to a mechanical ventilator, urinary catheterization, endotracheal intubation, and ICU stay autonomously forecasted the occurrence of hospital-acquired nosocomial infections in the ICU. Recognizing these risk factors highlights the significance of implementing focused preventive measures. To deepen our comprehension of the various risk factors and their correlations, there is a necessity for additional prospective observational studies, preferably carried out across various centers. This would contribute valuable insights to the ongoing efforts in refining strategies for the prevention and management of nosocomial infections in critical care settings. The study reveals a concerning prevalence of multidrug resistance in various bacterial species. This underscores the immediate requirement for coordinated initiatives in antimicrobial stewardship, the exploration and creation of novel antibiotics, and worldwide cooperation to tackle the rising issue of antibiotic resistance. The intricacy of observed resistance mechanisms highlights the significance of continuous monitoring and the formulation of inventive strategies to counter multidrug-resistant pathogens. Navigating this period of growing antibiotic resistance necessitates a comprehensive strategy involving healthcare practitioners, researchers, policymakers, and the public to uphold the efficacy of antimicrobial agents and guarantee viable treatment alternatives for bacterial infections in the coming years.

## Ethics approval and consent to participate

The study was approved by the Faculty of Medicine Beni Suef University Research Ethics Committee (FMBSUREC/02012024/Bani Melhem).

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## وبانيات عدوى المستشفيات في وحدة العناية المركزة بمستشفى بني سويف وإستراتيجيات مكافحتها

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### الملخص العربي

تنتشر العدوى المكتسبة من المستشفيات بشكل أكبر في وحدة العناية المركزة مقارنة بمناطق المستشفيات الأخرى. تهدف هذه الدراسة إلى توضيح علم الأوبئة وخصائص العدوى المكتسبة من وحدة العناية المركزة، مع التركيز على تأثير عوامل الخطر المختلفة. خللت هذه الدراسة بأثر رجعي بيانات 174 مريضاً في وحدة العناية المركزة تم إدخالهم بين ديسمبر 2022 ونوفمبر 2023. كان التركيز الأساسي على علم الأوبئة للعدوى البكتيرية، بما في ذلك معدل حدوثها والكائنات المسببة ومواقع العدوى. بالإضافة إلى ذلك، تهدف الدراسة إلى تحديد عوامل الخطر المرتبطة بالعدوى المكتسبة من وحدة العناية المركزة. من بين 174 مريضاً، أصيب 98 (56.3%) بعدوى مكتسبة من المستشفى في وحدة العناية المركزة. كانت *Acinetobacter baumannii* العامل المسبب الأساسي (19.0%)، وكانت عدوى المسالك البولية هي النوع الأكثر شيوعاً (29.0%). عانى المرضى المصابون بعدوى المستشفيات من إطالة مدة التهوية الميكانيكية وإقامة وحدة العناية المركزة. ومع ذلك، لم تختلف معدلات الوفيات بشكل كبير بين المرضى المصابين وغير المصابين. وشملت عوامل الخطر المستقلة التي تم تحديدها من خلال التحليلات المتعددة المتغيرات التنبيب، وقسطرة البول، والتهوية الميكانيكية التي استمرت كل منها لأكثر من 7 أيام، بالإضافة إلى الإقامة في وحدة العناية المركزة لأكثر من 7 أيام. تسلط هذه الدراسة الضوء على حدوث كبير للعدوى المكتسبة من المستشفيات في وحدة العناية المركزة وتحدد عوامل الخطر المحددة المرتبطة باكتسابها في بيئة الرعاية الحرجة.