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RESEARCH ARTICLE

Airborne and Surface Cross-Contamination of Bacteria Resistant to Carbapenem in Critical Units of a Teaching Hospital

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Abstract Hospital-acquired infections (HAIs) have been significantly exacerbated by the escalating environmental contamination of critical units in hospital settings. The surfaces equipment, as well as the surrounding air, may serve as a source for multidrug-resistant organisms (MDRO) to spread and causes HAIs. Among the MDROs, Carbapenem Resistant Enterobacterales (CREs) are the main cause of concern due to their potential to contribute to high morbidity and mortality rates, causing outbreaks, and spreading resistance to other bacteria. This study was conducted to investigate the presence of CRE cross-contamination in critical units at a teaching hospital located on the East Coast of Malaysia. The total colony forming unit (CFU) of microbial were compared in different critical units and between morning and evening sessions. A total of 304 environmental samples comprised of airborne and swab samples were collected, while the temperature, relative humidity and number of occupants in the units were recorded. Gram staining, oxidase tests, biochemical tests and disk diffusion tests using carbapenem antibiotics were carried out to screen and identify the presence of carbapenem-resistance bacteria, specifically CREs. The findings showed the total CFU, which indicates the extent of microbial contamination, varies across different critical units. The presence of carbapenem-resistance bacteria (17 isolates) were detected in the environment at Ward X (n=8, 47.1%), intensive critical units (n=4, 23.5%), Neuro-ICU (n=2, 11.8%), and surgical intensive care units (n=3, 17.6%). Most of the critical units exhibited no notable disparity in the total CFU of microbials collected between morning and evening sessions ($p>0.05$) except the Neuro-ICU. In conclusion, as there was a presence of CREs in the critical unit environments of the teaching hospital, therefore, strengthening surveillance policy on the hygienic condition of the critical units and routine sampling is highly suggested to reduce the risk of HAIs outbreaks related to CREs.

Keywords: Carbapenem Resistant Enterobacterales, hospital-acquired infections, airborne, surface swab.

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Introduction

The global dissemination of multidrug-resistant organisms (MDROs) has emerged as a critical public health issue, leading to increased morbidity and mortality worldwide. Over recent decades, MDROs have proliferated in hospitals and medical centres, significantly contributing to therapeutic failures in both hospital-acquired and community infections. MDROs were responsible for at least 1.27 million deaths

globally in 2019, highlighting their devastating impact [1]. MDROs thrive due to selection pressures arising from antibiotic misuse and overuse among immunocompromised or severely ill patients. Bacterial colonization further facilitates their widespread dissemination. The lack of effective treatment options for MDROs perpetuates their presence, prolongs disease episodes, escalates healthcare costs, and poses a substantial mortality risk [2].

Carbapenem Resistant Enterobacterales (CREs) are a notable group within MDROs, showing resistance to all carbapenems. These Enterobacteriaceae family members are difficult to treat and most commonly afflict patients in healthcare facilities, with 70% to 90% of cases arising in acute and long-term care. Alarming trends show increasing CRE infections within hospitals, challenging treatment due to their significant antimicrobial resistance (AMR), thus limiting the access to effective antibiotics. Moreover, CREs can also transfer their resistance to other bacteria via mobile genetic elements, exacerbating outbreaks [3,4]. On other occasions, the Infection Control Unit of Malaysia's Ministry of Health categorized CREs as one of the national surveillance organisms where the data from 2015 showed an increase in CRE incidence from 0.02 to 0.05 per 100 admissions since 2013 [5]. Recognizing its severity, both the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) classify CREs as a 'critical priority threat' requiring immediate attention [6,7].

Hospital environments play a crucial role in the transmission of diseases among patients. These dynamic spaces are influenced by various factors, including seasonal variations, weather conditions, indoor ventilation systems, moisture intrusion, microbial loads from the outdoors, and human activities. Collectively, these elements create conditions conducive to foster microbial contamination and growth [8]. Hence, the risk of contracting a microbial pathogen in this environment is significant. For instance, critical unit environments are recognized as potential reservoirs for opportunistic and pathogenic microbial strains that can persist and proliferate on medical equipment and in the surrounding environment, where these bacteria are capable of causing infections or diseases in humans, especially those with compromised immune systems. Bioaerosols pose an additional risk of contracting a microbial pathogen in hospital settings. Moreover, the hygienic conditions of hospital sites, patient rooms, and medical equipment may significantly impact the composition of the microbial community, as pathogens can persist on room surfaces and equipment for extended periods, serving as potential sources of infections [9]. These biological agents may be transmitted to patients via personnel gloves, visitor hands, or dust. Once deposited on surfaces, they can become contaminated and subsequently disperse through natural convection or air conditioning systems [10]. Despite the critical role of environmental contamination, there remains a scarcity of data specifically addressing the influence of these factors on the dissemination of CREs.

Similarly, previous studies conducted by the CDC underscore the critical importance of timely identification in managing CRE infections. Failure to recognize CREs promptly, especially in high-risk wards of medical institutions such as intensive care units (ICUs), can result in missed opportunities for intervention before these organisms spread extensively [11]. Promptly identifying all instances of CREs is essential for understanding the depth of the issue and ensuring effective implementation of infection control and outbreak management procedures. However, existing studies often fall short in investigating the timely identification of CRE infections across diverse clinical environments. To address these gaps, this study aims to investigate the presence and contributing factors of CREs cross-contamination within the hospital environment of different critical care units in a selected teaching hospital.

Materials and Methods

This cross-sectional study utilized a purposive sampling method which involved sample collection at targeted six critical units of a selected teaching hospital located in the East Coast of Malaysia, specifically Ward X, intensive critical units (ICUs), neuro-intensive care units (NICUs), cardiovascular-intensive care units (CICUs), coronary care units (CCUs) and surgical-intensive care units (SICU) from August 2023 to January 2024. The rationale behind the selection of critical unit wards at the selected hospital was rooted in the acknowledgment that both patients and healthcare personnels are more vulnerable to contracting CREs within the hospital's critical care units [9-11]. Previous study reported a prevalence of 5.74% CRE isolated from the clinical specimens at the same hospital with *Klebsiella pneumonia* as predominant species (388/408, 95%) [12]. Preceding the collection, a preliminary walkthrough survey of these units was performed to gather work task information data and pinpoint potential sampling locations with differing microbial exposure risks.

The collection of samples involved two methods: (i) the Duo SAS Super 360 Air Sampler (SAS VWR, Italy), which actively collected bioaerosol samples from the air [13]; and (ii) a site-specific surface swabs method. The collected air samples impacted on two types of agar plates which were nutrient agar (NA) and MacConkey agar (MAC) were then incubated overnight (24 hours) prior to conducting CFU counts. Similarly, the surface swabs underwent a serial broth microdilution technique following 48 hours of incubation. Subsequently, the samples were cultured on Nutrient agar (NA) and MacConkey (MAC) agar plates to determine CFU counts. Bacterial identification tests were then conducted on the positive cultures to determine the species of Enterobacteriaceae. Following that, disc diffusion tests were carried out with carbapenem antibiotics including meropenem, imipenem and ertapenem to identify the presence of any CRE organisms. Simultaneously, the temperature and relative humidity were assessed in every ward using the Extech Hygro-Thermometer (Extech Instruments). Aside from that, the number of occupants present in each ward was recorded. Overall, these samples were taken twice on the same day, specifically during the morning, approximately from 8.30 a.m. until 10.00 a.m., whereas the evening sampling was conducted from 2.00 p.m. until 3.30 p.m. as these were the times when each shift starts or ends, therefore to reduce any potential biological variation that could impact the outcomes of the research.

Data Collections

Bioaerosol Sampling and Analysis

The active sampling technique was applied to collect air samples by using a Bioaerosol Duo SAS Super 360 Air Sampler operated at a substantial flow rate of 180 L/min for 5 min, according to the manufacturer's instructions and protocols. There are two types of sampling mediums utilized namely, NA specifically for the investigation of bioburden inside the ward and MAC agar for the detection of CREs. The bioaerosol air sampler was strategically placed at a randomly selected location within the wards, specifically at a height ranging from 1.0 to 1.5 metres, which corresponds to the breathing zone [14]. In preparation for each sampling, the bioaerosol air sampler underwent disinfection of its interior and exterior using a solution consisting of 70% alcohol and precautionary measures were taken to mitigate the risk of cross-contamination including the utilization of gloves, facemasks and disposable plastic aprons. Subsequently, the sampling media petri plate was cautiously placed into the bioaerosol air sampler, followed by turning on the bioaerosol air sample. Then, the sampled air is drawn into the sampler through a perforated plate with 219 small holes and laminar air flow is directed onto the agar surface of a contact petri plate for microbiological examination. Following the completion of the sampling process, the plates were removed and well labelled, and then the samples were meticulously sealed with parafilm to minimize any potential exposure to the surrounding air, hence mitigating the risk of contamination. Afterwards, the collected samples were transported to the laboratory in a cooler box filled with ice packs.

The samples were incubated at 37°C for 24 hours prior to CFU counts. The microbial load was expressed as colony-forming units per cubic metre of air (CFU/m^3) following the following formula [14]:

$$\text{CFU/m}^3 = \frac{(N \times 1000)}{V * T} \quad (1)$$

Where,

CFU/m^3 = Concentration of colony forming unit (CFU) in indoor air;
N = colonies counted on petri dish (CFU);
V = the volume of air sampled in liters (litre);
T = duration of sampling (minute).

However, this formula is only valid if N is less than Nh, where Nh is the number of holes on the sampling head. If in the cases N is equal to or greater than Nh, the sample dish can be considered as overloaded with organisms. After a 24-hours incubation period, the bacterial and fungal colonies that grew on each plate were counted using a colony counter in order to determine the CFU count (CFU/m^3). The quantification of CFU counts (CFU/m^3) was performed at precise intervals to prevent the possible overgrowth of bacteria and fungi. The aforementioned methods were repeated in order to obtain triplicate samples in each ward at three distinct areas that were randomly selected for both morning and evening sessions.

Surface Swab Sampling and Analysis

After walkthrough surveys were conducted at the critical units, four distinct surface sampling sites were chosen based on their relevance and representativeness of the survey's objectives, concentrating on

high-touch or high-risk areas as well as medical equipment or materials, which included a personal table, curtain, bed auto-crank and bed hand reels. For each sample, a sterile cotton swab that had been moistened in 5 mL of sterile water was used for swabbing an area of 10 × 10 cm^2 via a surface swab template of the selected surface site.

The selected surface area was swabbed in three different directions or orientations with consistent pressure, which are horizontally, vertically and diagonally, covering the entire surface area of the swab template. Following the swabbing procedure, the obtained swab sample was immediately transferred into sterile test tubes containing 10 mL of Brain Heart Infusion Broth (BHIB). The test tubes were securely sealed with their caps and appropriately labelled. Then, the collected samples were transported to the laboratory in a cooler box containing ice packs to preserve the viability and integrity of the microorganisms. The samples were incubated at 37°C for 48 hours, in preparation for further analysis. Following a 48-hour incubation period, the BHIB culture solution was subjected to vigorous mixing using a vortex for 10 seconds, ensuring that the microorganisms in the solution were all uniformly dispersed. Next, a serial dilution was performed using this BHIB culture solution for the purpose of CFU counting on NA and MAC plates, starting with a 10^{-1} dilution and thereafter proceeding with dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Each dilution involved taking 1.0 mL from the previous tube, transferring it to the next tube, and mixing well. This process was repeated until a 10^{-7} dilution was achieved, using a new sterile pipette tip for each transfer. Once the dilution series was prepared, 0.1 mL of each dilution tube with a dilution factor of 10^{-6} and 10^{-7} was carefully pipetted and dropped onto the NA and MAC agar plates respectively. These media were aseptically plated and evenly distributed throughout the plates using the spread plate culturing technique. Then, both the NA and MAC agar plates were incubated at 37 °C for 24 hours. After the incubation period, the colonies grown on the plate were counted for CFU by using the formula shown below, which indicates the bacteria present in the series of suspension samples.

$$\text{CFU/mL} = \frac{Average\ Colony\ Count}{Dilution\ Factor} \quad (2)$$

The aforementioned methods were conducted twice daily in order to obtain the samples for both morning and evening sessions.

Screening and Identification of Bacteria

The macroscopic examination of different bacterial colonies grown on MAC agar plates was observed and recorded. Re-subculture on the MAC agar was carried out when mixed colonies were observed in order to obtain pure colonies. The pure bacterial colonies were then proceeded for further tests such as Gram stain, oxidase test, and identification test using biochemical tests such as Triple Sugar Iron Agar (TSI) test, Urease test, Citrate test, Sulfide Indole Motility (SIM) test, Methyl Red Voges-Proskauer (MRVP), Analytical Profile Index (API® 20E) test and Disc Diffusion test using carbapenem antibiotics on MHA plates.

Measurements of Zone Inhibition and Interpretation

Following an incubation period of 16–18 hours, the MHA plate was examined to determine the presence of a confluent lawn of growth and a uniformly circular zone of inhibition. Then, the measurements of zone inhibition were conducted using a manual approach, employing a ruler to determine the diameter of the zone of inhibition to the nearest whole millimetre (mm). The plate was held over the back and facing upwards towards the light while measuring the diameter of the zone of inhibition. The recorded zone diameter was compared with those in the chart of standardized antibiotic panel results for a specific organism, which may be reported as resistant, intermediate, or susceptible, based on the carbapenem breakpoint published by CLSI [15].

Data Analysis

In this study, IBM Statistical Package for the Social Science (SPSS) version 27 was used to analyze the data. Frequency, percentage, mean, median, standard deviation and interquartile range (IQR) were used for descriptive analysis. Non-parametric tests such as Mann-Whitney test, Kruskal Wallis test and Spearman Correlation were used for inferential analysis. The p-value of < 0.05 was taken as statistically significant at the confidence level of 95%.

Results and Discussion

Airborne and Surface Microbial Contamination in Different Critical Units

Table 1 presents the median values of total CFU of microbial contamination across various critical units. It compares two sample types: air samples (CFU/m³) and surface swab samples (CFU/mL) on their respective agar plates. Among air samples on nutrient agar, Ward X had the highest median CFU (1197.5 CFU/m³), followed by Neuro-ICU (199.0 CFU/m³), with ICU having the lowest (0.5 CFU/m³). On MAC agar, Ward X had the highest median CFU (4.0 CFU/m³). Overall, the median CFU of air samples was higher on nutrient agar (70.0 CFU/m³) than on MAC (0.0 CFU/m³). For surface swab samples on nutrient agar, the CCU had the highest median CFU (4.2 x 10^8 CFU/mL), followed by Neuro-ICU (4.0 x 10^8 CFU/mL). SICU had the lowest median CFU on nutrient agar (1.9 x 10^8 CFU/mL). The difference in total CFU between the highest and lowest medians on nutrient agar was 2.3 x 10^8 CFU/mL. Besides, the median CFU of surface swab samples was slightly higher on nutrient agar (2.4 x 10^8 CFU/mL) than on MacConkey Agar (1.1 x 10^8 CFU/mL), mirroring the trend observed in air samples.

These findings indicate that the median of total CFU on NA exhibited a higher CFU compared to the MAC agar. The disparity may arise from the discerning nature of the media, whereby NA permits a wider array of bacteria and fungi to flourish in comparison to MAC agar. Crucially, the findings showed that there was significant microbial contamination (e.g Ward X had exceed the permissible level of 500 CFU/m^3, $p < 0.05$) within the critical units, surpassing the permitted threshold for microbial levels in hospital environments. Thus, it constitutes a high risk of occurrence of HAIs. Nevertheless, an excess of bacterial counts does not necessarily imply a health risk but serves as an indicator for further investigation. Previous study conducted by AlRayess *et al*. [16], have reported findings similar to this current study. The investigation at the ICUs of the American University of Beirut Medical Centre (AUBMC) revealed significant insights into the bacterial composition within these crucial healthcare environments. The study found a substantial variation in the overall bacterial load in these ICUs, ranging from 20.4 to 134.3 CFU/m³. Interestingly, some ICUs exhibited particulate matter levels that exceeded the international guidelines for 24-hour exposure.

Table 1. Median (IQR) of colony forming unit (CFU) of microbial contaminations at different critical units

Critical units	Air samples (CFU/m^3)		Surface swabs (CFU/mL)	
	NA	MAC	NA	MAC
ALL WARDS	70.0 (25.2- 246.0)	0.0 (0.0- 2.0)	2.4 x 10^8 (1.8 x 10^8- 1.4 x 10^9)	1.1 x 10^8 (0.005 x 10^8-0.3 x 10^9)
Ward X	1197.5 (1042.3 – 1307.0)	4.0 (0.0 – 22.0)	2.4 x 10^8 (1.8 x 10^8 – 1.4 x 10^9)	1.5 x 10^8 (0.8 x 10^8 – 0.9 x 10^9)
ICU	0.50 (0.0- 12.0)	Nil	2.3 x 10^8 (1.3 x 10^8 – 1.0 x 10^9)	1.6 x 10^8 (8.8 x 10^8- 0.6 x 10^9)
Neuro-ICU	199.0 (101.3 - 578.0)	0.0 (0.0- 0.3)	4.0 x 10^8 (2.3 x 10^8- 1.9 x 10^9)	0.08 x 10^8 (0.0- 0.2 x 10^9)
CICU	58.5 (44.3 - 89.3)	0.5 (0.0- 3.0)	2.5 x 10^8 (2.2 x 10^8 – 1.6 x 10^9)	0.0 (0.0- 0.05 x 10^9)
CCU	55.5 (11.5 - 107.8)	0.0 (0.0- 1.3)	4.2 x 10^8 (2.2 x 10^8 – 2.1 x 10^9)	0.0 (0.00- 0.2 x 10^9)
SICU	43.5 (27.8 - 61.5)	1.5 (0.0- 15.5)	1.9 x 10^8 (1.0 x 10^8- 0.7 x 10^9)	1.0 x 10^8 (0.5 x 10^8- 0.2 x 10^9)
p value	0.001*	0.057	0.081	0.001*

IQR – Interquartile range, Statistical test: Kruskal Walis, *p<0.05

Based on the data presented in the table, there was a significant difference in the total CFU of airborne bacteria among the critical units for NA ($p<0.05$). This comparison analysis was subsequently subjected to Post hoc- Multiple Mann-Whitney tests with Bonferroni's Correction. The results indicated significant differences in the total CFU of airborne bacteria between Ward X (1197.5, IQR 1042.3 – 1307.0) and ICU (0.50, IQR 0.0 - 12.0); Ward X (1197.5, IQR 1042.3 – 1307.0) and CICU (58.5, IQR 44.3 - 89.3); Ward X (1197.5, IQR 1042.3 – 1307.0) and CCU (55.5, IQR 11.5 - 107.8); Ward X (1197.5, IQR 1042.3 – 1307.0) and SICU (43.5, IQR 27.8 - 61.5); ICU (0.50, IQR 0.0 - 12.0) and Neuro-ICU (199.0, IQR 101.3 - 578.0); ICU (0.50, IQR 0.0 - 12.0) and CICU (58.5, IQR 44.3 - 89.3); Neuro-ICU (199.0, IQR 101.3 - 578.0) and SICU (43.5, IQR 27.8 - 61.5). The disparity may arise from the discerning composition of airborne bacteria, whereby gram-positive bacteria often more resilient in airborne conditions than gram-negative bacteria due to differences in the cell wall structure making them more amenable to long-distance transportation. Consequently, a significant variation in the total CFU of airborne bacteria was observed across the critical units on NA, whereby this basic medium allows a broader range of bacteria and fungi to thrive compared to MAC agar.

Nevertheless, there was a significant difference in total CFU of surface swab bacteria between the critical units for MAC agar ($p<0.05$). The results indicated significant differences in the total CFU of surface swabs bacteria between Ward X (1.5 x 10^8; IQR 0.8 x 10^8 – 0.9 x 10^9) and CICU (0.0, IQR 0.0- 0.05 x

10^9); ICU (1.6 x 10^8, IQR 8.8 x 10^8- 0.6 x 10^9) and Neuro-ICU (0.08 x 10^8, IQR 0.0- 0.2 x 10^9); ICU (1.6 x 10^8, IQR 8.8 x 10^8- 0.6 x 10^9) and CICU (0.0, IQR 0.0- 0.05 x 10^9); CICU (0.0, IQR 0.0- 0.05 x 10^9) and SICU 1.0 x 10^8 (0.5 x 10^8, IQR 0.2 x 10^9). Indeed, gram-negative bacilli are frequently found on inanimate surfaces due to their ability to form biofilms and persist for extended periods. These bacteria have a thin peptidoglycan cell wall enveloped by an outer membrane, which serves as an additional layer of defense. This structure provides increased protection and resilience against various environmental factors, potentially giving them a survival advantage on inanimate surfaces compared to gram-positive bacteria. Due to this reason, the total CFU of surface swab bacteria on MAC agar showed a significant difference between the critical units, $p < 0.05$

Airborne and Surface Swabs Microbial Contamination in Different Critical Units During Morning and Evening Sessions

Table 2 displays the comparison between the total CFU of airborne bacteria (CFU/m^3) and surface swabs bacteria (CFU/mL), on NA and MAC agar between the morning and evening sessions. The data from the table indicate that only Neuro-ICU exhibited statistically significant differences between the morning and evening sessions, where the median for total CFU of airborne bacteria at Neuro-ICU in the morning (335.0, IQR 286.00-0.00) was significantly higher compared to the total CFU of airborne bacteria in the evening (104.0, IQR 93.0-0.0) ($p<0.05$). Microbial counts can fluctuate at different times due to a variety of factors, including environmental conditions, human activity, and sampling methods. In addition to human activity, environmental conditions in the Neuro-ICU may also contribute to the variations in the total microbial count between morning and evening. Despite the consistency in temperature and relative humidity, a variation in the amount of light exposure in this unit was observed. During the evening session, it was noted that nearly all areas within these units were exposed to daylight. These conditions have the potential to inactivate many microorganisms and fungi, potentially leading to a reduction in microbial community viability [17]. Consequently, the median total CFU of airborne bacteria in the evening session was lower compared to the morning session. The impacts of daylight exposure on microbial load were studied by Fahimipour *et al.* [18], which reported similar findings.

Table 2. Comparison between CFU of microbial contaminations on NA and MAC agar between the morning and evening sessions

Samples	Plate	Units	Median for CFU (IQR)		P value
			Morning	Evening	
Air samples (CFU/m^3)	NA	Ward X	1088 (905.00- 0.00)	1307 (1088.0- 0.0)	0.346
		ICU	0.00 (0.00- 0.00)	1.00 (0.00- 0.00)	0.817
		NeuroICU	335.00 (286.00- 0.00)	104.00 (93.0- 0.0)	0.050*
		CICU	77.00 (39.00- 0.00)	54.00 (46.0- 0.0)	0.513
		CCU	13.00 (7.00- 0.00)	87.00 (24.0- 0.0)	0.513
		SICU	39.00 (29.00- 0.00)	48.00 (24.0- 0.0)	0.827
	Total CFU NA		65.5 (25.0- 477.5)	73.5 (24.0- 108.3)	0.874
	MAC	Ward X	3.0 (0.0-0.0)	19.0 (0.0- 0.0)	0.376
		ICU	Nil	Nil	1.000
		Neuro ICU	Nil	Nil	0.317
		CICU	Nil	1.0 (0.0-0.0)	0.817
		CCU	Nil	1.00 (0.0-0.0)	0.121
		SICU	1.0 (0.0- 0.0)	15.0 (0.0-0.0)	0.376
	Total CFU MAC		0.0 (0.0- 1.25)	0.0 (0- 5.25)	0.367
Surface swabs (CFU/mL)	NA	Ward X	3.5 x 10^8 (1.9 x 10^8 – 1.6 x 10^9)	2.2 x 10^8 (1.8 x 10^8 – 1.3 x 10^9)	0.591
		ICU	4.1 x 10^8 (1.5 x 10^8 -1.0 x 10^9)	2.0 x 10^8 (0.8 x 10^8 – 0.9 x 10^9)	0.401
		Neuro ICU	4.0 x 10^8 (1.7 x 10^8 – 1.8 x 10^9)	7.7 x 10^8 (2.4 x 10^8 – 1.9 x 10^9)	0.753
		CICU	6.5 x 10^8 (2.2 x 10^8 – 1.6 x 10^9)	2.5 x 10^8 (1.3 x 10^8 – 1.6 x 10^9)	0.834
		CCU	4.2 x 10^8 (2.2 x 10^8 – 2.2 x 10^9)	6.5 x 10^8 (2.2 x 10^8 – 1.7 x 10^9)	0.713
		SICU	1.9 x 10^8 (9.6 x 10^8 – 0.7 x 10^9)	1.8 x 10^8 (0.5 x 10^8 - 0.7 x 10^9)	0.834
	Total CFU NA		2.5 x 10^8 (1.9 x 10^8 – 1.5 x 10^9)	2.4 x 10^8 (1.8 x 10^8 – 1.3 x 10^9)	0.564
	MAC	Ward X	1.7 x 10^8 (1.0 x 10^8 – 0.9 x 10^9)	1.4 x 10^8 (0.4 x 10^8 – 0.9 x 10^9)	0.580
		ICU	1.5 x 10^8 (0.9 x 10^8 – 0.6 x 10^9)	1.6 x 10^8 (0.9 x 10^8 – 0.7 x 10^9)	0.916
		Neuro ICU	0.08 x 10^8 (0.007 x 10^8 – 0.066 x 10^9)	0.3 x 10^8 (0.00- 0.6 x 10^9)	0.915
		CICU	0.2 x 10^8 (0.0- 0.2 x 10^9)	0.00 (0.00-0.013 x 10^9)	0.228
		CCU	0.00 (0.0- 0.2 x 10^9)	0.7 x 10^8 (0.0 – 0.4 x 10^9)	0.469
		SICU	0.8 x 10^8 (0.4 x 10^8 – 0.2 x 10^9)	1.7 x 10^8 (0.5 x 10^8 – 0.6 x 10^9)	0.401
	Total CFU MAC		0.8 x 10^8 (0.05 x 10^8 – 0.3 x 10^9)	1.2 x 10^8 (0.0 x 10^8 – 0.5 x 10^9)	0.807

IQR – Interquartile range, Statistical test: Mann-Whitney, *$p<0.05$

The Presence of Isolated Bacteria Colonies in Different Critical Units

Table 3 reveals that out of 304 samples, 101 (33.2%) isolates were selected for further species identification tests. Among these, gram negative bacteria were the most common, amounting for 88 isolates (87.1%), while gram-positive bacteria made up 13 isolates (12.9%). This finding is contradicted with the previous report of airborne microbial contamination in the operation theatre that found majority were gram positive bacteria (95.6%) [13]. The gram-negative bacteria were subsequently identified using biochemical sets and API® 20E tests as confirmation test for bacterial identification process to determine their respective species. However, none of the biochemical tests and API® 20E tests were tested for 13 (12.9%) *Bacillus* spp. The observations and interpretations of the biochemical test reactions and the API® 20E tests were conducted in accordance with the established guidelines outlined by the Medical Microbiology Department of the Ministry of Health, Malaysia. As illustrated in Table 3, there were 71 isolates (70.3%), demonstrated susceptibility to three types of classes of carbapenem antibiotics; meropenem, imipenem, and ertapenem. This is similar to our recent findings of Carbapenem Resistant *Klebsiella pneumoniae* (although in clinical samples) showed susceptibility to with ertapenem having the highest susceptibility followed by imipenem and meropenem [19]. In contrast, the current study showed that 17 isolates (16.8%), exhibited resistance to these antibiotics. None of the disk diffusion tests using carbapenem antibiotics were tested for *Bacillus* spp. which accounted for 13 isolates (12.9%).

Table 3. The presence of isolated bacteria colonies in critical units

Tests		Frequency (%)
Gram-stain	Gram-positive bacteria	13 (12.9)
	Gram-negative bacteria	88 (87.1)
TSI	A/A/G	60 (59.4)
	A/K	17 (16.8)
	K/K	11 (10.9)
	NA	13 (12.9)
Citrate	POS	82 (81.2)
	NEG	6 (5.9)
	NA	13 (12.9)
Urease	POS	45 (44.5)
	NEG	43 (42.6)
	NA	13 (12.9)
SIM	NEG/NEG/NEG	53 (52.5)
	NEG/POS/NEG	7 (6.9)
	NEG/POS/POS	10 (9.9)
	NEG/NEG/POS	18 (17.8)
	NA	13 (12.9)
MRVP	POS	21 (20.8)
	NEG	67 (66.3)
	NA	13 (12.9)
Disk diffusion	Susceptible	71 (70.3)
	Resistant	17 (16.8)
	NA	13 (12.9)

TSI - Triple Sugar Iron Agar, SIM - Sulfide Indole Motility, MRVP - Methyl Red Voges-Proskauer

Based on findings detailed in Table 4, the predominant bacterial species identified out of the total 304 bacteria isolated were *Klebsiella pneumoniae* (*K. pneumoniae*) (n=34, 33.7%). This was followed by 13 *Bacillus* spp. (12.9%), and 12 *Acinetobacter baumannii* (*A. baumannii*) (11.9%). Both *Enterobacter* spp. and *Pseudomonas aeruginosa* (*P. aeruginosa*) exhibited a frequency of 9 isolates, corresponding to 8.9%, each. Additionally, *Klebsiella oxytoca* (*K. oxytoca)* was identified in 7 isolates (6.9%), and *E. coli* in 6 isolates (5.9%). *Klebsiella ozaenae* (*K. ozaenae*) and *Citrobacter* spp. each had 4 isolates (4%), and *Pseudomonas* spp. was found in 3 isolates (3%). This discovery aligns with a prior investigation conducted in Florence, Italy, which involved 494 patients in intensive care units (ICUs). Among these patients, 46 experienced HAIs. The predominant pathogens responsible for these infections were strains of gram-negative bacteria, namely *K. pneumoniae* (30%), *A. baumannii* (20%), *E. coli* (20%), *P. aeruginosa* (17%), and other gram-negative bacteria such as *K. oxytoca* and *Enterobacter* spp. (13%) [20].

Table 4. Types of isolated bacteria at different sampling locations within critical units

Isolated bacteria	Frequency of bacteria (%)						
	Ward X (n=84)	ICU (n=44)	Neuro-ICU (n=44)	CICU (n=44)	CCU (n=44)	SICU (n=44)	Total (N=304)
A. baumannii	6 (7.1)	2 (4.5)	4 (9.1)	Nil	Nil	Nil	12 (11.9)
Bacillus spp.	5 (6.0)	2 (4.5)	Nil	2 (4.5)	Nil	4 (9.1)	13 (12.9)
E. coli	2 (2.4)	2 (4.5)	Nil	Nil	Nil	2 (4.5)	6 (5.9)
K. oxytoca	2 (2.4)	2 (4.5)	3 (6.8)	Nil	Nil	Nil	7 (6.9)
K. pneumoniae	16 (19.0)	8 (18.2)	1 (2.3)	5 (11.5)	Nil	4 (9.1)	34 (33.7)
K. ozaenae	Nil	Nil	Nil	Nil	2 (4.5)	2 (4.5)	4 (4.0)
P. aeruginosa	2 (2.4)	Nil	Nil	1 (2.3)	3 (6.8)	3 (6.8)	9 (8.9)
Pseudomonas spp.	Nil	Nil	Nil	Nil	1 (2.3)	2 (4.5)	3 (3.0)
Enterobacter spp.	3 (3.6)	Nil	Nil	1 (2.3)	2 (4.5)	3 (6.8)	9 (8.9)
Citrobacter spp.	Nil	Nil	4 (9.1)	Nil	Nil	Nil	4 (4.0)

The Presence of Carbapenem Resistant Bacteria Isolated from Environmental Samples in Different Critical Units

Among the 101 isolated bacteria, 17 (16.9%) isolates were resistant to carbapenem antibiotics, thereby classified under carbapenem resistance bacteria. Of these, a total of 8 (47.1%) were found at Ward X, 4 (23.5%) at ICU, 2 (11.8%) at Neuro-ICU, and 3 (17.6%) at SICU. No carbapenem-resistant bacteria were identified at the CICU and CCU.

Table 5 shows that the majority of carbapenem-resistant bacteria in the critical units were *K. pneumoniae* (8 isolates, 47.1%), followed by *A. baumannii* (6 isolates, 35.3%), and *P. aeruginosa* (3 isolates, 17.6%). Among the 8 carbapenem resistance organisms at Ward X, 4 (50%) of them were identified as *A. baumannii* and 4 (50%) *K. pneumoniae*. Besides, *K. pneumoniae* has been identified as the bacterial species responsible for all 4 (100%) isolated carbapenem resistance bacteria at the ICU. Moreover, *A. baumannii* species was identified as the causative agent of 2 (100%) isolated carbapenem resistance bacteria detected in the Neuro-ICU, whereas *P. aeruginosa* was found to be responsible for 2 (100%) isolated carbapenem resistance bacteria in the SICU. *K. pneumoniae* is among the most prevalent bacteria causing CRE infections in healthcare settings [21]. A study by Chang *et al*. [22] reported that *K. pneumoniae* has emerged as a significant pathogen of global concern due to the rising incidences of hypervirulent and carbapenem-resistant strains. Similarly, Kamio & Espinoza identified *K. pneumoniae* as the predominant bacterium causing CRE infections, accounting for approximately 60% of CRE infections in Japanese hospital settings during the surveillance period [23]. This assertion is corroborated by a recent study in which *K. pneumoniae* was identified as responsible for eight isolated CRE species in critical units specifically, 4 of them were detected in Ward X while another 4 isolates were detected in the ICU. Importantly, all the carbapenem-resistant bacteria were found in surface swab samples, except for one instance where a CRPA was isolated from an air sample. This observation is consistent with the understanding that bacterial transmission primarily occurs through contact with contaminated instruments or surfaces [24].

Table 5. Types and frequency of carbapenem resistant bacteria's species in different critical units

Critical units	n	Organism	Frequency (%)
Ward X	8	*A. baumannii*	4 (50)
		K. pneumoniae	4 (50)
ICU	4	*K. pneumoniae*	4 (100)
Neuro-ICU	2	*A. baumannii*	2 (100)
SICU	3	*P. aeruginosa*	3 (100)

Correlation Between Temperature, Relative Humidity and Occupancy with Bacterial Counts in Critical Units

The recorded temperature and relative humidity measurements at critical units mostly fell within the acceptable range set by the Ministry of Health (MOH) Malaysia. The standard temperature level ranged from 23 °C to 26 °C, while the acceptable range for relative humidity was between 40% and 70% [25]. However, the occupancy of each critical unit varies based on the ward's capacity and ability to meet the demand for critical care services, ranging from 13 to 43 occupants.

The association between the contributing factors, which are temperature, relative humidity, and number of occupants, with the total CFU of bacterial contamination within critical units was investigated using Spearman's Rho Correlation test. Table 6 displays data indicating that none of the air samples or surface swabs samples demonstrated significant correlations between temperature, relative humidity and number of occupants with the total CFU of bacterial contamination, as indicated by the p-value > 0.05. However, a weak positive correlation was observed between both the total CFU of air and surface swabs bacteria with these factors, except for surface swabs bacteria with temperature, which showed a moderate positive correlation (r-value of 0.32). Previous studies have shown that temperature plays a significant role in microbial contamination by affecting microbial load and diversity. For instance, Qiu *et al*. found a negative correlation between ventilation rate and relative humidity with microbial levels, while temperature showed a positive correlation [26]. Similarly, Monteiro *et al*. found a significant correlation between temperature and relative humidity with airborne bioburden, but not with occupancy [27].

Microbial activity is influenced by relative humidity and temperature. As relative humidity increases, so does microbial activity. High relative humidity promotes biofilm development on surfaces, providing a habitat for microorganisms and enabling bacteria to survive in hostile environments [28]. In contrast, in dry environments, microorganisms experience desiccation and cellular dehydration, leading to shrinkage. Microbial reproduction ceases when relative humidity falls below 60% [29]. Despite these findings, this study showed no significant correlations between the total CFU of microbial contamination and environmental factors. This could be due to other factors impacting microbial growth rate, such as pH, water activity, oxygen level, pressure, and radiation [30]. Occupancy can also impact microbial contamination levels in hospital environments, depending on factors such as the type and activity of the occupants and the origin and dispersion of germs [31]. However, this study found a weak correlation between occupancy and the total CFU of microbial contamination in the critical units of the hospital.

Table 6. Correlation between temperature, relative humidity, occupancy with total bacterial counts

Types of samples		Contributing factors		
		Temperature (^{O}C)	Relative humidity (%)	Occupancy
Air samples (CFU/m^3) (n=232)	Correlation coefficient (r)	0.073	0.025	0.127
	p value	0.265	0.703	0.053
Surface swabs samples (CFU/mL) (n=72)	Correlation coefficient (r)	0.320	0.085	0.047
	p value	0.060	0.478	0.697

Statistical test – Spearman's Rho, *p<0.05

Conclusions

In summary, the study underscores significant microbial contamination disparities across the hospital's critical units. It evaluated the total CFU in air and surface bacteria samples, considering environmental factors influencing microbial growth and spread. This ongoing monitoring is vital for maintaining a hygienic environment and ensuring patient and staff well-being. The study also revealed the presence of CREs in the environmental surroundings of different wards within the critical units. Notably, 17 carbapenem-resistant bacteria strains were isolated, eight of which were CREs, with *K. pneumoniae* being the causative agent. These findings, despite being within microbial load limits, suggest potential risks to critical units patients and staff. Moreover, no significant differences in total CFU of bacteria were observed between morning and evening sessions across units, except for Neuro-ICU. The study found no correlation between the total CFU of bacteria and environmental factors, specifically temperature, humidity, and occupancy, indicating the need for further research. Understanding environmental pathogens' origins and spread is crucial for effective preventive measures. The study provides insights into local pathogen prevalence and resistance, aiding in timely interventions to reduce colonization, initiate empirical antibiotic treatment for HAIs, and ultimately decrease mortality rates.

Infection prevention and control (IPC) encompassing interventions such as surveillance, standard precautions, hand hygiene, and environmental cleaning, that has already been imposed in this hospital following the MOH requirement is proven crucial in preventing the further spread of CRE [12]. The study recommends enhancing routine hygiene monitoring in critical units to ensure its safety. This can be achieved through regular cleaning, disinfection, hand hygiene, proper waste disposal, use of PPE, air ventilation checks, and audits. The collective responsibility of preventing infection spread and ensuring well-being requires everyone's participation. Clinicians and researchers should be cognizant of the risk of pathogen cross-transmission from air or surfaces to implement appropriate infection control measures. Furthermore, hospital infection control committees should also adopt active environmental monitoring for IPC, as demonstrated in this study, to effectively investigate, control, and minimize HAIs, particularly

those originating from air and contaminated surfaces. Additional research is needed to understand virulence and resistance determinants, genetic lineage, transmission mechanisms, diagnostic techniques, antibacterial treatment targets, and preventive measures. These efforts are vital in mitigating HAIs, ultimately reducing morbidity and mortality rates

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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