

Metagenomic Analysis of Contaminated Lift Buttons Reveals Prevalent Pathogens with Antimicrobial Resistance Genes: A Study in a Public Hospital in Pahang, Malaysia

# Izzati Muhammad, MSc

Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia izzati.97.muhammad@gmail.com

## Raja Noor Zaliha Raja Abd Rahman, PhD

Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia <u>rnzaliha@upm.edu.my</u>

## \*Norsyuhada Alias, PhD

Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia norsyuhada\_alias@iium.edu.my

## Niza Samsuddin, PhD

Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia <u>niza\_shamsuddin@iium.edu.my</u>

## Norhidayah Kamarudin, MMed

Department Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia norhidayahkamarudin@iium.edu.my

\**Corresponding author:* Norsyuhada Alias, <u>norsyuhada\_alias@iium.edu.my</u>

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# Abstract:

Introduction: Hospital lift buttons are potential reservoirs for microbial contamination, contributing to the transmission of hospital-acquired infections (HAIs). Despite routine cleaning efforts, concerns persisted regarding the prevalence of contaminants on these surfaces, particularly in healthcare settings within Southeast Asian countries. This study aimed to investigate the prevalence and microbial diversity of contaminants on lift buttons in a public hospital (PH) located in Pahang, Malaysia, during the coronavirus disease 2019 pandemic. Methods: Purposive swab sampling was conducted thrice at two-week intervals. Standard plate count and metagenomic analysis were employed to determine the prevalence of contaminants and identify the diverse microbial communities, respectively. Analysis of variance and Bonferroni test evaluated at alpha value less or equal to 0.05, were performed to determine the significance of the findings. **Results:** The investigation revealed a notable prevalence of contaminants at 30.4% on both interior and exterior lift buttons, with no significant disparity observed between lifts in high-risk and moderate-risk areas (p>0.05). Metagenomic analysis revealed Firmicutes as the dominant phylum, with *Staphylococcus* and *Bacillus* being the most prevalent genera. Analysis of the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways highlighted the importance of ABC transporter and two-component systems, where key genes involved in the iron complex transport, antimicrobial resistance, and multidrug efflux enriched, crucial for the microbial survival. **Conclusion:** These findings underscored the complexity of microbial ecosystems and their adaptive mechanisms in response to environmental pressures, while emphasizing the importance of implementing effective infection control measures to mitigate the risk of lift buttons-associated HAIs. Future research should broaden the geographical scope to encompass diverse hospital settings and explore the interaction of microbial communities' functional capabilities with the hospital environment, offering insights into optimizing hygiene practices and targeted intervention.

**Keywords:** lift button, hospital, KEGG, metagenomic, antimicrobial resistance genes, hospital-acquired infection

## Introduction:

Lift in healthcare facilities stand as indispensable conduits of vertical mobility, facilitating the transportation of patients, medical and essential resources. Despite staff, their significance, lifts, as one of the high-touch surfaces, often go unnoticed as potential vectors of microbial silently contributing contamination, to the transmission of infectious agents within hospitals. Hospital-acquired infections (HAIs) are persistent challenges in healthcare settings, often caused by various pathogens, including those found on lift buttons.

The emergence of HAIs is not a recent concern, as this issue has been globally prevalent since the 1900s. A study conducted by Goh et al. (2023) revealed that the prevalence of HAIs in Southeast Asian countries stood at 22% between 1990 and 2022, reaching the upper limit of the worldwide HAI rate (7-22%) reported by WHO (Kilpatrick et al., 2011). WHO's data in year 2022 highlighted that the infection rate for HAIs was one in 100 patients in low- and middle-income countries, with an average mortality rate of one in every ten affected patients (WHO, 2022).

The source of HAIs extends beyond the wellknown ESKAPE pathogens, namely Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. Additional pathogens have been recognized in patients with HAIs, many of whom develop pathogenicity due to compromised immune systems (AMBOSS, 2020). Various risk factors contribute to these infections, including, but not limited to, age (particularly over 70 years old), extended hospital stays, antibiotic usage, and metabolic diseases (AMBOSS, 2020). HAIs are often associated with specific conditions such as central line-associated bloodstream infection, catheterassociated urinary tract infection, surgical site infection, and ventilator-associated pneumonia, which are among the most prevalent causes of these infections (CDC, 2014).

Lift buttons are not excluded from being contaminated with agents responsible for HAIs. A study conducted in Tehran, Iran, discovered the presence of methicillin-resistant *S. aureus* on hospital lift buttons (Abbasian et al., 2018). Similarly, research in China revealed the isolation of carbapenemresistant *A. baumannii* on lift buttons (Liu et al., 2017). Despite hospitals implementing thorough cleaning protocols to combat HAIs, the reduction in HAI trends across Southeast Asian countries from 1990-2014 to 2014-2022 was marginal, with only 0.01% (Goh et al., 2023). This percentage poses a challenge to healthcare departments, suggesting potential shortcomings in disinfection techniques and cleaning routines.

Understanding the microbial contamination of lift buttons is crucial for infection control measures, given their frequent use due to the constant flow of individuals, numerous touchpoints and enclosed space (Gooch and Wadhwa, 2020). Particularly in Southeast Asian countries, most studies conducted in hospital environments are more focused on clinical samples, leaving a gap in the study of fomites like lift buttons, (Sani et al., 2014; Sukri et al., 2022).

Recognizing the need to address this gap, the study embarked on an exploration of the prevalence and metagenomic profiles of microbial contamination on the lift buttons of a public hospital (PH) in Pahang, Malaysia, a country with a standard ideal population (Department of Statistics Malaysia, 2022). The objectives encompass analysing the prevalence of contaminants and understanding the dominance patterns and the survival capabilities of varies microbial species harbouring hospital lift buttons. By into the intricacies of lift delving button contamination, the study aimed to contribute valuable insights to fortify infection control strategies in regional healthcare settings. This investigation served

as a critical step towards understanding and mitigating the potential infectious threats present in a hospital infrastructure.

## Methodology:

#### **Study Location**

The public hospital was located in a coastal and urban environment in Pahang. The hospital, one of the largest healthcare facilities in the region, had over 500 beds with more than 50% bed occupancy rates. It consisted of more than 20 different departments and units. The hospital served a diverse population from both urban and rural areas by providing a range of healthcare services, from primary care to specialized treatments and outpatient services (Mahdi Yahya Mohsen et al, 2016).

## **Sampling Procedure**

This study focused on evaluating the microbial contamination of hospital lift buttons under defined parameters. Eligible lifts included those accessible to all patient floors with a dimension that could fit a standard hospital bed, excluding lifts transporting COVID-19 related matters. Systematic selection of lift buttons centred around floors or areas with documented reports of contaminants, encompassing main entrances (ME, floor G), operation theatres (OT, floor 3), intensive care units (ICU, floor 3), paediatric wards (PW, floor 6), and general wards (GW, floor 8) (De Paula Menezes et al., 2022; Doughty et al., 2022; Olise and Simon-Oke, 2018).

The sampling process used the technique adapted from Carrascosa et al. (2018), where a cotton swab moistened with 1mL of 0.1% peptone water (Merck, Germany) was used along a 14 cm<sup>2</sup> stencil for 10 seconds. The collection of control samples was implemented after disinfecting the lift buttons with 70% ethanol. A collection of 100 lift buttons, including both interior (horizontal and vertical panels) and exterior buttons was obtained in a single sampling procedure (Figure 1). The sampling process was conducted three times at two-week intervals during the COVID-19 Movement Control Order (March-April 2021).

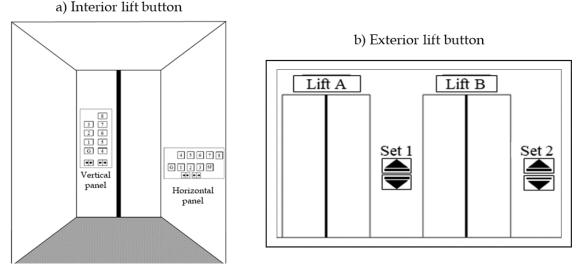


Figure 1: Location of interior (vertical and horizontal panels) and exterior lift buttons. The vertical panel is situated within the lift beside the door, whereas the horizontal panel is positioned on the side of the lift, closer to the bottom. Exterior lift buttons are arranged in sets, typically consisting of an up button and a down button.

#### **Standard Plate Count**

A 10  $\mu$ L aliquot was aliquoted and spread evenly on the surface of nutrient agar plates and incubated for 24-48 hours at 37°C (Elsergany et al. 2015; Mohd Fauzi, 2018). The prevalence of bacteria and colony forming units per millilitre (CFU/mL) were calculated using the formula from the Glasgow et al. (2013) and Appeh et al. (2022), respectively. The analysis of variance (ANOVA) test was used to measure the association between lifts, lift buttons panels and the selected floors, while the Bonferroni test was used as a post hoc test. Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS) software version 27.0, where the analyses were evaluated at an alpha value less or equal to 0.05.

#### **Metagenomic Analysis**

Similar volume of samples was aliquoted and pooled into 10 mL of tryptic soy broth (Merck, Germany). The tubes were incubated at 37°C with aeration of 200 rpm for 24-40 hours (Valeriani et al., 2018), followed by pelleting down at 13,100 x g for 5 minutes and removing the supernatant. The genomic DNA of the samples was performed using DNeasy® UltraClean® Microbial Kit by Qiagen (Hilden, according the manufacturer's Germany) to instructions. The quality and quantity of the extracted DNA were evaluated through gel electrophoresis using 1% agarose gel (Vivantis, Malaysia) and spectrophotometer readings.

DNA samples with concentration of >45ng/ $\mu$ L were sequenced using the Oxford Nanopore Technologies (ONT) MiNION flowcell for long reads, following the manufacturer's instructions (Oxford Nanopore, United Kingdom). The data obtained in raw fast5 files were subjected to base-called in high accuracy mode using Guppy v 4.4.1. The reads obtained were filtered based on their quality and read length to ensure a longer read with qscore of 7 and above. The reads were assembled using SPAdes with the default setting (Siew et al., 2022; Wick et al., 2017).

The sequences obtained were uploaded to Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) (The Metagenomics RAST server; https://www.mg-rast.org) in fastq format with its metadata for annotation and analysis of the sequences (Meyer et al., 2008). The reads were taxonomically annotated by similarity searching against the RefSeq and KO database with default parameters. Percentage of taxonomy abundance was calculated based on the number of reads per taxa of interest over total number of reads obtained.

#### **Results:**

# Quantification of Contaminants Isolated from the Lift Buttons

Out of the 46 lift buttons sampled, 28 cultured colonies were isolated, resulting in a prevalence of 30.4%. A total of 440 CFU/mL was isolated from the interior lift buttons (Table 1). Although floor 6 (PW) had the highest number of colonies, there was no difference in the mean across the different floors (p>0.05). Similarly, there was no significant difference observed in the means of lifts, floors and panel of interior lift buttons (p>0.05).

The exterior lift buttons exhibited a distinct outcome compared to the interior ones, with only 120 CFU/mL of bacteria isolated. Although lift button set 2 had more colonies than set 1, there was no difference in their mean counts. A comparison of the exterior lift button surfaces showed that floors 3 (OT & ICU) and 7 (GW) had higher colony counts than other floors, but the difference was not statistically significant.

I ili dataila			Average colony forming unit per mL (CFU/mL ± SD)						
Lift details		Floor G	Floor 3	Floor 6	Floor 7	Floor 8	Total		
Interior lift button	Lift A	Vertical panel	$20 \pm 28.3$	$0 \pm 0.0$	$30 \pm 42.4$	$0 \pm 0.0$	$40 \pm 28.3$	- 110 ± 15.2	
		Horizontal panel	$20 \pm 28.3$	$0 \pm 0.0$	$0 \pm 0.0$	$0 \pm 0.0$	$0 \pm 0.0$		
	Lift B	Vertical panel	$30 \pm 14.1$	$20 \pm 28.3$	$110 \pm 99.0$	$10 \pm 14.1$	$0 \pm 0.0$	- 240 ± 32.0	
		Horizontal panel	$10 \pm 14.1$	$30 \pm 42.4$	$0 \pm 0.0$	$10 \pm 14.1$	$20 \pm 28.3$		
	Lift C	Vertical panel	$0 \pm 0.0$	$0 \pm 0.0$	$20 \pm 28.3$	$0 \pm 0.0$	$20 \pm 28.3$	$-90 \pm 9.9$	
		Horizontal panel	$0 \pm 0.0$	$20 \pm 28.3$	$10 \pm 14.1$	$20 \pm 28.3$	$0 \pm 0.0$	- 90 ± 9.9	
ſft	Set 1	Up button	$0 \pm 0.0$	$0 \pm 0.0$	$0 \pm 0.0$	$0 \pm 0.0$	NA	- 50 ± 11.9	
terior li button		Down button	NA	$30 \pm 42.4$	$0 \pm 0.0$	$0 \pm 0.0$	$20 \pm 0.0$		
Exterior lift button	Set 2	Up button	$10 \pm 14.1$	$10 \pm 14.1$	$0 \pm 0.0$	$30 \pm 42.4$	NA	$70 \pm 0.0$	
	Se	Down button	NA	$0 \pm 0.0$	$10 \pm 14.1$	$10 \pm 14.1$	$0 \pm 0.0$	$-70 \pm 9.9$	
Total			$90 \pm 11.3$	$110 \pm 12.9$	$180 \pm 33.9$	$80 \pm 10.3$	$100 \pm 14.9$	$560 \pm 18.7$	

Table 1: Quantification of bacteria on lift buttons across different floors in a public hospital (PH).

NA = Data not available; SD = Standard deviation

#### **Metagenomic Findings**

Analysis of the metagenome showed that there were 2 628 047 number of reads, which approximates to a 1650 species count based on the rarefraction curve. The investigation of taxonomy abundance revealed bacteria as the highest contributor of species (Figure 2). The rank phylum illustrated Firmicutes as the amplest the metagenome followed in by Proteobacteria and Actinobacteria. The rank class was dominated by Bacilli with Clostridia as the second tier. Unclassified viruses and Saccharomycetes contributed less than 0.05% to the metagenome. The rank order was dominated by Bacillales, one of the order ranks for the class Bacilli. *Staphylococcaceae* and *Bacillaceae* were two of the highest prevalent distributors in the rank family while other families contributed only 1.15% to the metagenome. Deeper analysis of the genus rank showed that *Staphylococcus* contributed almost three-quarter of the species in the rank. *Bacillus* species came in second as the most found species in the metagenome followed by *Geobacillus, Anoxybacillus* and other species (Figure 3).

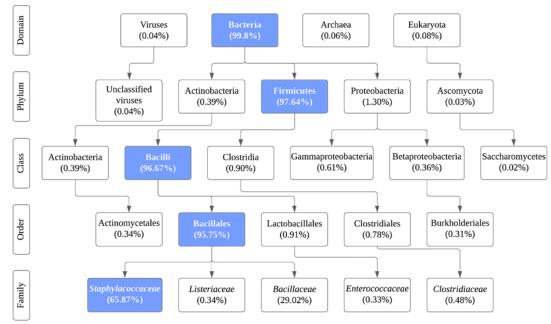


Figure 2: Taxonomy abundance at different hierarchy in PH metagenome. The blue box shows the highest abundance of species per rank. The figure includes only the top five or top six species in each rank. Species that reveal low taxonomy abundance are excluded.

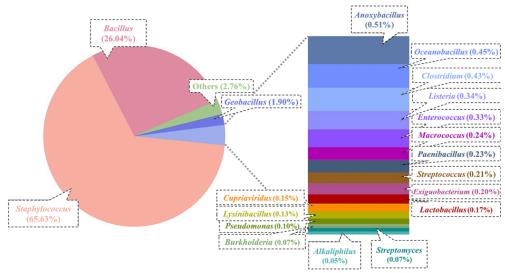


Figure 3: Taxonomic data of rank genus in PH metagenome. *Staphylococcus* and *Bacillus* made up around 90% of the species in rank genus. Other species listed total up to 5.58% with an average of 0.33% per species, excluding the segment labelled "others".

Functional protein				
Field	Group	Pathway	Percentage of abundance (%)	
EIP	Membrane transport	ABC transporters (PATH: ko02010)	7.9	
GIP	Translation	Aminoacyl-tRNA biosynthesis (PATH: ko00970)	4.7	
EIP	Signal transduction	Two-component system (PATH: ko02020)	4.4	
Met	Amino acid metabolism	Alanine, aspartate and glutamate metabolism (PATH: ko00250)	3.8	
Met	Amino acid metabolism	Glycine, serine and threonine metabolism (PATH: ko00260)	3.7	
Met	Nucleotide metabolism	Purine metabolism (PATH: ko00230)	2.9	
GIP	Translation	Ribosome (PATH: ko03010)	2.5	
Met	Carbohydrate metabolism	Pyruvate metabolism (PATH: ko00620)	2.4	
Met	Amino acid metabolism	Cysteine and methionine metabolism (PATH: ko00270)	2.3	
GIP	Replication and repair	DNA replication (PATH: ko03030)	2.2	
Met	Carbohydrate metabolism	Pentose phosphate pathway (PATH: ko00030)	2.2	
Met	Amino acid metabolism	Arginine and proline metabolism (PATH: ko00330)	2.2	
Met	Amino acid metabolism	Valine, leucine and isoleucine degradation (PATH: ko00280)	2.2	
Met	Carbohydrate metabolism	Glycolysis / Gluconeogenesis (PATH: ko00010)	2.0	
EIP	Membrane transport	Phosphotransferase system (PATH: ko02060)	2.0	

Table 2. Abundance of KEGG pathways in the hospital samples

EIP = Environmental Information Processing; GIP = Genetic Information Processing; Met = Metabolism; PATH = the pathway number annotated by the database. The table showed a part of the pathways analysed by the website. The total abundance of the pathway analysed by the website was 842, 746.

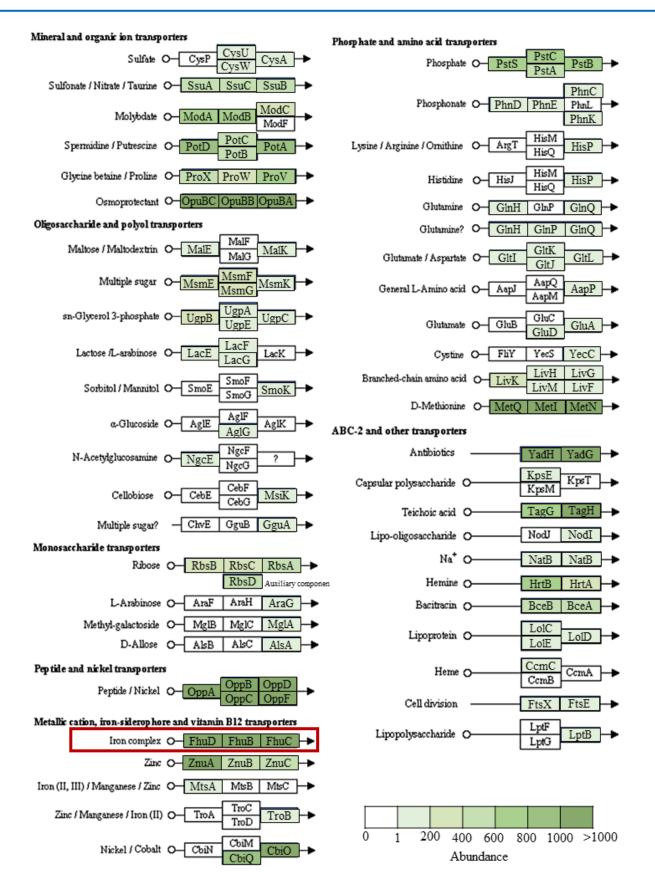


Figure 4: Genes enriched in the family of the ABC transporters pathways. The intensity of the colour green reflected the abundance of the enriched genes. The most enriched gene was *fluD* (highlighted in red box), which expressed as substrate-binding protein in iron complex transport system.

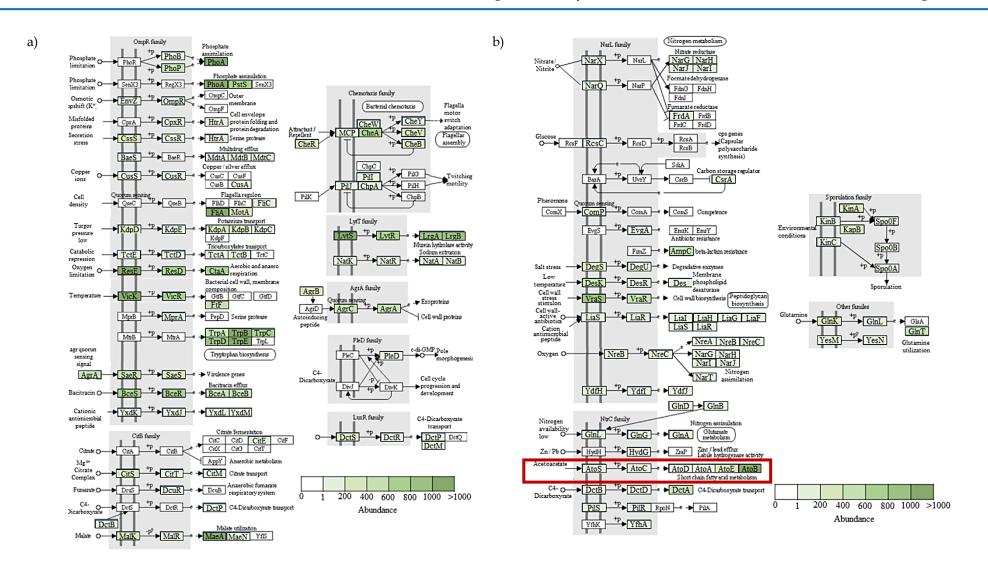


Figure 5: Pathways with enriched genes involving major family such as (a) OmpR and (b) NarL, under the two-component system. The intensity of the colour green reflected the abundance of the enriched genes. The most enriched gene in this system was *ato*B (highlighted in red box), which expressed as acetyl-CoA C-acetyltransferase. This protein plays a part in short chain acid metabolism under the NtrC family, a regulator that response to nitrogen level.

#### **KEGG Pathway Analysis**

A Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway was annotated using the plugins available from the analysis website. It should be noted that the analysis is not limited to a single organism and would involve all species in the metagenome. The pathway annotated by the website yielded fields and groups, as seen in Table 2. The system categorised the pathways into six different fields which were cellular processes, environmental information processing, genetic information processing, human diseases, metabolism and organismal systems. Most of the pathways shown were metabolic, while some of them were part of the genetic and environmental information processing fields. Other fields were less abundant and thus, were not highlighted in this study.

A total of 137 different pathways were created from 36 different groups or subsystems. The highest number of pathways expressed attributed to amino acid metabolism, totaling up to 22% of the whole annotated subsystem. The highest abundance of proteins was annotated from the ATP synthase binding cassette (ABC) transporters pathway, with notable differences from other pathways. This pathway along with the two-component system were investigated in greater depth since they play an important role in survivability of the species inhabiting the hospital lift buttons.

Each pathway consisted of several different gene sets which would be upregulated, downregulated or remained unchanged, depending on the internal and external stimulus received by the species. Figure 4 shows the enriched genes sets with different abundance grouped under the ABC transporters pathway. The abundance reflects the number of species with specific enriched genes. The most enriched genes in the pathway were *fhuD* and *fhu*B, proteins identified in the iron complex transport system. The hierarchy was followed by oppA (peptide/nickel transport system) and fhuC (iron complex transport system). Some of the genes enriched in the pathway, such as yadH, yadG, bceB and bceA, are involved in transporting antibiotics and other antimicrobial related matters from the cell.

The two-component system have 11 family categorised under it where OmpR and NarL family have a significant role in the antimicrobial resistant properties of the cell, as displayed in Figure 5. Several genes are enriched under the OmpR family including genes involved in multidrug efflux, virulence genes and bacitracin efflux. The gene enriched in the NarL family that have a similar effect on antimicrobial resistant properties is *amp*C, which promotes the resistance of the organism to beta-lactam antibiotics. The environment on lift buttons may also enrich the genes related to salinity, temperature, oxygen concentration and nutrient, which aids in prolonging the organisms survivability.

## **Discussion:**

#### Prevalence Patterns of Contamination on Lift Buttons

The investigation on prevalence of contaminants on a public hospital's lift buttons vielded distinct outcomes compared to previous studies in other countries. It should be noted that there was an absence of pre-pandemic data on bacteria prevalence on lift buttons in the studied region, hindering a conclusive comparison over time. A study on two hospitals, located in Kenya and Nepal, reported a bacteria prevalence of 66.3-80.8% isolated from fomites in 2018, a pre-pandemic era (Bhatta et al., 2018; Maina et al., 2018). Later, other studies conducted revealed contaminants prevalence of approximately 50.0%, isolated from fomites in Egyptian and Chinese hospitals (El-Masry and Taha, 2022; Qin et al., 2022). This fluctuating trend between 2018 and 2022 suggest significant progress in addressing contaminants over time.

One important change during the pandemic was a more stringent cleaning routine, as mandated by the updated guidelines (MOH, 2021). The guidelines maintained similar equipment, chemicals and cleaning steps as before but with improved regularity. Daily cleaning routines utilized multipurpose detergent, while incident and decontamination routines employed broad-spectrum chlorine-based disinfectants. (Amir, personal communication, March 13, 2021). Cleaning frequency was determined by the lift's location, with low-risk areas needing weekly cleaning and moderate-risk areas requiring daily attention (Hashmi, 2014). However, there remained a lack of standardization in interpreting cleaned environmental surfaces in healthcare settings (CDC, 2015). Innovative approaches such as integrating ultra-violet light technology and voice-controlled systems in the lifts could supplement existing cleaning routines, offering touch-free alternatives and continuous disinfection to enhance infection control measures in healthcare settings.

Restrictions on visitor access during sample collection, coupled with the provision of hand sanitizer inside and outside lifts, were implemented in

hospitals during the pandemic to reduce contamination from human touch (Department of Educational 2022). Additionally, Health, environmental factors such as temperature and climate of the lifts also affect the colonisation and localisation of the bacteria. Some HAIs-causing agents, including K. pneumonia and A. baumannii exhibited higher incidence density in warmer weather while S. aureus and E. faecium thrive better in cold environments (Schwab et al., 2014). Hospital lifts typically maintain a constant comfortable temperature ranging from 15-32°C with adequate ventilation, creating an environment conducive to the proliferation of mesophilic bacteria (Parker et al., 2016)

The investigation into bacterial prevalence on lift buttons unveiled varied patterns across different floors housing diverse departments, challenging the notion that high-risk areas would consistently exhibit fewer contaminated lift buttons compared to moderate-risk areas Hashmi (2014). For instance, floors housing OT and PW showed higher bacteria prevalence compared to others. This was similarly seen in research conducted in a Nigerian hospital, where ICU exhibited significantly higher bacteria prevalence compared to PW (Olise and Simon-Oke, 2018). Even carbapenem-resistant A. baumannii was isolated from fomites in the ICUs, which showed sterility was compromised (Doughty et al., 2022). This deviation from expected sterility levels warrants further exploration into contamination dynamics within healthcare settings.

## Microbial Diversity and its Survival Mechanisms

The metagenomic analysis of PH lift buttons unveiled diverse microbes with varying contamination levels. Utilizing genomic DNA for analysis provided insights into both viable and nonviable cells, encompassing a range of aerobic and anaerobic organisms. These contaminants could stem from various sources, including human contact and environmental factors, highlighting the potential pathways for contamination of lift buttons. Notably, previous research on fomite contamination, particularly lift buttons, is limited, and the application of metagenomic analysis in assessing microbial communities hospital on lift buttons is unprecedented, positioning this study as a pioneer in the field (Sukri et al., 2022).

Among the contaminants isolated from PH, included bacteria with several genera known for their association with HAIs. *Staphylococcus* was recognised as one of the most prevalent species in hospitals due to its association with human skin microflora (Ehlers and Merrill, 2022; Bhatta et al., 2018). Additionally, *Bacillus* known for its ability to form endospores, has exhibited resilience against disinfectants and environmental stressors (Ulrich et al., 2018). Actinomycetales, known for its antibiotic-producing capability, was also identified, posing potential risks for chronic, non-contagious infection due to trauma or surgery (Okulicz et al., 2022). Despite regular cleaning and disinfection routines, these species persist on lift buttons, suggesting the development of resistance to disinfectants over time.

The survival of contaminants on the lift buttons, even in low concentrations, hinges greatly on their survival mechanisms. Pathways such as ABC transport and two-component systems play crucial roles in normalizing and regulating responses to and internal stimuli. Resistance external to antimicrobial agents, such as bacitracin and cationic antimicrobial peptides, was observed among species in the metagenome. Bacitracin is an over-the-counter tropical antibiotic ointment and the resistance to it is documented in B. subtilis (Radeck et al., 2016). Cationic antimicrobial peptide, a natural antibiotic produced by living things, consist of short, hydrophobic molecules that harbour a net positive charge that act by cell membrane disruption, making it almost impossible to be resisted by the microbes (Zasloff, 2002). However, relative resistance to these peptides was observed in human gut microflora, suggesting the potential for resistance development in other microbial populations (Ge et al. 1999).

## **Conclusion:**

In conclusion, the investigation into the contaminant's prevalence on lift buttons in this public hospital revealed intriguing findings that enlighten the microbial colonisation and their survival mechanisms in a hospital environment. A notable prevalence of contaminants was observed despite regular cleaning routines, with 30.4% of interior lift buttons and 120 CFU/mL of bacteria isolated from the exterior lift buttons. Remarkably, there was no substantial difference in contaminant prevalence observed between lifts situated in high-risk and moderate-risk highlighting uniform areas, contamination level across the sampled areas. Metagenomic analysis provided valuable insights into the microbial diversity on lift buttons, with Firmicutes identified as the dominant phylum. The genus-level analysis revealed Staphylococcus and Bacillus as the most prevalent species, underscoring their persistence despite cleaning efforts. Furthermore, the annotation of KEGG pathways shed light on the metabolic

pathways and survival mechanisms employed by these microbial communities. A closer examination of the ABC transporters pathway highlighted the role of genes involved in iron complex transport and antimicrobial resistance, suggesting adaptive responses to environmental pressures. Similarly, the two-component system revealed enrichment of genes associated with multidrug efflux and antimicrobial resistance, indicative of the microbial community's ability to adapt to external stimuli. However, it is important to acknowledge that the study only focused on one hospital in a specific region, limiting the diversity captured across various healthcare facilities. Additionally, the absence of pre-pandemic data on contaminant prevalence and a detailed exploration of cleaning schedules in prior research limits the study's capacity to make comprehensive comparisons. Overall, these findings underscore the complex interplay between environmental factors, microbial colonization, and survival strategies of microbial contaminants on lift buttons in hospital settings. Understanding these dynamics is crucial for implementing effective infection control measures and mitigating the risk of HAIs. Future studies could broaden the geographical scope to encompass diverse hospital settings and to conduct in-depth analyses of cleaning routines, which would provide valuable insights for optimizing hygiene practices on frequently touched surfaces like lift buttons. Likewise, further research into the functional capabilities of these microbial communities and their interactions with the hospital environment is warranted to inform targeted interventions and improve patient safety.

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## **References:**

Abbasian, S., Farahani, N. N., Mir, Z., Alinejad, F., Haeili, M., Dahmardehei, M., Mirzaii, M., Khoramrooz, S. S., Nasiri, M. J., & DarbanSarokhalil, D. (2018). Genotypic characterization of Staphylococcus aureus isolated from a burn centre by using agr, spa and SCCmec typing methods. New Microbes and New Infections, 26, 15–19. https://doi.org/10.1016/j.nmni.2018.08.001

- AMBOSS (2020). Hospital-acquired infections. <u>https://www.amboss.com/us/knowledge/hospi</u> <u>tal-acquired-infections/</u> (Accessed on 01-06-2024)
- Appeh, O. G., Egwuatu, T. F., Nwankwo, E., & Ibe, A.
  C. (2022). Bacterial contamination of microphones used in places of worship in Umuahia, Abia state, Nigeria. Suan Sunandha Science and Technology Journal, 06(2), 15–22. <u>https://li02.tci-thaijo.org/index.php/ssstj/article/view/346</u>
- Bhatta, D. R., Hamal, D., Shrestha, R., Subramanya, S. H., Baral, N., Singh, R. K., Nayak, N., & Gokhale, S. (2018). Bacterial contamination of frequently touched objects in a tertiary care hospital of Pokhara, Nepal: how safe are our hands? Antimicrobial Resistance and Infection Control, 7(1). <u>https://doi.org/10.1186/s13756-018-0385-2</u>
- Carrascosa, C., Sanjuán, E., Millán, R., Martín, S., Saavedra, P., Raposo, A., del Rosario-Quintana, C., & Jaber, J. R. (2019). Is the use of supermarket trolleys microbiologically safe? Study of microbiological contamination. Journal of Applied Animal Research, 47(1), 17–23. https://doi.org/10.1080/09712119.2018.1555091
- Centers for Disease Control and Prevention (2014). Healthcare-Associated Infections (HAIs). <u>https://www.cdc.gov/hai/index.html</u> (Accessed on 01-06-2024)
- Centers for Disease Control and Prevention (2015). Environmental sampling. <u>https://www.cdc.gov/infectioncontrol/guideline</u> <u>s/environmental/background/sampling.html</u> (Accessed on 01-06-2024)
- De Paula Menezes, R., De Andrade Marques, L., Silva, F. F., Silva, N. B. S., Alves, P. G. V., De Souza Bessa, M. A., De Araújo, L. B., Penatti, M. P. A., Pedroso, R. D. S., & Von Dolingër De Brito Röder, D. (2022). Inanimate Surfaces and Air Contamination with Multidrug Resistant Species of Staphylococcus in the Neonatal Intensive Care Unit Environment. Microorganisms, 10(3), 567. <u>https://doi.org/10.3390/microorganisms1003056</u> <u>7</u>

Department of Educational Health. (2022). Infografik COVID-19 - Info Sihat [Infographic]. Ministry of Health, Malaysia. htt<u>ps://www.infosihat.gov.my/infografik-covid-</u>19.html (Accessed on 01-06-2024)

- Department of Statistics Malaysia. (2022). Vital Statistics Malaysia 2022 (ISSN: 0127-466X). <u>https://open.dosm.gov.my/publications/vitalsta</u> <u>tistics\_2022</u> (Accessed on 26-07-2024)
- Doughty, E. L., Liu, H., Moran, R. A., Hua, X., Ba, X., Guo, F., Chen, X., Zhang, L., Holmes, M. A., Van Schaik, W., McNally, A., & Yu, Y. (2022). Extensive acquisition of carbapenem-resistant Acinetobacter baumannii in Intensive Care Unit patients is driven by widespread environmental contamination. medRxiv (Cold Spring Harbor Laboratory). https://doi.org/10.1101/2022.05.19.22275186
- Ehlers, S., & Merrill, S. A. (2022). Staphylococcus saprophyticus Infection, StatPearls [Internet], Treasure Island, Florida-United States <u>https://www.ncbi.nlm.nih.gov/books/NBK4823</u> <u>67/?report=reader</u>
- El-Masry, E. A., & Taha, A. E. (2022). Role of hospital environmental surfaces in the transmission of the severe acute respiratory syndrome Coronavirus-2. Journal of Infection in Developing Countries, 16(9), 1432–1438. <a href="https://doi.org/10.3855/jidc.16572">https://doi.org/10.3855/jidc.16572</a>
- Elsergany, M., Moussa, M., Ahsan, A., Khalfan, A., & Eissa, A. (2015). Exploratory study of bacterial contamination of different surfaces in four shopping malls in Sharjah, UAE. Journal of Environmental and Occupational Science, 4(2), 101. <u>https://doi.org/10.5455/jeos.20150611030039</u>
- Ge, Y., MacDonald, D., Holroyd, K. J., Thornsberry, C., Wexler, H. M., & Zasloff, M. (1999). In vitro antibacterial properties of pexiganan, an analog of magainin. Antimicrobial Agents and Chemotherapy, 43(4), 782–788. <u>https://doi.org/10.1128/aac.43.4.782</u>
- Glasgow, L., Forde, M., Antoine, S. C., Pérez, E. C., & Indar, L. (2013). Estimating the burden of acute gastrointestinal illness in Grenada. Journal of Health Population and Nutrition. <u>https://www.ncbi.nlm.nih.gov/pmc/articles/P</u> <u>MC4022000/</u>
- Goh, L. P. W., Marbawi, H., Goh, S. M., Asis, A. K. B. A., & Gansau, J. A. (2023). The prevalence of hospital-acquired infections in Southeast Asia (1990-2022). Journal of Infection in Developing

Countries, 17(02), 139–146. https://doi.org/10.3855/jidc.17135

- Gooch, C. M., & Wadhwa, R. (2023). Body fluid exposures. StatPearls [Internet], Treasure Island, Florida-United States <u>https://www.ncbi.nlm.nih.gov/books/NBK5578</u> <u>50/#\_NBK557850\_pubdet\_/</u>
- Hashmi, A. (2014). Hospital Environmental Cleaning & Disinfection, Procedures & Practices [Powerpoint slides]. SlideShare. <u>https://www.slideshare.net/anjumhashmi61/ho</u> <u>spital-environmental-cleaning-disinfection</u> (Accessed on 01-06-2024)
- Kilpatrick, C., Allegranzi, B., & Pittet, D. (2011). WHO First Global Patient Safety Challenge: Clean Care is Safer Care, Contributing to the training of healthcare workers around the globe. International Journal of Infection Control, 7(2). https://doi.org/10.3396/ijic.v7i2.011.11
- Liu, W. J., Fu, L., Huang, M., Zhang, J. P., Wu, Y., Zhou, Y., Zeng, J., & Wang, G. X. (2017). Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant Acinetobacter baumannii. Journal of Medical Microbiology, 66(1), 13–17. https://doi.org/10.1099/jmm.0.000403
- Mahdi Yahya Mohsen, S., Hamzah, H. A., Muhammad Imad Al-Deen, M., & Baharudin, R. (2016). Antimicrobial Susceptibility of Klebsiella pneumoniae and Escherichia coli with Extended-Spectrum β-lactamase associated Genes in Hospital Tengku Ampuan Afzan, Kuantan, Pahang. The Malaysian Journal of Medical Sciences, 23(2), 14–20. <u>https://www.ncbi.nlm.nih.gov/pmc/articles/P</u> <u>MC4976709/</u>
- Maina, S. M., Nyerere, A., & Ngugi, C. W. (2018). Isolation of bacterial diversity present in medical waste and health care settings in hospitals in Kenya. African Journal of Microbiology Research, 12(26), 606-615. https://doi.org/10.5897/ajmr2018.8876
- Meyer, P., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., & Edwards, R. A. (2008). The metagenomics RAST server a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics, 9(1).

https://doi.org/10.1186/1471-2105-9-386

- Ministry of Health Malaysia. (2021). Garis panduan pembersihan dan disinfeksi di tempat awam (Annex 36). <u>https://covid-19.moh.gov.my/garispanduan/garis-panduan-kkm</u> (Accessed on 01-06-2024)
- Mohd Fauzi, F. S. (2018). Isolation of bacteria from lift buttons of Library and Kulliyyah of Nursing at IIUM Kuantan [Degree thesis]. IIUM Repository. <u>http://irep.iium.edu.my/65831/</u> (Accessed on 26-07-2024)
- Okulicz, J. F., Polenakovik, H., & Polenakovik, S. (2022). Actinomycosis: background, pathophysiology, epidemiology. <u>https://emedicine.medscape.com/article/211587</u> <u>-overview?form=fpf</u> (Accessed on 01-06-2024)
- Olise, C. C., & Simon-Oke, I. A. (2018). Fomites: Possible vehicle of nosocomial infections. Journal of Public Health Catalogue, 01(01), 16–16. <u>https://doi.org/10.35841/public-health-</u> <u>nutrition.1.1.11-16</u>
- Parker, N., Mark, S., Anh-Hue, T. T., Philip, L., & Brian, M. F. (2016), "9.4 Temperature and Microbial Growth", Microbiology, OpenStax, Houston-Texas <u>https://openstax.org/books/microbiology/page</u> <u>s/9-4-temperature-and-microbial-growth</u> (Accessed on 01-06-2024)
- Qin, M., Chen, P., Deng, B., He, R., Wu, Y., Yang, Y., Deng, W., Ding, X., Yang, F., Xie, C., Yang, Y., & Tian, G.-B. (2022). The emergence of a multidrugresistant and pathogenic ST42 lineage of Staphylococcus haemolyticus from a hospital in China. Microbiology Spectrum, 10(3), e0234221. https://doi.org/10.1128/spectrum.02342-21
- Radeck, J., Gebhard, S., Orchard, P., Kirchner, M., Bauer, S., Mascher, T., & Fritz, G. (2016). Anatomy of the bacitracin resistance network in Bacillus subtilis. Molecular Microbiology, 100(4), 607–620. https://doi.org/10.1111/mmi.13336
- Sani, N. a. M., Sapri, H. F., Neoh, H. M., & Hussin, S. (2014). First report on the molecular epidemiology of Malaysian Staphylococcus epidermidis isolated from a University Teaching Hospital. BMC Research Notes, 7(1). https://doi.org/10.1186/1756-0500-7-597
- Schwab, F. J., Gastmeier, P., & Meyer, E. (2014). The warmer the weather, the more Gram-Negative bacteria - Impact of temperature on clinical isolates

in intensive care units. PLOS ONE, 9(3), e91105. https://doi.org/10.1371/journal.pone.0091105

- Siew, S. W., Yen, C. M., Marshall, I. P. G., Hamid, H. A., Kamal, S. S., Nielsen, D. S., & Ahmad, H. F. (2022). Gut microbiome and metabolome of sea cucumber (Stichopus ocellatus) as putative markers for monitoring the marine sediment pollution in Pahang, Malaysia. Marine Pollution Bulletin, 182, 114022. https://doi.org/10.1016/j.marpolbul.2022.114022
- Sukri, A., Zaki, H. H. M., & Zin, N. M. (2022). Antibiotic Resistance Patterns of Coagulase-Negative Staphylococcus (CoNS) Isolates from a Major Teaching Hospital in Kuala Lumpur, Malaysia. Sains Malaysiana, 51(3), 865–872. <u>https://doi.org/10.17576/jsm-2022-5103-20</u>
- Ulrich, N., Nagler, K., Laue, M., Cockell, C. S., Setlow, P., & Moeller, R. (2018). Experimental studies addressing the longevity of Bacillus subtilis spores – The first data from a 500-year experiment. PLOS ONE, 13(12), e0208425. <u>https://doi.org/10.1371/journal.pone.0208425</u>
- Valeriani, F., Crognale, S., Protano, C., Gianfranceschi, G., Orsini, M., Vitali, M., & Spica, V. R. (2018). Metagenomic analysis of bacterial community in a travertine depositing hot spring. PubMed, 41(2), 126–135.

https://pubmed.ncbi.nlm.nih.gov/29498741

- Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology, 13(6), e1005595. <u>https://doi.org/10.1371/journal.pcbi.1005595</u>
- World Health Organization. (2022). WHO launches first ever global report on infection prevention and control. World Health Organization. https://www.who.int/news/item/06-05-2022who-launches-first-ever-global-report-oninfection-prevention-andcontrol#:~:text=The%20impact%20of%20healthcar e%20associated,care%20unit%20die%20each%20y ear/;%202022 (Accessed on 01-06-2024)
- Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389– 395. <u>https://doi.org/10.1038/415389a</u>