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Research Article

Identification of Carbapenemase Gene in Sinks of the General Hospital in Makassar City, Indonesia

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Abstract

Background: Carbapenem-resistant bacteria pose a significant threat to healthcare systems. Sinks can harbor germs and serve as a source of transmission.

Objectives: This study aims to determine the presence of carbapenemase in sinks located in the emergency room (ER), intensive care unit (ICU), and inpatient rooms in general hospitals in Makassar city.

Methods: This cross-sectional observational study examined 63 sink swab specimens from 19 general hospitals in Makassar, Indonesia. The specimens were cultured for gram-negative bacteria, and the resulting colonies were analyzed for bacterial strains and carbapenemase genes (blaKPC, blaNDM, blaVIM, blaIMP, and blaOXA) using PCR.

Results: The study found that 52.6% of hospitals had sinks contaminated with carbapenemase, distributed as 15% in ERs, 21% in ICUs, and 29% in inpatient rooms. The identified carbapenemases were Ambler class B (Metallo-β-lactamase) and class D, with imipenem (IMP)-resistant *Pseudomonas* carbapenemase being the most common. *Enterobacter* sp. and *Klebsiella* sp. accounted for 37.1% of the gram-negative bacteria found in sinks. This combination exhibited the highest diversity of resistance genes, with all four carbapenemase genes (blaIMP, blaVIM, blaOXA, blaNDM) detected.

Conclusions: Carbapenemase contamination is widespread in hospital sinks. Infection control strategies must be strengthened to reduce nosocomial transmission.

Keywords: Carbapenem-Resistant Bacteria, Carbapenemases, Hospital Sinks, Polymerase Chain Reaction

1. Background

Carbapenem-resistant Enterobacteriaceae (CRE) are categorized as critical priority pathogens by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) due to their high resistance to last-resort antibiotics and their role in hospital-acquired infections (1, 2). The key mechanism behind this resistance is the production of carbapenemase enzymes, such as *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi Metallo-β-lactamase (NDM), Verona Integron-Mediated Metallo-β-lactamase (VIM), Imipenem (IMP), and Oxacillinase (OXA), which hydrolyze carbapenems and render them ineffective (3, 4).

Healthcare-associated infections (HAIs) can arise from both direct clinical interventions and exposure to contaminated hospital environments (5). Over the past two decades, more than 30 studies have reported carbapenem-resistant organisms in hospital water systems, particularly in faucets, sinks, and drains (6). Sinks have emerged as critical reservoirs for multidrug-resistant organisms (MDROs), including Carbapenemase-Producing Enterobacteriaceae (CPE), Carbapenem-Resistant *Acinetobacter baumannii* (CRAB),

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and *Pseudomonas aeruginosa* (CRPA), all of which pose a transmission risk to patients (6, 7).

Sink drains are increasingly recognized as environmental niches for persistent colonization by resistant bacteria (6-8). Transmission from sinks to patients may occur via splash-back or aerosolized droplets during faucet use, which can contaminate nearby surfaces and equipment (8). A study by Kotay et al. demonstrated that droplet-mediated dispersion is a major transmission route from contaminated sink traps (8). Moreover, documented outbreaks have shown recontamination of sinks even after replacement, suggesting biofilm persistence in drainage systems (9).

Carbapenemase genes are frequently located on mobile genetic elements such as plasmids, facilitating their spread between bacteria and across healthcare environments (6, 10). Molecular methods like PCR have proven effective in detecting these genes with high sensitivity and specificity, despite their cost and technical demands (11). In Indonesia, environmental surveillance of carbapenemase in hospital settings remains limited. This study addresses that gap by investigating the presence and distribution of carbapenemase genes in sinks from various wards in general hospitals in Makassar.

2. Objectives

The aim of this study was to determine the presence of carbapenemase in sinks in the emergency room (ER), intensive care unit (ICU), and inpatient rooms using molecular methods in general hospitals in Makassar city.

3. Methods

This cross-sectional observational study analyzed 63 sink specimens collected from 19 general hospitals in Makassar City, Indonesia. Samples were processed at Hasanuddin University Hospital's Microbiology Laboratory. Eligible sinks were functional and located in ERs, ICUs, or inpatient wards. Non-functional sinks or those without running water were excluded. Ethical approval was obtained from Hasanuddin University's Faculty of Medicine IRB (Approval No. UH24050398).

3.1. Sink Sampling

From 19 hospitals, at least three sinks were sampled — one each from the ER, ICU, and inpatient ward. Sink drains were swabbed using COPAN Flocked Eswabs per manufacturer instructions. Samples were transported in Amies medium on ice and cultured on MacConkey agar (12).

3.2. Laboratory Methods

Swabs were processed within 24 hours by streaking on MacConkey agar and incubated overnight at 37°C. Colonies were examined for lactose fermentation. PCR was performed on grown colonies to identify gram-negative bacteria (*Escherichia coli*, *Enterobacter* sp., *Klebsiella* sp., *K. pneumoniae*, *P. aeruginosa*) and detect carbapenemase genes (blaIMP, blaVIM, blaNDM, blaOXA, blaKPC).

DNA was extracted using the gSYNC™ kit. PCR mixtures contained GoTaq Master Mix, primer sets specific for carbapenemase genes or bacterial identification, nuclease-free water, and DNA template, with a final volume of 50 µL. Amplification used a BioRad Thermal Cycler with the following protocol: Initial denaturation at 94°C for 2 minutes; 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds; and a final extension at 72°C for 10 minutes. PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide at 100 V for 30 minutes (12-15).

3.3. Data Collection

Swab samples from sinks were collected from all hospitals between July and August 2024. Staff knowledgeable about hospital characteristics were interviewed, and photographs of sampled sinks were taken.

3.4. Data Analysis

Descriptive analysis was used to characterize the sinks in the hospitals in the study. Statistical analysis was conducted using Microsoft Excel.

4. Results

This study involved 19 general hospitals out of 25 general hospitals in Makassar city. Most of them were class B public hospitals, namely 11 hospitals (57.9%). A total of 7 (36.8%) were class C hospitals, and 1 (5.3%) was a class A hospital.

4.1. Sink Characteristics

Most of the sink swab samples were taken from inpatient rooms, totaling 24 locations. About 90% of the sinks were clean and equipped with hand soap facilities. Additional facilities such as paper towels were available at 28 sinks (44.44%). The main function of the sinks in this study was to wash the hands of health workers. A total of 21 sinks (33.33%) were located close to the

Figure 1. Sinks with patient bed distance > 2 meters, with curtain

patient's bed, mostly with a distance of more than 2 meters and equipped with a dividing curtain (Figure 1). The majority of the sinks, 90%, were made of ceramic material. Table 1 provides more information about the characteristics of the sinks that were the subject of this study.

4.2. Hospitals Contaminated with Gram-Negative Bacteria and Carbapenemase Genes in Sinks

This study showed that of the 19 public hospitals studied, 10 hospitals (52.6%) were found to have carbapenemase genes in sink swab samples. This figure presents the distribution of gram-negative bacterial isolates cultured on MacConkey agar. The most prevalent combinations were *Enterobacter* sp. and *Klebsiella* sp. (37.11%) and *Enterobacter* sp. alone (35.05%). Other notable combinations included *Enterobacter* sp. with *K. pneumoniae* (18.56%) and *Enterobacter* sp. with *P. aeruginosa* (3.09%). A small percentage of isolates were solely *Klebsiella* sp. (1.03%) or yielded no detectable bacteria (2.06%). The total number of isolates analyzed was 97. These results highlight the dominance of *Enterobacter* and *Klebsiella* species in the sampled environments, which are common opportunistic pathogens in healthcare settings (Figure 2).

This figure illustrates the distribution of carbapenemase gene types across different hospital sink locations: Inpatient rooms, intensive care rooms, and ERs. The most frequently detected gene was blaKPC, followed by blaIMP, blaVIM, blaNDM, and blaOXA. The data suggests variability in gene prevalence by location, with inpatient and intensive care rooms showing higher detection rates for certain genes (e.g., blaKPC). This underscores the potential role of hospital sinks as reservoirs for carbapenem-resistant genes, particularly in high-risk areas like ICUs (Figure 3).

4.3. Distribution Pattern of Carbapenemase Genes in Sinks

The results showed that the distribution of carbapenemase genes in sinks in general hospitals in Makassar city was spread across 8 different sub-districts. The carbapenemase gene contamination of these hospital sinks showed a varied distribution pattern, indicating variations in the types of resistant bacteria that may be related to the conditions and infection prevention practices in each region.

The VIM gene was found in sinks in hospitals located in Tamalanrea, Ujung Pandang, and Manggala sub-districts, indicating the potential spread of highly resistant bacteria in these sub-districts. The IMP-resistant *Pseudomonas* carbapenemase gene was detected in hospital sinks located in four sub-districts, namely Biringkanaya, Tamalanrea, Ujung Pandang, and Manggala, which may indicate the presence of more widespread contamination in different parts of the city, especially for this imipenem-resistant gene.

In addition, the OXA gene, which also contributes to carbapenem antibiotic resistance, was found in hospitals located in Ujung Tanah, Rappocini, and Tamalate sub-districts, suggesting the spread of OXA gene contamination in these areas. The NDM gene, which is notorious for its high resistance and global

Table 1. Sink Characteristics

Sink Characteristics	Amount (N = 63)	%
Sink location		
Emergency room	20	31.7
Intensive care unit	19	30.2
Surgical ward	24	38.1
Cleanliness of the sink		
Sink appears clean	57	90.48
Sink does not appear clean	6	9.52
Availability of hand soap		
Soap cleanser available at sink	59	93.65
Soap cleanse unavailable	4	6.35
Availability of tissues		
Tissues available at sink	28	44.44
Tissues unavailable	35	55.56
Sink function		
Only staff handwashing	47	74.6
Handwashing staff and patient	16	25.4
Washing medical devices	15	23.81
Presence of bed near the sink		
There is a bed near the sink	21	33.33
No bed is near the sink	42	66.67
Presence of curtain/bed divider with sink		
There is a curtain between the bed and the sink	14	66.67
No curtain between the bed and sink	7	33.33
Distance between sink and patient bed (m)		
< 2	6	28.57
> 2	15	71.43
Sink material		
Ceramics	57	90.48
Stainless steel	6	9.52

spread, was found in the sinks of hospitals located in Wajo and Tamalanrea sub-districts.

4.4. Proportion of Contaminated Sinks by Location and Types of Carbapenemase in Hospital Sinks

The figure compares the number of sinks testing positive or negative for carbapenemase genes across inpatient rooms, intensive care rooms, and ERs. Inpatient rooms had 7 positive and 17 negative sinks, intensive care rooms had 4 positive and 15 negative sinks, and ERs had 3 positive and 17 negative sinks. While the majority of sinks were negative, the presence of positive sinks in all locations — especially inpatient and intensive care rooms — indicates persistent contamination risks. The high number of negative sinks suggests potential success in infection control measures, but the positive cases warrant further attention (Figure 4).

This figure correlates specific gram-negative bacterial isolates with detected carbapenemase genes. The gene blaKPC was the most common, followed by blaNDM, blaOXA, blaVIM, and blaIMP. The isolates most frequently associated with these genes were *Enterobacter* sp. with *K. pneumoniae*, *Enterobacter* sp. with *Klebsiella* sp., and *Enterobacter* sp. alone. This suggests that *Enterobacter* and *Klebsiella* species are key carriers of carbapenemase genes, which could contribute to the spread of antibiotic resistance in clinical environments (Figure 5).

5. Discussion

The increasing use of antibiotics has led to an increase in the number of antibiotic-resistant bacteria, such as carbapenemase-producing gram-negative bacteria. In Indonesia, research on carbapenemase-producing bacteria in the hospital environment is still

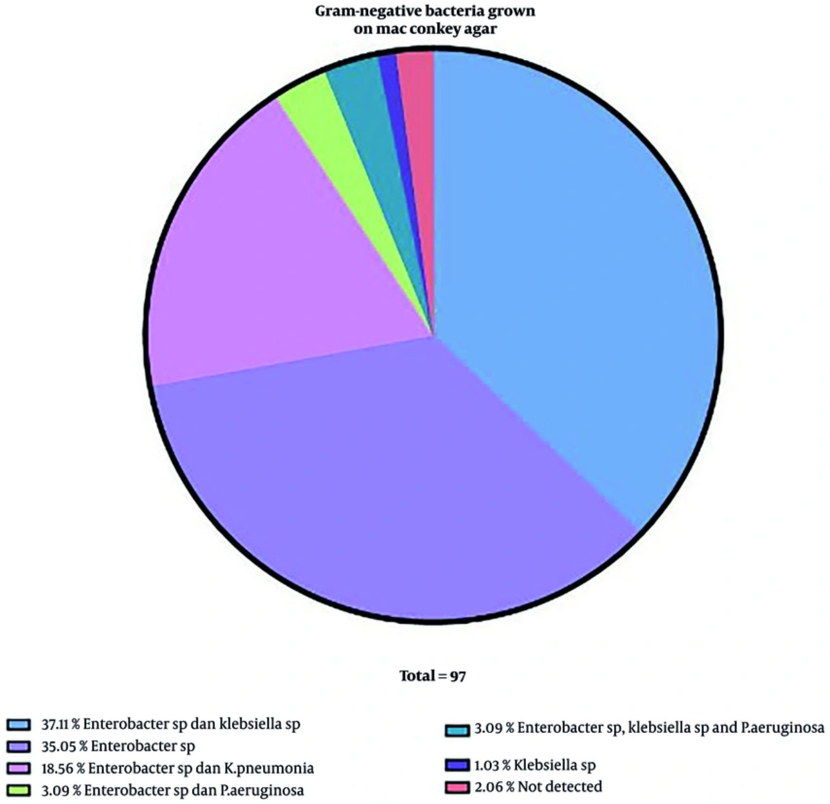

Figure 2. Gram-negative bacteria grown on MacConkey agar

very limited. Meanwhile, the hospital environment can be a source of resistant bacteria (16). Our findings show that 10 out of 19 general hospitals (52.6%) harbored carbapenemase genes in sink swab samples, distributed across 8 sub-districts. This underscores a significant environmental reservoir of resistance within hospitals, particularly concerning given the critical role of carbapenems in managing severe infections. The widespread distribution of genes reflects a diverse resistance pattern that likely corresponds with local antibiotic usage and infection control practices (9).

The highest contamination rates were observed in sinks located in inpatient rooms (29%), followed by ICUs (21%) and emergency departments (15%). Inpatient rooms typically experience more prolonged stays, higher foot traffic, and possibly less stringent environmental cleaning protocols compared to ICUs. These factors increase the risk of biofilm development and cross-contamination, as also reported in prior studies (17).

Pathogenic bacteria are often present in sinks in healthcare settings, including bacteria that have genes for resistance to multiple antibiotics. Sinks have been identified as a source of hospital-to-patient transmission and infection outbreaks, especially sinks located less than 1 meter from the patient, which can serve as an important reservoir for multidrug-resistant pathogens such as Carbapenem Resistant Organisms (CRO) (18). The physical proximity of patient beds to sinks was another noteworthy risk factor. In 21 hospitals, beds were located near sinks, with at least one hospital having a distance of less than 2 meters — conditions that facilitate bacterial transmission via splash or aerosol. Previous studies in Belgium and South Africa support this mechanism, where carbapenemase genes such as blaNDM and blaVIM were detected in sink aerosols despite no direct link to colonized patients (19, 20). Thus, sinks not only act as passive reservoirs but may

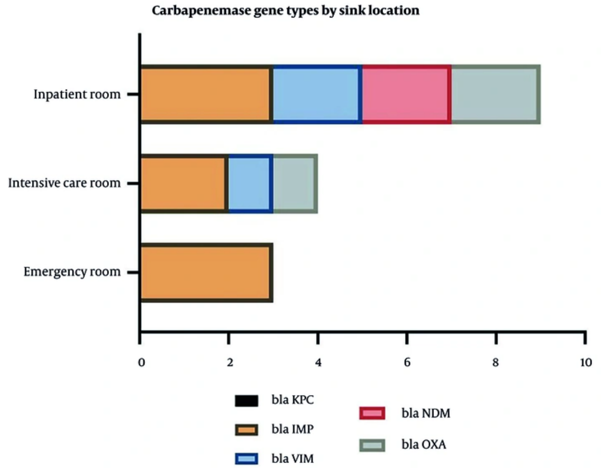

Figure 3. Carbapenemase gene types by sink location

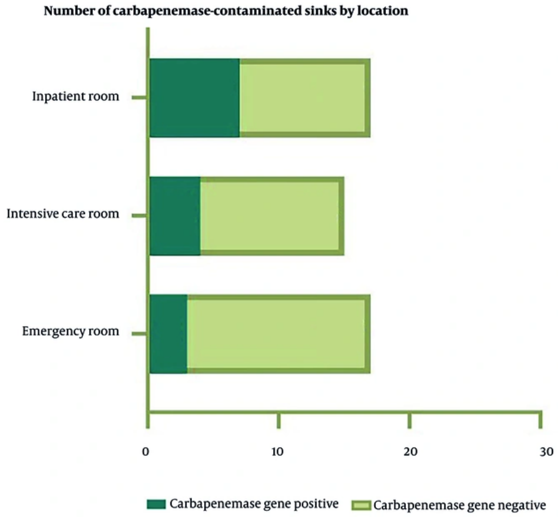

Figure 4. Number of carbapenemase-contaminated sinks by location

actively contribute to HAIs through environmental contamination.

Supporting this, the study identified a variety of carbapenem-resistant species, including *P. aeruginosa*, *K. pneumoniae*, *Enterobacter* spp., and *A. baumannii*. These findings are consistent with previous multicenter studies in Ohio and Asia that reported frequent colonization of sinks with CPE and non-fermenters (6, 21-23). The presence of such organisms in moist environments such as P-traps and drains can lead to persistent colonization and periodic release of contaminated aerosols, especially during water use.

Notably, blaIMP was the most prevalent gene identified, found predominantly in ICU and inpatient areas. Other Metallo-β-lactamase genes (blaVIM, blaNDM) and the Ambler class D gene blaOXA were also

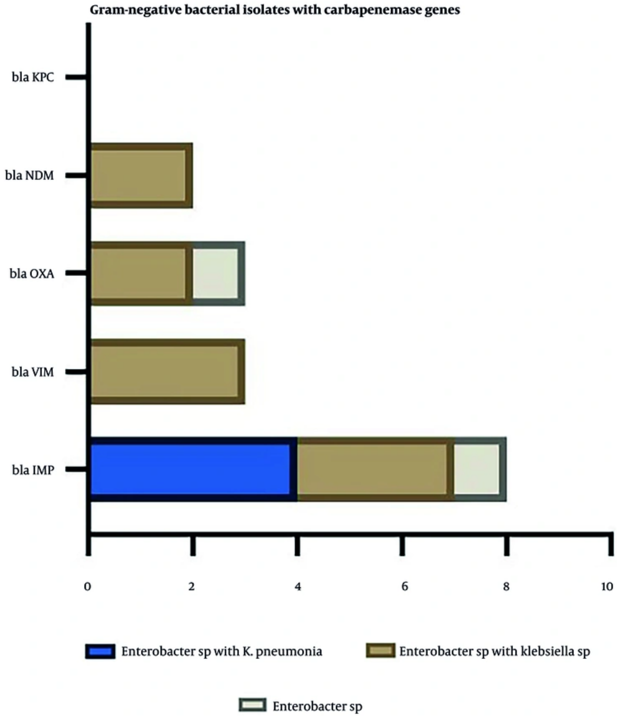

Figure 5. Gram-negative bacterial isolates with carbapenemase genes

detected. Interestingly, the class A gene blaKPC was not found in any sample, which contrasts with findings from Israel and parts of the US, where blaKPC is more dominant (9, 24, 25). This suggests regional differences in resistance gene epidemiology, possibly driven by distinct antimicrobial stewardship and infection control protocols.

Our findings align with surveillance data from Southeast Asia, where blaNDM, blaIMP, and blaVIM are the most commonly reported carbapenemase genes (25). For instance, a Philippine study from 2013 - 2016 identified blaNDM in 33% of carbapenem-resistant isolates. In India, blaNDM and blaOXA-23 dominate among Enterobacteriaceae and *A. baumannii*, respectively.

The study conducted on sink swab samples from general hospitals in Makassar City identified significant carbapenemase contamination, predominantly in hospitalization areas. Carbapenemase genes detected were categorized under Ambler class B (Metallo-β-lactamases: IMP, VIM, NDM) and class D (OXA), with the IMP gene being the most prevalent. The absence of the Ambler class A KPC gene was notable.

5.1. Conclusions

This study demonstrates that more than half of the general hospitals in Makassar have sinks contaminated with carbapenemase genes, particularly the IMP gene, with the highest contamination occurring in inpatient areas. The most common bacteria found were *Enterobacter* sp. and *Klebsiella* sp., which harbored diverse resistance genes. These findings underscore the role of hospital sinks as environmental reservoirs for antibiotic-resistant bacteria and highlight the urgent need for robust infection control and sink maintenance strategies. Strengthening surveillance and applying strict disinfection protocols are essential to prevent nosocomial transmission of carbapenem-resistant organisms.

5.2. Limitations

This study has several limitations. First, the relatively small sample size may limit the generalizability of the findings to all hospitals in Indonesia. Secondly, not all factors that may affect contamination (such as cleaning schedules, sink materials, and usage practices) could be controlled or collected equally. In addition, the association between the presence of resistance genes and hospital factors was not statistically analyzed due to the descriptive study design.

Continuous surveillance of the epidemiological dynamics of carbapenemase genes in sinks is critical to understanding their dissemination within our hospital. Enhanced focus on strengthening nosocomial infection prevention and control strategies is imperative. Furthermore, the investigation of additional carbapenemase-producing genes is warranted to comprehensively address the potential threats posed by antimicrobial resistance.

Footnotes

Authors' Contribution: Study concept and design: A. R. S. and F. H.; Statistical analysis: Z. S. A.; Analysis and interpretation of data: Z. S. A.; Drafting and critical revision of the manuscript: Y. D. P., A. R. R., F. S., Y. D. R., L. T., and B. M.; Supervision: A. R. S. and F. H. All authors reviewed, approved, and agreed to the final version of the manuscript for publication

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: No new data were created or analyzed in this study. Data sharing does not apply to this article.

Ethical Approval: The study was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Hasanuddin University (Ethics Board approval number: UH24050398).

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