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ORIGINAL RESEARCH ARTICLE

Prevalence of Biofilm-forming and Carbapenemase-producing Gram-negative Bacilli Colonizing Indwelling Urinary Catheters of Patients

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ABSTRACT

The increasing prevalence of biofilm-forming and carbapenemase-producing Gram-negative Bacilli (GNB) in catheterized patients is linked to significant clinical challenges, including persistent infections, prolonged hospital stays, and higher colonization rates. To address these issues, establishing localized antimicrobial resistance (AMR) surveillance data is critical for guiding effective patient management and implementing robust infection prevention and control strategies. This study analyzed 200 indwelling urinary catheters (IUCs) from hospitalized patients using standard microbiological techniques, with isolates characterized using the VITEK 2 system. Phenotypic screening for biofilm production and carbapenem resistance was conducted using the Congo Red Assay and the Carba NP Test, respectively. Carbapenemase genes were identified via Polymerase Chain Reaction (PCR) with specific primers. Of the 200 samples, 164 (82.0%) were positive for GNB, with 87 (43.5%) identified as biofilm producers. Carbapenem resistance was confirmed in 89 (44.5%) isolates using CRE agar and the Carba NP Test. The distribution of carbapenemase genes among GNB isolates was as follows: *bla*$_{KPC}$ (29.5%), *bla*$_{NDM}$ (100%), *bla*$_{VIM}$ (13.5%), *bla*$_{OXA}$ (100%), and *bla*$_{IMP}$ (20.0%). Antibiogram analysis revealed high resistance rates (54.5%-100%) to amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, ceftriaxone, meropenem, and ertapenem. However, susceptibility was observed to nitrofurantoin, ciprofloxacin, and Gentamicin, suggesting their potential as empiric treatments for GNB infections in catheterized patients. The study highlights the importance of adhering rigorously to structured catheter care protocols, including daily assessment of catheter necessity and strict adherence to aseptic techniques during insertion and maintenance. Regular training for healthcare staff on best practices for catheter management is essential to reduce colonization rates and improve clinical outcomes for patients at risk of carbapenem-resistant GNB (CR-GNB) infections. These findings highlight the urgent need for localized AMR surveillance and targeted infection control measures to mitigate the growing threat of multidrug-resistant GNB in healthcare settings.

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INTRODUCTION

Indwelling urinary catheters are a vital component of clinical care, used to manage urinary incontinence and retention, measure urine output, and support patients recovering from surgical procedures or various medical conditions (Drake *et al.*, 2024). It is estimated that approximately 25% of hospitalized patients require the use of urinary catheters; however, this intervention is associated with a heightened risk of urinary tract infections (UTIs). Research indicates that 70-80% of nosocomial infections acquired within healthcare facilities are linked to catheter-associated urinary tract infections (CAUTIs) (Asmare *et al.*, 2024; Puro *et al.*, 2022). Furthermore, short-term use of indwelling catheters is correlated with an increased incidence of UTIs that may persist even after catheter removal. In instances of long-term catheterization, the occurrence of bacteriuria is anticipated

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in a significant majority of users. Consequently, healthcare professionals must be vigilant in addressing potential complications such as symptomatic UTIs and recurrent catheter blockages. By adopting comprehensive management strategies, providers can optimize patient outcomes and enhance the quality of care for individuals who depend on indwelling urinary catheters.

Indwelling urinary catheters are widely recognized for their role in promoting bacterial colonization within the body, which can lead to hospital-associated infections (Niveditha *et al.*, 2012; Stickler *et al.*, 2014; Stærk *et al.*, 2024). The most common pathogens responsible for catheter-associated urinary tract infections (CAUTIs) are Gram-negative bacilli (GNB), including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Acinetobacter baumannii* (Flores-Mireles *et al.*, 2019). Recent studies have shown that *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa* account for 13.3–53.3%, 8–25%, 18.5–40.5%, and 7.1–20.2% of CAUTIs, respectively (Asmare *et al.*, 2024; Oumer *et al.*, 2021; Awoke *et al.*, 2019; Kolpa *et al.*, 2018). The risk of urinary tract infections (UTIs) is linked to the duration of catheter use. The prevalence of bacteriuria increases daily by an estimated 3% to 10%, and nearly all patients with an indwelling catheter will develop bacteriuria after 30 days of catheterization (Kulbay *et al.*, 2024; Warren, 2001).

The frequent use of catheterization in healthcare settings presents a significant challenge, as bacteria have the propensity to colonize and contaminate these devices, resulting in the formation of biofilms. The duration for which a urinary catheter remains in place correlates with an increased likelihood of biofilm development by these organisms, potentially predisposing patients to both asymptomatic and symptomatic urinary tract infections (UTIs). Catheter-associated urinary tract infections (CAUTIs) are identified as the most prevalent adverse events associated with indwelling urinary catheter use (Wald, 2008; Tambyah, 2000). Notably, CAUTIs are often asymptomatic, and only 3% of affected individuals will progress to develop bacteremia with a urinary isolate (Nakawuki *et al.*, 2022).

Bacteria can proliferate in biofilms on the surfaces of catheters, which are organized communities of microorganisms encased in a self-produced extracellular polymeric matrix (Niveditha *et al.*, 2012). The formation of these biofilms poses significant challenges in healthcare environments, resulting in persistent and recurrent infections as well as device-related infections. Biofilm-producing bacteria possess the ability to persist on both abiotic and biotic surfaces. This bacterial population may transition between indwelling urinary catheters and vulnerable patients, or alternatively, from patients to catheters. The presence of a catheter increases the patient's susceptibility to infections by facilitating access for uropathogens to the bladder through both its external and internal surfaces, where biofilms can establish on the catheters and the uroepithelium (Stickler, 2008). Although exposure to antibiotics may temporarily delay the onset of bacteriuria in catheterized patients, such effects are typically short-term. The presence of biofilm-forming bacteria is of considerable medical concern, as they frequently exhibit reduced susceptibility to antimicrobial agents. Furthermore, the close spatial arrangement of cells within a biofilm can promote the exchange of plasmids, thereby enhancing the dissemination of antimicrobial resistance (Niveditha *et al.*, 2012).

Several studies have highlighted the complexity of bacterial populations found in the urine and blood cultures of catheterized patients. Notably, there has been a significant rise in the presence of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae species (Bebell *et al.,* 2017; Tolera *et al.,* 2018; Musinguzi *et al.,* 2019; Amisu *et al.,* 2019). These developments indicate a growing resistance to multiple antibiotic classes.

Carbapenems represent one of the limited treatment options available for multidrug-resistant (MDR) Gram-negative bacilli, including those that produce beta-lactamases genotype. The presence of a urinary catheter is envisaged as a significant risk factor for the development of carbapenem-resistant Gram-negative bacilli (CR-GB) bacteremia. Additional risk factors encompass the utilization of central venous catheters, the presence of other indwelling devices, and patient admission to intensive care units (ICUs). The transmission of carbapenemase-producing Enterobacterales (CPE) can occur both between hospitals and within individual healthcare facilities. Transferring patients from institutions with high rates of CPE to different facilities may lead to the introduction of these organisms into previously unaffected areas. Carbapenem-resistant strains, particularly *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, pose substantial challenges in terms of treatment due to their intrinsic resistance mechanisms, capacity to acquire further resistance genes, and their ability to form biofilms. These biofilms can impair immune system clearance and compromise the efficacy of antibiotics. Currently, the emergence of carbapenemase production is recognized as a significant mechanism of drug resistance. Pathogens that produce carbapenemase, including *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM), present critical challenges in the management of patients with urinary catheters. These enzymes confer resistance to carbapenems, which are vital for the treatment of severe Gram-negative infections, and are often encoded on mobile genetic elements that facilitate rapid dissemination. The rising prevalence of these pathogens has been linked to adverse clinical outcomes, including persistent infections, prolonged hospital stays, and higher rates of colonization.

Research indicates that the proliferation of carbapenemase-producing bacteria in Nigeria is contributing to a significant increase in mortality rates associated with various bacterial infections within the region (Nomeh *et al.*, 2022; Ilang *et al.*, 2023a; Joseph *et al.*, 2023; John-Onwe *et al.*, 2023).

Indwelling urinary catheters (IUCs) can contribute to urinary incontinence and retention, and their use is linked

to the risk of device-related healthcare-associated infections (HCAIs). Low-income countries, including Nigeria, encounter significant challenges in implementing effective preventive measures. These challenges encompass inadequate environmental hygiene, characterized by microbial colonization of drainage bags, catheter insertions performed outside of sterile operating environments, and non-compliance with catheter care protocols. Additionally, factors such as poor infrastructure, insufficient medical equipment, understaffing, overcrowding, and the prevalence of rapidly deteriorating underlying health conditions exacerbate the risk of biofilm formation and the transmission of carbapenemase-producing Gram-negative bacilli. Such factors may lead to colonization of indwelling urinary catheters and pose enhanced risks to less susceptible patients. Currently, there is a dearth of epidemiological data concerning biofilm-forming and carbapenemase-producing organisms among patients with IUC in Nigeria. Previous studies on catheter-associated urinary tract infections (CAUTI) (Amisu *et al.*, 2019; Sharif *et al.*, 2020; Bashir *et al.*, 2020) have primarily overlooked the prevalence of biofilm-forming and carbapenemase-producing bacteria in these patients. Consequently, significant knowledge gaps remain regarding the prevalence of these organisms and their associated antibiotic resistance profiles within Nigeria. To facilitate the effective management of patients with IUC and to implement comprehensive infection prevention and control measures, it is imperative to establish current and localized antimicrobial resistance (AMR) surveillance data. Such information is vital for addressing the spread of AMR associated with indwelling urinary catheters.

METHODS AND MATERIALS

Inclusion and Ethical Consideration

Patients who were not catheterized were excluded from this study. The research was conducted within the Departments of Obstetrics and Gynaecology, Intensive Care Unit (ICU), and Surgical services. Approval was obtained from the Institutional Ethical Committee of the University of Calabar Teaching Hospital, located in Cross River State, Nigeria, at latitude 4° 57.4' N and longitude 80° 19' 10.2" E (Edemekong *et al.,* 2025), prior to the collection of samples. This study complied with the ARRIVE guidelines (Animal and Human Research: Reporting of *In Vivo* and *In Vitro* Experiments) for the reporting of experiments involving both animal and human subjects (https://arriveguidelines.org). To protect participant confidentiality, all identifiable information was anonymized during data collection and analysis.

Sample collection and Gram-negative bacilli Identification

A total of 200 indwelling urinary catheters were collected from hospitalized patients over three months. All patients admitted to the wards were eligible for study participation within 48 hours after admission. The urinary catheters were removed as directed by the reviewing medical personnel during their rounds. The tip of each catheter was aseptically cut about 2-3 cm below the balloon point with a disinfected pair of scissors and immediately placed into Brain-Heart infusion broth. This sample was transported in a cooler box to the microbiology laboratory within 30 minutes to maintain sample integrity, following standard protocols. The samples were then sent to the Microbiology Laboratory at the University of Calabar, where cultures were performed to identify the bacterial species. Bacterial culture isolations were carried out using standard techniques. After overnight incubation, a loopful of the catheter tip turbidity broth culture was seeded onto CHROMagar™ *Acinetobacter*, cetrimide agar, MacConkey agar, and *Klebsiella* ChromoSelect Selective Agar (Sigma-Aldrich, Darmstadt, Germany). These were incubated at 37°C for 24 to 48 hours. The colonial appearances, which included red, green, pink, and purple (mucoid) growths, indicated the presence of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae*, respectively. To proceed with identification, the isolated colonies were further screened using the Gram staining technique and various biochemical tests, including the oxidase test, indole test, catalase test, urease test, citrate test, coagulase test, and triple sugar iron (TSI) test, along with the TSI slant and butt test (Tille, 2015). The bacteria were also identified using the VITEK-2 Automated System (BioMérieux, France).

Biofilm Production Assay (Qualitative Assay)

Congo red Method

By growing isolates on Congo red agar (CRA), the phenotypic characterization of Gram-negative bacilli strains that produce biofilms was carried out. The CRA was made by mixing 37 g/L of Brain Heart Infusion broth (bioMérieux, France) with 50 g/L of sucrose, 10 g/L of agar, and 0.8 g/L of Congo red dye (Peter *et al.*, 2022). After being autoclaved separately, Congo red was added to the mixture that had cooled to 55°C. After being streaked onto the agar, the isolates were cultured for 24 hours at 37°C. When bacterial polysaccharides interact with the Congo red dye, biofilm-producing bacteria form black colonies, but non-biofilm-producing bacteria stay red. Following incubation, bacteria that produced biofilms were identified by their strong black, black, or reddish-black colonies with a hard, crystalline texture. Conversely, isolates exhibiting Bordeaux-red or smooth red colonies were designated as non-biofilm producers (Peter *et al.*, 2022).

Antibiotic resistance testing

In this study, the antibiotic resistance profiles of Gram-negative bacilli (GNB) isolates were evaluated against twelve antibiotics. The antibiotics tested included amoxicillin-clavulanic acid (20/30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), nitrofurantoin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), gentamicin (5 µg), ciprofloxacin (5 µg), ertapenem (10 µg), imipenem (10 µg), and meropenem (10 µg) (Oxoid, UK). Susceptibility testing was conducted on Mueller-Hinton agar (Sigma-Aldrich, Darmstadt, Germany) using the Kirby-Bauer disk diffusion method,

following the standardized guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2021; Edemekong *et al.*, 2025). Bacterial growth around the antibiotic discs was observed, and the zones of inhibition were measured using a metric ruler. The results were interpreted in accordance with the 2021 CLSI guidelines.

Detection and identification of carbapenemase production

The initial screening for carbapenemase production in Gram-negative bacilli (GNB) was conducted based on the susceptibility profiles of the isolates to imipenem and meropenem. Isolates of GNB that demonstrated resistance to ertapenem, imipenem, and/or meropenem were identified as potential carbapenemase producers. These isolates were subsequently evaluated using a chromogenic medium known as Chromatic Carbapenem-resistant Enterobacteriaceae agar, which consists of a Chromatic Carbapenem-resistant Enterobacteriaceae agar base supplemented with 1% Chromatic CRE (Liofilchem, Province of Teramo, Italy) (CLSI, 2021). In this evaluation, two to three separate colonies of GNB isolates were suspended in 3 mL of normal saline and inoculated onto the Chromatic CRE agar. The confirmation of carbapenemase production was established by observing the growth of specific pigmented colonies of GNB on the Chromatic CRE agar. The identification of bacterial species was performed in accordance with the chromogenic color code specified in the manufacturer's instructions (Liofilchem, 2020).

Quality control

This was done to ensure the accuracy and reliability of the test. The culture media were assessed for sterility by incubating the batch of sterilized solidified media at 37 °C for 24 hours, and their quality was checked by seeding the agar plates with the control bacteria strain. For the carbapenemase production positive strain, *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC BAA 1705 [American Type Culture Collection (ATCC)] were used, while *K. pneumoniae* ATCC BAA 1706 was used for the carbapenemase production negative control.

Carba NP Test

Carbapenemase production was also confirmed via the Carba NP test, a rapid colorimetric assay based on the hydrolysis of carbapenem by carbapenemase enzymes (Bouslah *et al.,* 2020). Isolates that produce carbapenemase turn the solution from red to yellow, indicating positive results.

Molecular Detection of Carbapenemase Genes

All carbapenem-resistant isolates underwent molecular analysis to detect specific carbapenemase-encoding genes using PCR. The following genes were targeted: (i) *bla*$_{KPC}$, (ii) *bla*$_{NDM}$, (iii) *bla*$_{VIM}$, (iv) *bla*$_{OXA}$, and (v) *bla*$_{IMP}$ (Saleem *et al.,* 2025; Shahid *et al.,* 2023; Nomeh *et al.,* 2023a). PCR was conducted via the following protocol. First, we performed DNA extraction via a standard boiling method. Second, we performed PCR amplification using specific primers for the abovementioned genes. Finally, the amplified products were run on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light

RESULTS

1.1 *Distribution of Gram-negative bacilli*

The distribution of Gram-negative bacilli (GNB) across the analyzed samples revealed a high prevalence rate of 164 (82.0%) in catheter tips collected from hospitalized patients. Among the identified isolates, *Escherichia coli* was the most predominant, accounting for 35.0% of cases. This was followed by *Klebsiella pneumoniae* at 19.5% and *Pseudomonas aeruginosa* at 16.5% as shown in Table 1. In contrast, *Acinetobacter baumannii* was the least frequently isolated, representing only 11.0% of the GNB identified. These findings highlight the significant role of catheter-associated colonization by GNB in hospital settings, with *E. coli* emerging as the leading pathogen in this context.

1.2 Frequency of Biofilm-forming Gram-negative Bacilli

A total of 87 (43.5%) Gram-negative bacilli were biofilm producers, as shown in Figure 1. In different wards, the prevalence was as follows, as presented in Figure 1. Surgical (n=31/15.5%): *Acinetobacter baumannii* (n=3/1.5%), *Pseudomonas aeruginosa* (n=6/3.0 %), *E. coli* (n=15/7.5%), *Klebsiella pneumoniae* (n=7/3.5%). Obstetrics and Gynaecology, (n=47/23.5%), *Acinetobacter baumannii* (n=5/2.5 %), *Pseudomonas aeruginosa* (n=10/5.0%), *E. coli* (n=26/13.0%), *Klebsiella pneumoniae* (n=12/6.0%). ICU (n=9/4.5%), *Acinetobacter baumannii* (n=0/0.0%), *Pseudomonas aeruginosa* (n=3/1.5 %), *E. coli* (n=4/2.0%), *Klebsiella pnuemoniae* (n=2/1.0%).

1.3 Antibiotic Resistance proportion of Gram-negative bacilli

The Antibiotic resistance and Susceptibility of Gram-negative bacilli are presented in Figure 2. The Antibiotic Resistance proportion of Gram-negative bacilli was as follows:

Acinetobacter baumannii (n=22), amoxicillin-clavulanic acid R=16(72.7%), S=6(27.3%), ceftriaxone R= 22(100%), cefotaxime R=13 (59.1%), S=8(36.3%), ceftazidime R=(100%), S=0(0.0 %) cefepime R= 10(45.5%), S=12(54.5%), nitrofurantoin R=4 (18.2%), S=18(81.8%), trimethoprim-sulfamethoxazole R= (100%), S=0(0.0 %), Gentamicin R= 2 (9.1%), S=20(90.9%), ciprofloxacin R=0(0.0 %) S=22(100%), Ertapenem R= 15 (68.2%), S=7(31.8%), imipenem R= 13 (59.1%), S=9 (40.9%), meropenem R= 12 (54.5%), S=10 (45.5%).

Pseudomonas aeruginosa (n=33), amoxicillin-clavulanic acid R=33(100%) S=0(0.0 %), ceftriaxone R=30 (90.9%) S= 3(9.1 %), cefotaxime R=21 (63.6%), S=12(36.3%), ceftazidime = 33(100%) S=0(0.0 %), cefepime R= 33(100%) S=0(0.0 %), nitrofurantoin R=9 (27.3%), S=24(72.7%), trimethoprim-sulfamethoxazole R= (100%), S=0(0.0 %), Gentamicin R=0(0.0 %), S=33

(100%), ciprofloxacin R=0(0.0 %), S=33(100%), Ertapenem R=33 (100.%) S=0(0.0 %, imipenem R= 18 (54.5%), S=15 (45.5%), meropenem R= 33 (100%), S=0(0.0%).

E. coli (n=70), amoxicillin-clavulanic acid R=70(100%), S=0(0.0%), ceftriaxone R=70(100%), S=0(0.0%), cefotaxime R=70(100%), S=0(0.0%), ceftazidime R=70(100%), S=0(0.0%), cefepime R=41 (58.6%), S=29 (41.4%), nitrofurantoin R=17 (24.3%), S=43(61.4%), trimethoprim-sulfamethoxazole R= 70(100%) S=0(0.0%), Gentamicin R=15(21.4%), S=55 (78.57%), ciprofloxacin S=70(100%) S=0(0.0%), Ertapenem R=70(100 %, S=0 (0.0.%), imipenem R= 41 (58.6%), S=29 (41.4%), meropenem R= 51 (72.9%), S=19(27.1%).

Klebsiella pnuemoniae (n=39), amoxicillin-clavulanic acid R=29 (74.4%), S=10(25.6%), ceftriaxone R=39(100%), S=0(0.0%), cefotaxime R=25(64.1%), S=14(35.9%), ceftazidime R=39(100%), S=0(0.0%), cefepime R=21 (53.8%), S=18 (46.2%), nitrofurantoin R=4(10.3%), S=35(89.7%), trimethoprim-sulfamethoxazole R= (100%) S=0(0.0%), Gentamicin R=15(38.5%), S=24 (61.5%), ciprofloxacin R= 16(41.0%) S=23(59.9%), Ertapenem R=39(100 %, S=0 (0.0 %), imipenem R= 18 (46.2%), S=22 (56.4%), meropenem R= 31 (79.5%), S=8(20.5%).

1.4 Phenotypic Carbapenem-resistant Enterobacteriaceae agar and Carba NP Test confirmed Carbapenem-resistant Gram-negative bacilli

In Figure 3, Carbapenem-resistant Enterobacteriaceae agar and Carba NP Test confirmed 89 (44.5%) Gram-negative bacilli that are carbapenem-resistant. This comprises *Acinetobacter baumannii* (n=12/%), *Pseudomonas aeruginosa* (n=18/9.0 9.0%), *E. coli* (n=41/20.5%), and *Klebsiella pneumoniae* (n=18/9.0 9.0%). In different wards, the isolation rate of Gram-negative bacilli was as follows.

Surgical (n=17/8.5): *Acinetobacter baumannii* (n=2/1.0 %), *Pseudomonas aeruginosa* (n=5/2.5 %), *E. coli* (n=8/4.0 %), *Klebsiella pneumoniae* (n=2/1.0 %).

Obstetrics and Gynaecology (n=29/14.5 %): *Acinetobacter baumannii* (n=3/1.5%), *Pseudomonas aeruginosa* (n=10/5.0 %), *E. coli* (n=10/5.0 %), *Klebsiella pneumoniae* (n=6/3.0 %).

Intensive Care Unit (n=43/21.5 %): *Acinetobacter baumannii* (n=7/3.5 %), *Pseudomonas aeruginosa,* (n=3/1.5%), *E. coli* (n=23/11.5 %), *Klebsiella pnuemoniae* (n=10//5.0 %).

The Overall occurrence of Carbapenemase Genes in Gram-negative bacilli: *bla*$_{KPC}$ 59(29.5 %), *bla*$_{NDM}$, 89(100 %), *bla*$_{VIM}$, 27 (13.5 %), *bla*$_{OXA}$ 89(100 %), and *bla*$_{IMP}$ 40 (20.0 %) is presented in Figure 4.

1.5 Prevalence of Carbapenemase Genes in Gram-negative bacilli from different hospital wards

According to hospital wards, the prevalence of the carbapenemase gene was as follows:

Surgical

bla$_{KPC}$ (n=16/8.0)-*Acinetobacter baumannii* (n=1/%), *Pseudomonas aeruginosa,* (n=5/%), *E. coli* (n=8/4%), *Klebsiella pnuemoniae* (n=2/ %).

bla$_{NDM}$ (n=17/8.5 %)- *Acinetobacter baumannii* (n=2/1.0 %), *Pseudomonas aeruginosa,* (n=5/2.5 %), *E. coli* (n=8/4.0 %), *Klebsiella pnuemoniae* (n=2/1.0 %).

bla$_{VIM}$ (n=7/3.5)-*Acinetobacter baumannii* (n=0/0.0 %), *Pseudomonas aeruginosa,* (n=2/ %), *E. coli* (n=4/%), *Klebsiella pnuemoniae* (n=1/ %).

bla$_{OXA}$ (n=17/ 8.5)- *Acinetobacter baumannii* (n=2/%), *Pseudomonas aeruginosa,* (n=5/%), *E. coli* (n=8/4%), *Klebsiella pnuemoniae* (n=2/ %).

bla$_{IMP}$ (n=10/5.0)-*Acinetobacter baumannii* (n=2%), *Pseudomonas aeruginosa,* (n=3/%), *E. coli* (n=3/%), *Klebsiella pnuemoniae* (n=2/%).

Obstetrics and Gynaecology

bla$_{KPC}$ (n=21/10.5 %)- *Acinetobacter baumannii* (n=3/1.5%), *Pseudomonas aeruginosa,* (n=3/%), *E. coli* (n=9/%), *Klebsiella pnuemoniae* (n=6/%).

bla$_{NDM}$ (n=29/14.5 %)-*Acinetobacter baumannii* (n=3/1.5%), *Pseudomonas aeruginosa,* (n=10/5.0 %), *E. coli* (n=10/5.0 %), *Klebsiella pnuemoniae* (n=6/3.0 %).

bla$_{VIM}$ (n=7/3.5)-*Acinetobacter baumannii* (n=0/0.0%), *Pseudomonas aeruginosa,* (n=2/ %), *E. coli* (n=3/ %), *Klebsiella pnuemoniae* (n=2/%).

bla$_{OXA}$ (n=29/14.5 %)- *Acinetobacter baumannii* (n=3/1.5%), *Pseudomonas aeruginosa,* (n=10/5.0 %), *E. coli* (n=10/5.0 %), *Klebsiella pnuemoniae* (n=6/3.0 %).

bla$_{IMP}$ (n=14/7.0) -*Acinetobacter baumannii* (n=1/%), *Pseudomonas aeruginosa,* (n=5/5.0 %), *E. coli* (n=3/5.0 %), *Klebsiella pnuemoniae* (n=6/3.0 %).

ICU

bla$_{KPC}$ (n=22/11.0 %)-*Acinetobacter baumannii* (n=5/%), *Pseudomonas aeruginosa,* (n=1/%), *E. coli* (n=9/11.5 %), *Klebsiella pnuemoniae* (n=7/ %).

bla$_{NDM}$ (n=43/21.5%)-*Acinetobacter baumannii* (n=7/3.5 %), *Pseudomonas aeruginosa,* (n=3/1.5%), *E. coli* (n=23/11.5 %), *Klebsiella pnuemoniae* (n=10//5.0 %).

bla$_{VIM}$ (n=13/6.5 %)- *Acinetobacter baumannii* (n=1/3.5 %), *Pseudomonas aeruginosa,* (n=0.0/%), *E. coli* (n=4/1 %), *Klebsiella pnuemoniae* (n=8/ %).

bla$_{OXA}$ (n=43/21.5%)- *Acinetobacter baumannii* (n=7/3.5 %), *Pseudomonas aeruginosa,* (n=3/1.5%), *E. coli* (n=23/11.5 %), *Klebsiella pnuemoniae* (n=10//5.0 %).

bla$_{IMP}$ (n=16/8.0)-*Acinetobacter baumannii* (n=2/%), *Pseudomonas aeruginosa,* (n=3/1.5%), *E. coli* (n=8/%), *Klebsiella pnuemoniae* (n=3/%).

Table 1: Distribution of Gram-negative bacilli in Catheter tip Samples

Gram-negative bacilli	Surgical (n=89)	Obstetrics and Gynaecology (n=50)	Intensive Care Unit (n=61)	Frequency (%)
Acinetobacter baumannii	6(3.0)	11(5.5)	5(2.5)	22(11.0)
E. coli	21(10.5)	35(17.5)	14(7.0)	70(35.0)
Pseudomonas aeruginosa	16(8.0)	14(7.0)	3(1.5)	33(16.5)
Klebsiella pnuemoniae	13(6.5)	19(9.5)	7(3.5)	39(19.5)
Total	**56(28.0)**	**79(39.5)**	**29(14.5)**	**164 (82.0)**

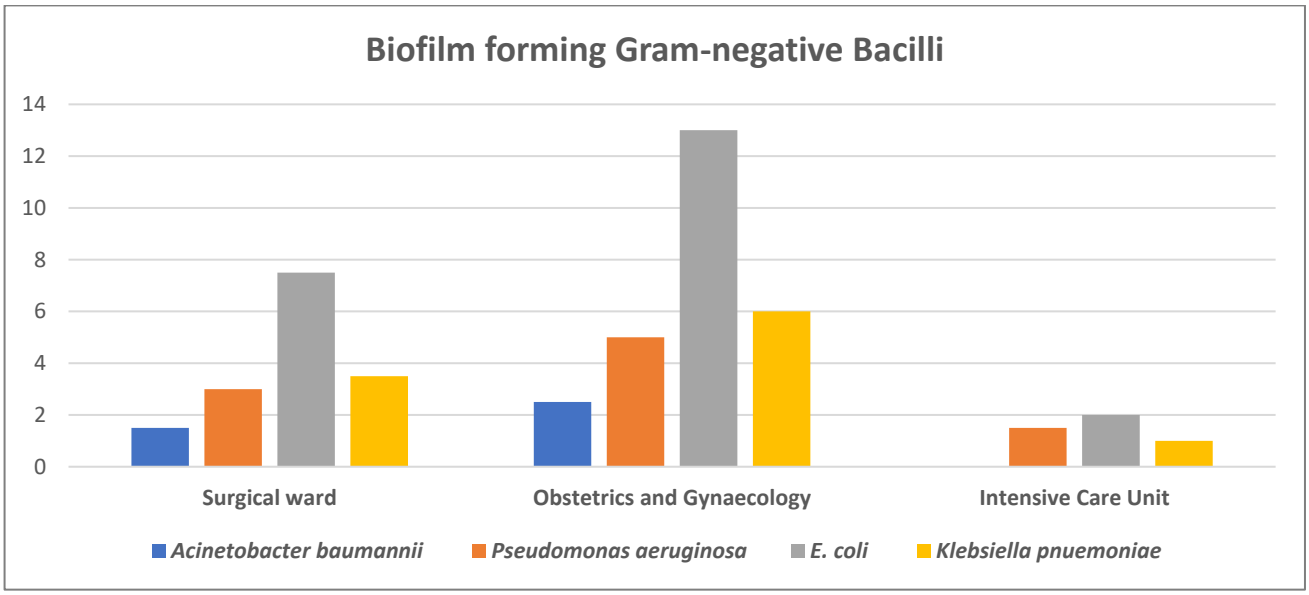

Figure 1: Frequency of Biofilm-forming Gram-negative Bacilli

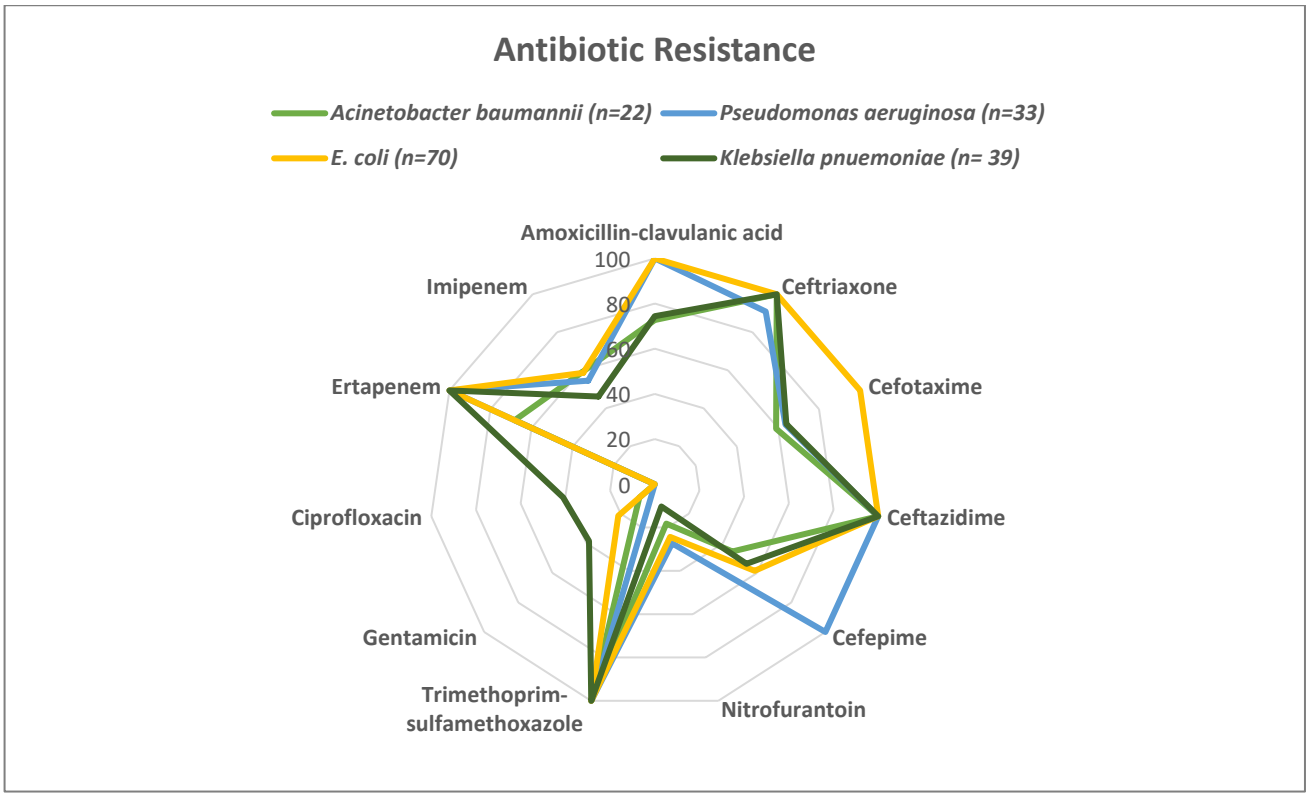

Figure 2: Radar chart showing the antibiotic resistance proportion of Gram-negative bacilli

Discussion

The study found that Gram-negative bacilli colonized 164 (82.0%) cases, of catheter tips. Among these, *Escherichia coli* was the most prevalent at 35.0%, followed by *Klebsiella pneumoniae*, with *Acinetobacter baumannii* being the least common at 11.0%. Research indicates that the Gram-negative bacilli identified in Table 1 are commonly isolated from urine samples of both catheterized and non-catheterized patients with urinary tract infections (UTIs), highlighting their potential as human uropathogens (Niveditha *et al.*, 2012; Sharif *et al.*, 2020; Nakawuki *et al.*,

2022; Asmare *et al.*, 2024). Even though the urine samples from these patients did not explicitly assess for asymptomatic bacteriuria (ASB) (Asmare *et al.*, 2024), the colonization of these bacteria in catheter tips raises concerns about the associated risk for both ASB and UTIs. It is essential to note that ASB has been frequently observed in all patients who were catheterized for 30 days or less (Warren, 2001; Luu *et al.*, 2022; Asmare *et al.*, 2024). The presence of these pathogens increases the likelihood of developing UTIs, which can lead to serious complications, especially in individuals with underlying health issues or compromised immune systems. While current literature extensively covers ASB and UTIs in catheterized patients, there remains a critical need for further research to investigate the clinical outcomes following the removal of indwelling urinary catheters (IUCs). Understanding these outcomes is crucial for developing effective management strategies and minimizing the risk of infection after catheter removal.

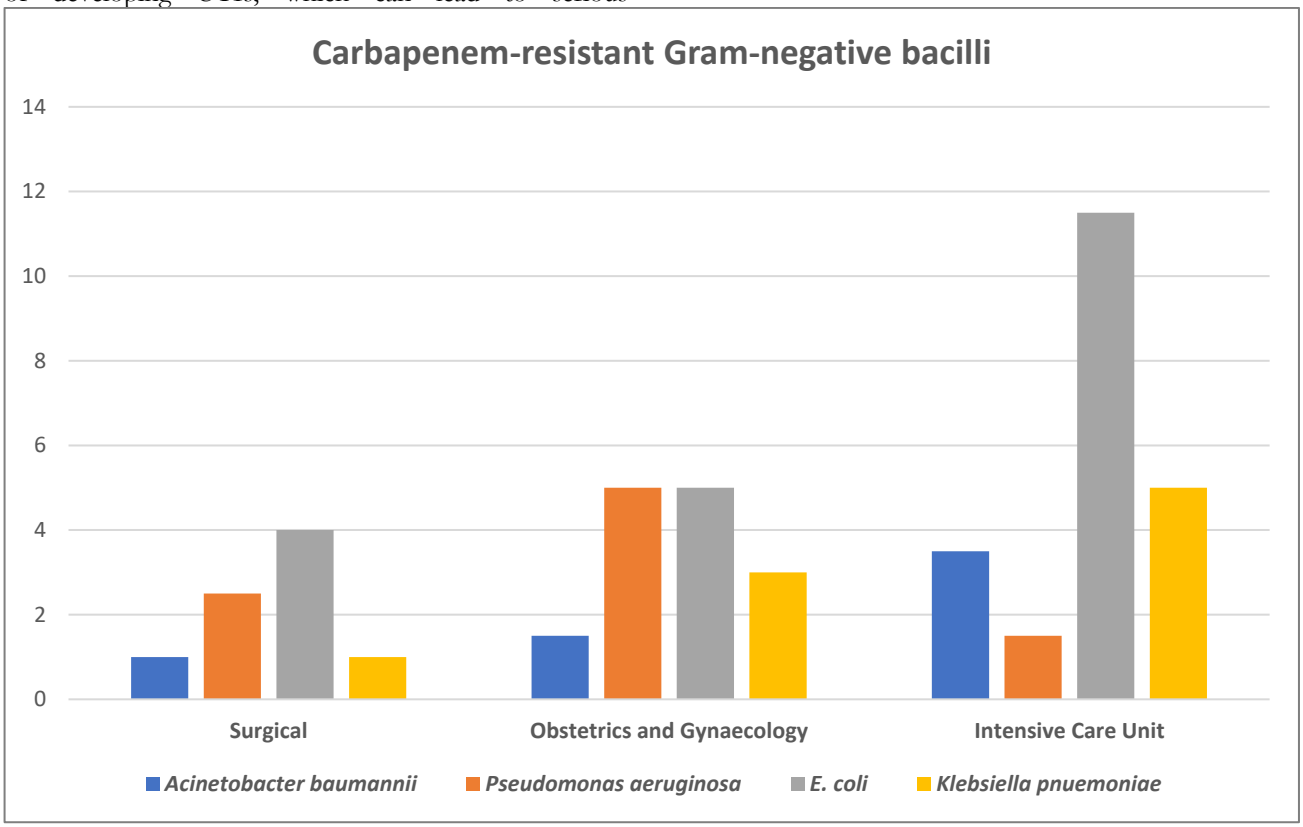

Figure 3: Phenotypic Carbapenem-resistant Enterobacteriaceae agar and Carba NP Test confirmed Carbapenem-resistant Gram-negative bacilli

The high prevalence of Gram-negative bacilli identified in our study suggests areas where improvements can be made. Factors such as host-related pathogen colonization, the need for improved urinary catheter maintenance, and more effective drainage bag care are crucial. Addressing these concerns could significantly reduce catheter colonization rates. Additionally, enhancing education around catheter care practices, particularly regarding the importance of hand hygiene when handling drainage bags, can further improve outcomes. By adhering to CDC guidelines for the prevention of catheter-associated urinary tract infections (CAUTIs) and promoting best practices in catheter care, we can work toward decreasing the risk of catheter-related infections (Gould *et al.*, 2019; Health Protection Surveillance Centre, 2011).

Regarding biofilm assay, the Congo Red method is recognized for its rapidity, sensitivity, and reproducibility. A notable advantage of this method is that the colonies remain viable on the medium. In the present study, 43.5% of Gram-negative bacilli exhibited *in vitro* biofilm production as determined using Congo Red agar. In comparison, a related study reported biofilm production rates of 60% and 73% in cases of catheter-associated urinary tract infections (Niveditha *et al.*, 2012; Reid, 1992). Biofilms represent the predominant phenotype of nearly all bacteria in their natural environments, irrespective of whether they are pathogenic or environmental. Significant quantities of Gram-negative bacilli were isolated from the catheter tip and shaft, demonstrating a strong capacity of these isolates to colonize foreign bodies within the bladder. The process of biofilm formation facilitates bacterial attachment and colonization on the catheter surface, which likely contributes to successful colonization in the affected patients.

Gram-negative bacilli that produce biofilms exhibit a notable resistance to immune factors and antibiotic therapy, frequently resulting in chronic urinary tract infections (UTIs). In general, free-floating bacterial cells can be effectively eliminated using antibiotic concentrations established through Minimum Inhibitory Concentration (MIC) studies, which indicate the lowest concentration of an antibiotic that prevents visible bacterial growth. However, to successfully eradicate cells embedded within biofilms, concentrations often

exceeding 100 times the MIC of the relevant antibiotics are necessary. This substantial increase in required antibiotic concentration arises not only from the protective nature of the biofilm matrix but also from its complex architecture, which serves as a barrier to drug penetration. Consequently, the effective management of such infections necessitates substantial efforts to develop innovative therapeutic agents that either inhibit biofilm formation or promote the detachment of these biofilms, which could greatly enhance treatment efficacy. Biofilms are implicated in a range of persistent infections that are not adequately addressed by traditional antibiotic therapies (Peter *et al.,* 2022). Their formation contributes to the propagation of antibiotic resistance among hospital-acquired pathogens, as biofilms can foster higher mutation rates and facilitate the transfer of genes related to resistance. Thus, overcoming infections linked to device-associated biofilm organisms often requires the removal of the infected implant in conjunction with antibiotic treatment (Peter *et al.,* 2022). Another key aspect of biofilm bacteria is their physiological heterogeneity. This characteristic influences bacterial growth rates and metabolic activities, driven by interbacterial signaling, toxin accumulation, and alterations in the local microenvironment (Uduku *et al.,* 2023). While these so-called persister cells are not inherently resistant to antibiotics, their association within biofilms contributes to their survival during antibiotic treatment. Addressing these challenges through innovative approaches could lead to more effective management of biofilm-related infections.

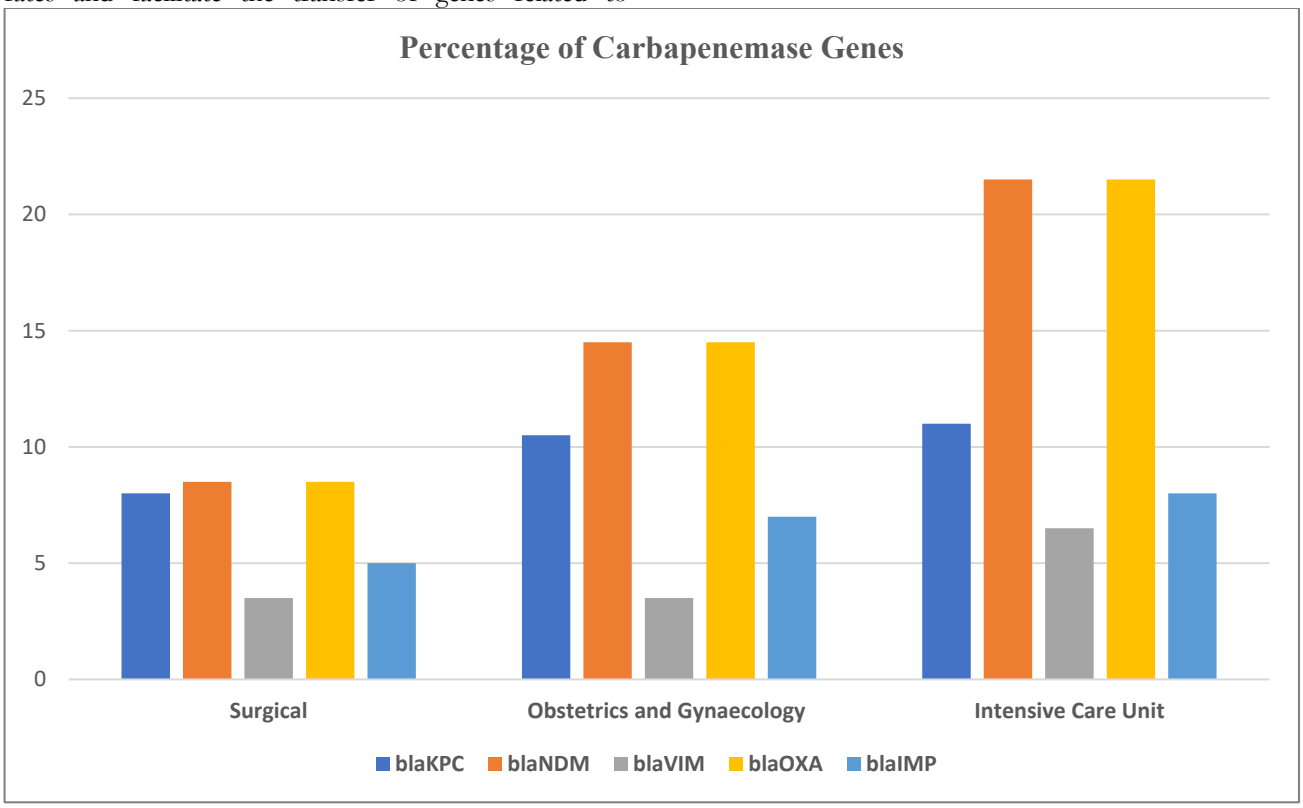

Figure 4: Prevalence of Carbapenemase Genes in Gram-negative bacilli from different hospital wards

The antibiotic resistance patterns illustrated in Figure 2 indicate that a significant proportion of the isolates in this study demonstrate resistance to one or more commonly utilized antibiotics, including cephalosporins, carbapenems, and trimethoprim-sulfamethoxazole, with resistance rates varying from 18.2% to 100%. Previous investigations have highlighted the implications of Gram-negative bacilli that exhibit resistance to the assessed antibiotics (Nomeh *et al.*, 2022; Ilang *et al.*, 2023a; Joseph *et al.*, 2023; John-Onwe *et al.*, 2023). This notable resistance pattern may arise from insufficiently guided antibiotic prophylaxis before catheterization, as well as ongoing empirical therapy for infections associated with Gram-negative bacilli, such as pneumonia, skin and soft tissue infections, or unspecified septicemia. Additionally, it is critical to identify the determinants of carbapenem resistance in bacterial pathogens. While numerous isolates express carbapenemase, others may develop resistance through alternative mechanisms, such as porin loss (Atrissi *et al.*, 2021) and biofilm formation, as observed in this study. Addressing these issues is imperative for enhancing treatment approaches and mitigating the impact of antibiotic resistance.

Additionally, Gentamicin, nitrofurantoin, and ciprofloxacin were effective against the Gram-negative bacteria. Their judicious application in the clinical care of these patients will reduce prolonged hospital stays and treatment costs.

Non-carbapenemase-producing strains that target carbapenems and β-lactam antibiotics are frequently associated with multidrug resistance (MDR). Mutations in AmpC and its regulatory genes are often linked to *Pseudomonas aeruginosa's* resistance to novel β-lactam antibiotics and β-lactamase inhibitor combinations (Barceló *et al.*, 2022). On the other hand, plasmid-encoded AmpC is primarily found in resistant Enterobacterales

(Philippon *et al.*, 2002). Among the efflux pump systems, the AcrAB-TolC RND system is the most commonly described (Chetri *et al.,* 2019). Additionally, mutations in porin proteins, particularly OmpC and OmpF, can inhibit the entry of carbapenems into the bacterial cell (Masi *et al.*, 2017).

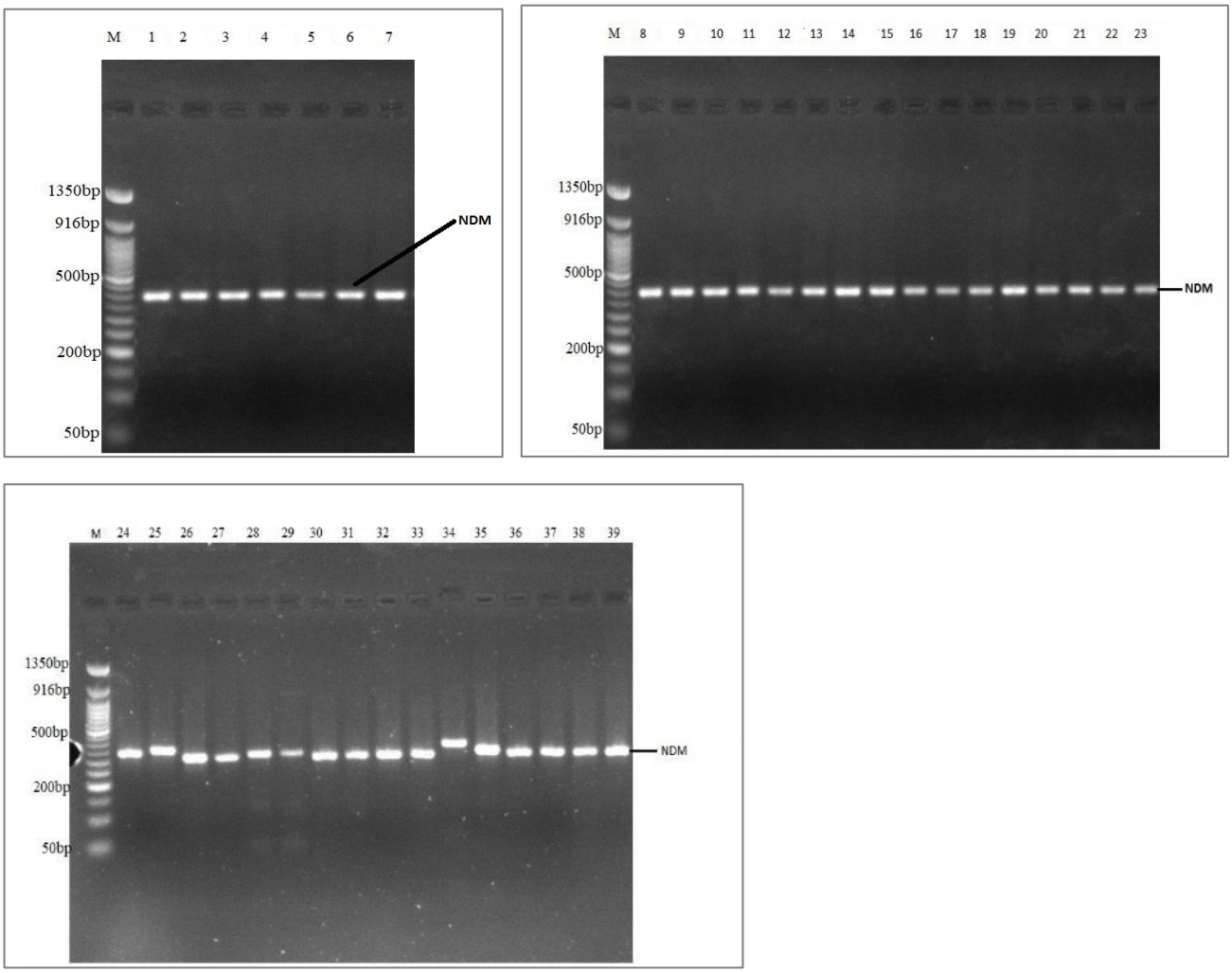

Figure 5(a): Gel image showing amplification of *bla*$_{NDM}$ at 350bp.
Lane M: Molecular size marker (50bp ladder, Invitrogen, U.S.A®) was used to estimate the base pair size of the amplicons **Lane 1-7=** *Acinetobacter baumannii,* **Lane 8-15=** *Pseudomonas aeruginosa,* **Lane 16-23=** *E. coli,* **Lane 24-39=** *Klebsiella pnuemoniae*

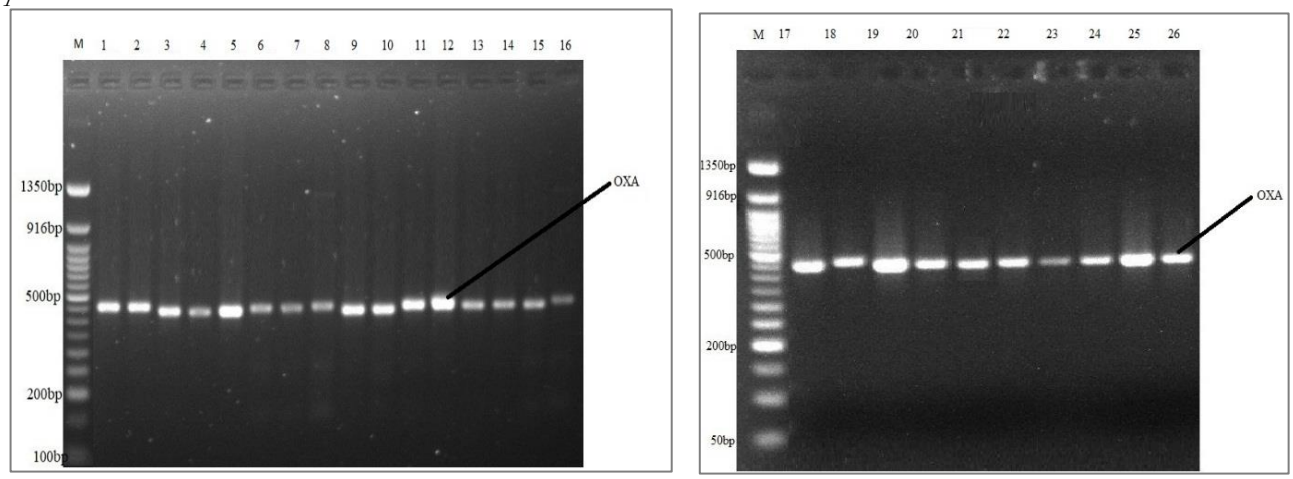

Figure 5(b): Gel image showing amplification of *bla*$_{OXA}$ at 450bp. A 50bp ladder was used to estimate the base pair size of the amplicons
Lane M: Molecular size marker (50bp ladder, Invitrogen, U.S.A®) was used to estimate the base pair size of the amplicons **Lane 1-5=** *Acinetobacter baumannii,* **Lane 6-11=** *Pseudomonas aeruginosa,* **Lane 12-16=** *E. coli,* **Lane 17-26=** *Klebsiella pnuemoniae*

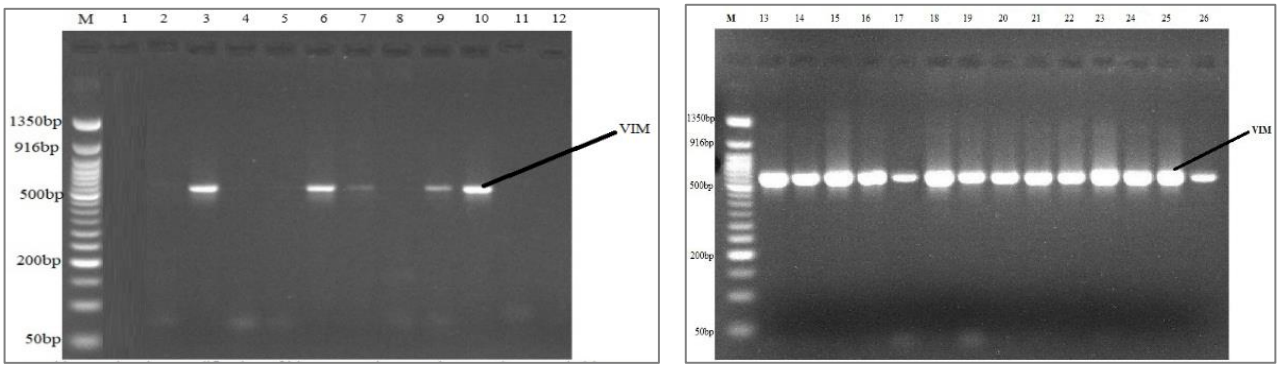

Figure 5(c): Gel image showing amplification of *bla*$_{VIM}$ at 550bp. A 50bp ladder was used to estimate the base pair size of the amplicons
Lane M: Molecular size marker (50bp ladder, Invitrogen, U.S.A®) was used to estimate the base pair size of the amplicons **Lane 1-7=** *Acinetobacter baumannii,* **Lane 8-12=** *Pseudomonas aeruginosa,* **Lane 13-19=** *E. coli,* **Lane 20-26=** *Klebsiella pnuemoniae*

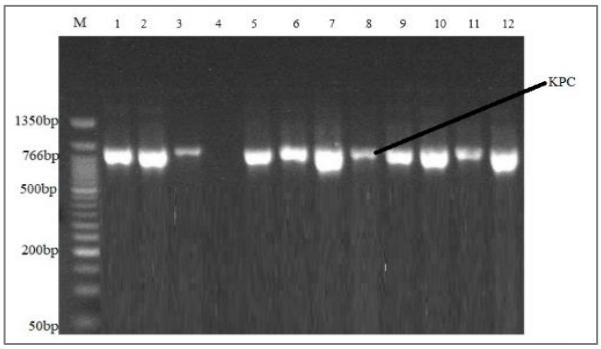

Figure 5(d): Gel image showing amplification of *bla*$_{KPC}$ at 800bp. A 50bp ladder was used to estimate the base pair size of the amplicons
Lane M: Molecular size marker (50bp ladder, Invitrogen, U.S.A®) was used to estimate the base pair size of the amplicons **Lane 1-7=** *Acinetobacter baumannii,* **Lane 8-12=** *Pseudomonas aeruginosa*

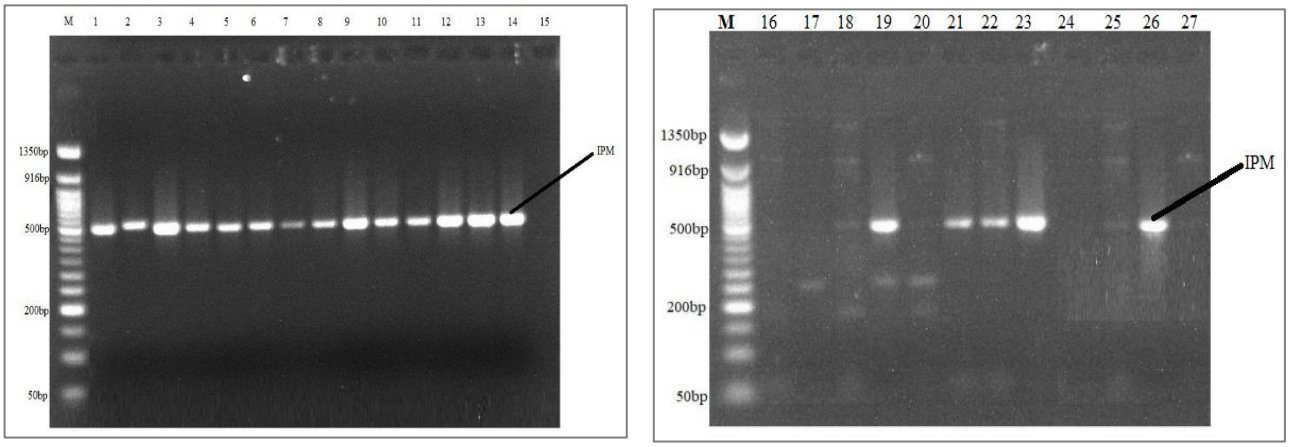

Figure 5(e): Gel image showing amplification of *bla*$_{IPM}$ at 500bp. A 50bp ladder was used to estimate the base pair size of the amplicons.
Lane M: Molecular size marker (50bp ladder, Invitrogen, U.S.A®) was used to estimate the base pair size of the amplicons **Lane 1-7=** *Acinetobacter baumannii,* **Lane 8-15=** *Pseudomonas aeruginosa,* **Lane 16-21=** *E. coli,* **Lane 22-27=** *Klebsiella pnuemoniae*

A total of 89 (44.5%) of Gram-negative bacilli were identified as carbapenemase producers through the CRE agar and Carba NP Test. Previous research conducted in Sudan, South Africa, Egypt, Uganda, and Nigeria has shown varying rates of carbapenem resistance among Gram-negative bacilli, reported at 83%, 68%, 62.7%, 28.6%, and 11.9%, respectively (Elbadawi *et al.*, 2021; Perovic *et al.*, 2016; Amer *et al.*, 2016; Okoche *et al.*, 2015; Yusuf *et al.*, 2014). Given the current trends, it is essential to acknowledge that carbapenem resistance is likely to increase in low- and middle-income countries (LMICs) in Africa, primarily due to the unrestricted use of these antibiotics. The CRE agar and Carba NP Test findings indicated resistance rates of *Acinetobacter baumannii* (*n*=12, 6.0%), *Pseudomonas aeruginosa* (*n*=18, 9.0%), *Escherichia coli* (*n*=41, 20.5%), and *Klebsiella pneumoniae* (*n*=18, 9.0%). These resistance patterns highlight a concerning trend, particularly since carbapenems are often used as a last line

of defense. To tackle this challenge, it is crucial to address the increasing use of carbapenems in both community and hospital settings, alongside improving infection control measures. By implementing effective infection prevention strategies and promoting antimicrobial stewardship programs, we can ensure the judicious use of antibiotics. Additionally, investing in the development of new antibiotics that target these resistant strains is an essential step forward. A collaborative and multifaceted approach is key in combating the rising threat posed by carbapenemase-producing *Acinetobacter*, *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas* species. Through concerted efforts, it is possible to improve public health outcomes and enhance treatment options for individuals affected by these resistant infections (Mulani *et al.*, 2019; Jean *et al.*, 2022).

In our study, the most frequently identified carbapenemase-encoding genes were *bla*$_{NDM}$ and *bla*$_{OXA}$, both found in 89 samples (100%). The New Delhi metallo-beta-lactamase (NDM), classified as group B in the Ambler classification, is an enzyme capable of degrading a wide range of beta-lactam antibiotics, including carbapenems. Notably, NDM was primarily identified in *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *Acinetobacter baumannii*. Our findings are consistent with those reported in Ethiopia, Mexico, Sudan, India, South Africa, Saudi Arabia, and other Middle Eastern countries (Gashaw *et al.*, 2024; Nieto-Saucedo *et al.*, 2023; Elbadawi *et al.*, 2021; Perovic *et al.*, 2016; Deshpande *et al.*, 2010; Kumarasamy *et al.*, 2010; Sonnevend *et al.*, 2015). NDM is particularly concerning due to its ability to rapidly spread among different bacterial species through horizontal gene transfer, which can lead to the emergence of extensively drug-resistant infections (Gashaw *et al.*, 2024). The *bla*$_{NDM}$ gene has demonstrated potential for rapid dissemination, as observed in Turkey and other countries worldwide (Karabay *et al.*, 2016; Gashaw *et al.*, 2024). Furthermore, its frequent association with different antibiotic resistance determinants indicates the potential for co-transfer, necessitating comprehensive strategies to address this pressing issue in antibiotic resistance management.

Our study highlights the global distribution of carbapenemases, with a particular focus on the predominance of the *bla*$_{OXA}$ gene in the Middle East and Africa (Suay-García *et al.*, 2019). We found an even distribution of *bla*$_{OXA}$ genes among various Gram-negative bacterial isolates, similar to the distribution of the *bla*$_{NDM}$ gene. The prevalence of the *bla*$_{OXA-51}$-like gene in our study aligns with previous research conducted in Egypt (Abouelfetouh *et al.*, 2019) and South Africa (Anane *et al.*, 2020), but it was higher than what was reported in a study from Jimma, which indicated a prevalence of 63.1% (Sewunet *et al.*, 2022). This increase may be attributed to the higher proportion of Gram-negative bacilli strains found in the catheter tip samples we evaluated, compared to earlier studies. We did not explore the regulatory mechanisms that may contribute to the increased expression of *bla*$_{OXA-51}$-like enzymes. As such, we can only hypothesize about their potential role in contributing to the phenotypically resistant isolates, which could be further influenced by factors such as changes in membrane permeability or the action of efflux pumps that expel antimicrobials from bacterial cells. Additionally, in the case of *Pseudomonas aeruginosa*, the carbapenem resistance could be as the result of the carbapenemases gene, and it is also more plausible that this resistance is primarily due to porin loss, a mechanism suggested by previous research (Atrissi *et al.*, 2021), which impairs the bacterium's ability to uptake carbapenem antibiotics effectively. This nuanced understanding of resistance mechanisms is crucial for developing targeted interventions to combat these multidrug-resistant pathogens.

We found the following carbapenemase-encoding genes: *bla*$_{KPC}$ in 59 cases (29.5%), *bla*$_{VIM}$ in 27 cases (13.5%), and *bla*$_{IMP}$ in 40 cases (20.0%). Notably, the presence of these genes is concerning due to their association with resistance to carbapenem antibiotics, which are often used as a last line of defense against serious infections caused by Gram-negative bacteria.

Despite the critical implications of these findings, the literature on the prevalence of such genes remains sparse. Noteworthy studies addressing this issue include those conducted by Garza-Ramos *et al.* (2023), Nomeh *et al.* (2023), Ilang *et al.* (2023b), Garza-González *et al.* (2023), Ogba *et al.* (2022), and López-García *et al.* (2018), which provide valuable insights into the dynamics of resistance mechanisms in clinical settings. Given the high lethality rate linked to infections from carbapenem-resistant Gram-negative bacilli (CR-GNB), underscores the importance of risk assessment at both the hospital and ward levels. This involves not only identifying the specific mechanisms of resistance but also ensuring meticulous care in the maintenance of urinary catheters and drainage bags.

By adhering to stringent catheter care protocols, we can significantly reduce the rates of urinary catheter colonizationa major contributor to infection risks. Moreover, strict adherence to the guidelines established by the Centers for Disease Control and Prevention (CDC) for preventing catheter-associated urinary tract infections (CAUTIs) is essential. These measures will play a vital role in mitigating the risk of catheter-related infections, thereby protecting patient safety and preventing the spread of nosocomial infections by enhancing overall infection control strategies within healthcare settings.

CONCLUSION

This study reports the biofilm and carbapenemase potential of Gram-negative bacilli colonizing the catheter tip of hospitalized patients. Drugs such as Gentamicin, nitrofurantoin, and ciprofloxacin have been proven effective and can serve as an empirical treatment for infections caused by Gram-negative bacilli colonizing the catheter tips of hospitalized patients. Also, rigorous adherence to structured catheter care protocols is a key strategy for reducing the prevalence of urinary catheter colonization of this strain, which serves as a significant gateway for infections. Regular training for healthcare

staff on best practices for catheter management, including daily assessment of the necessity of catheters and proper aseptic technique during insertion and maintenance, is vital. Furthermore, strict adherence to evidence-based guidelines regarding the prevention of catheter-associated urinary tract infections (CAUTIs) will enhance patient safety and quality of care. By adopting these detailed and constructive measures, we can effectively mitigate the risk of catheter-related infections and improve overall clinical outcomes for patients at risk of CR-GNB infections.

CONFLICT OF INTEREST

None

FINANCIAL SUPPORT

None

ETHICS STATEMENT

All experiments in this study were performed following ARRIVE guidelines regarding Animal and human subjects. Documentation is available from the corresponding author upon reasonable request.

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