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Detection of carbapenemase-producing strains of Gram-negative bacilli in University Hospital of Angre in 2021

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The spread of carbapenemase-producing Gram-negative bacilli (GNB) poses a significant threat to public health. In Africa, data on carbapenem-resistant bacteria are poorly defined. This study aimed to determine the prevalence of carbapenemase genes among GNB isolated from patient samples at Angre University Hospital. A cross-sectional study was conducted in 2021 at the medical biology department, where carbapenem-resistant Gram-negative bacilli from various clinical specimens were collected. Identification and antimicrobial susceptibility testing were performed using the Vitek automated system, and potential carbapenemase-producing GNB were confirmed by simplex polymerase chain reaction (PCR). Data were analyzed using EPI info 7 software. The prevalence of carbapenem-resistant bacteria (CR) was found to be 8.94% (16/179). Among these, 12 (75%) produced at least one carbapenemase gene, with the OXA-48 gene being predominant, followed by NDM in 25% (4/16) and 18.75% (3/16), respectively. The predominant species were *Klebsiella pneumoniae* (50%, 8/16) and *Escherichia coli* (25%, 4/16). CR bacteria were also resistant to gentamicin (81.25%), amikacin (31.25%), ciprofloxacin (62.50%), and levofloxacin (68.75%). The multidrug resistance rate was 75% (12/16). Detection of these highly resistant bacteria should be implemented routinely, as this will effectively contribute to strategies for combating antimicrobial resistance.

Key words: Gram-negative bacilli, carbapenemase, polymerase chain reaction, Abidjan.

INTRODUCTION

Bacterial infections caused by multidrug-resistant (MDR) Gram-negative pathogens have become a major global public health problem over the last two decades due to inadequate therapeutic options, leading to increased morbidity, mortality, and higher healthcare costs (Gaibani et al., 2022). Carbapenems (that is, imipenem,

meropenem, doripenem, and ertapenem) are a safe and highly effective class of antibiotics considered last-resort drugs for controlling MDR Gram-negative pathogens, including extended-spectrum beta-lactamase-producing Enterobacteriaceae. Due to their broad-spectrum activity, carbapenems are frequently used in the treatment of life-threatening infections (Haji et al., 2021). However, an increase in carbapenem resistance has been reported worldwide in recent years. Carbapenem resistance is primarily due to the expression of carbapenemase enzymes, efflux pumps, or porin loss. Among these, the production of carbapenemase enzymes is the most significant and challenging mechanism, as these enzymes are located on mobile genetic elements that can be easily transferred between bacteria such as *Pseudomonas* species, *Acinetobacter* species, *Escherichia coli*, and *Klebsiella* species, which the World Health Organization (WHO) designated as high-priority organisms in 2017 (Breijyeh et al., 2020; WHO, 2017). The first reports of carbapenem resistance in Enterobacteriaceae isolates occurred in the early 1990s, with resistance initially attributed to AmpC β-lactamase production and outer membrane protein loss (Lutgring, 2019). KPC-type carbapenemases (*Klebsiella pneumoniae* carbapenemase) were first described in 1996 in a strain of *K. pneumoniae* from North Carolina in the United States. The clinical impact and diversity of carbapenemases gradually increased, becoming significant in the mid-2000s. Since 2008, carbapenemases of the NDM (New Delhi Metallo-β-lactamase) type from the metallo-β-lactamase (MBL) family have become endemic in India and Pakistan (Aggoune et al., 2018) and have spread widely throughout the world. The most common carbapenemases in France are OXA-48 (Dortet et al., 2017). However, in Africa, the molecular epidemiology of carbapenemases is less well described. Nigeria and Senegal have reported carbapenemases in Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, with all carbapenemases described in Senegal classified as class D (Sekyere et al., 2016). In Côte d'Ivoire, there are few studies on MDR Gram-negative (MR BGN) infections.

Given the significant dissemination capacity of carbapenemase-producing strains and the therapeutic impasse they create in the event of infection, effective detection of carbapenemases has become a major issue. Clinical microbiology laboratories are on the front lines of the fight against the expansion of carbapenem-resistant strains and play several critical roles. They must identify these strains for both patient care and infection control purposes and provide hospital epidemiologists and public health institutions with prevalence data on carbapenem-resistant strains to help determine when outbreaks are occurring. The aim of this study was to determine the prevalence of carbapenemase genes among Gram-negative bacilli isolated from patients at Angre University Hospital.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted at the medical biology laboratory of Angre University Hospital, consisting of a retrospective analysis of the clinical and microbiological parameters of bacterial strains collected between January and December 2021. Primary culture, bacterial identification using standard biochemical tests, and antibiotic susceptibility testing by the VITEK MD 2 Compact® system (bioMérieux, France) were performed on these isolates at the medical bacteriology unit. All strains of Gram-negative bacilli isolated from various clinical samples from inpatients with reduced sensitivity to carbapenems were included in the study. These isolates were derived from pus, urine, blood, and bronchial secretions. Strain selection criteria were interpreted according to the recommendations of the microbiology guidelines of the Antibiogram Committee of the French Microbiology Society and the European Committee on Antimicrobial Susceptibility Testing (AC-FMS/EUCAST) (French Microbiology Society, 2019a). Strains were stored in duplicate in deep agar tubes at room temperature (25°C) and in brain heart broth with 20% glycerol at -80°C until further analysis, such as molecular testing. A predeveloped worksheet was used to collect information related to the sociodemographic characteristics of the patients, identified isolates, antibiotic susceptibility patterns of the isolates, and classes of carbapenemases.

Subculturing and disk diffusion susceptibility testing

Isolates were subcultured on nutrient agar (BA) and subsequently subjected to further susceptibility testing on Müller-Hinton agar for amoxicillin (20 µg), amoxicillin-clavulanic acid (20/10 µg), ticarcillin (75 µg), ticarcillin-clavulanic acid (75/10 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprim-sulfamethoxazole (TMP/SMX) (1.25/23.75 µg), ertapenem (10 µg), imipenem (10 µg), and meropenem (10 µg) (Bio-Rad®, France). All susceptibility results were interpreted according to the AC-FMS/EUCAST 2019 guidelines. Any strain with reduced sensitivity (intermediate/resistant) to at least one of the carbapenems was considered a suspected carbapenemase-producing strain (French Microbiology Society, 2019b). *E. coli* ATCC 25922 (sensitive), *K. pneumoniae* ATCC 700603 (producing extended-spectrum beta-lactamases, SHV-18), and *P. aeruginosa* ATCC 27853 (sensitive) were used as internal quality control strains.

Molecular analysis of carbapenemases genes

The main carbapenemase-encoding genes were detected by simplex polymerase chain reaction (PCR). All molecular/PCR tests (DNA extraction, amplification, and gel electrophoresis) were

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Table 1. Primers used to detect carbapenem resistance genes by conventional PCR (Poirel et al., 2011).

Gene	Primers	Sequences (5'-3')	Hybridization temperature	Fragment size (pb)
bla_{OXA-48}	OXA-48-F	GCGTGGTTAAGGATGAACAC	52°C	438
	OXA-48-R	CATCAAGTTCAACCCAACCG		
bla_{VIM}	VIM-F	GATGGTGTTTGGTCGCATA		390
	VIM-R	CGAATGCGCAGCACCAG		
bla_{IMP}	IMP-F	GGAATAGAGTGGCTTAAYTCTC		232
	IMP-R	GGTTTAAYAAAACAACCACC		
bla_{NDM}	NDM-F	GGTTTGGCGATCTGGTTTTC		621
	NDM-R	CGGAATGGCTCATCACGATC		
bla_{KPC}	KPC-F	CGTCTAGTTCTGCTGTCTTG		798
	KPC-R	CTTGTCATCCTTGTTAGGCG		

conducted at the bacteriological laboratory of the Faculty of Medical Sciences, Félix Houphouët Boigny University. Nucleic acids were extracted using a heat shock method: bacterial suspensions were obtained by vortexing young bacterial colonies (less than 24 h old) in an elution buffer and stored in Eppendorf tubes at -20°C for 15 min, then heated to 95°C for 15 min and centrifuged at 14,000 rpm for 10 min at room temperature. After centrifugation, the DNA contained in the supernatant was recovered in new Eppendorf tubes for amplification.

The presence of carbapenemase-encoding genes was determined using primers targeting *bla*VIM, *bla*IMP, *bla*KPC, *bla*OXA-48, and *bla*NDM (Poirel et al., 2011), obtained from Eurofins Genomics, Germany, as shown in Table 1. For amplification, five microliters of total DNA were added to a 20 µL reaction mixture containing 5X Hot Fire PolR premix (Solis Biodyne), 4 µL of AmpliTaq polymerase (Invitrogen, Germany), 13 µL of sterile water, and 0.75 µL of each primer. The amplification was performed using the MJ Research PTC-200 Thermal Cycler. The program consisted of denaturation at 94°C for 30 s, hybridization at 52°C for 40 s, and elongation at 72°C for 50 s. The cycles were repeated 36 times, with all primer sets undergoing a final extension at 72°C for 10 min (Poirel et al., 2011). The size of the amplification products was confirmed by gel electrophoresis on a 2% agarose gel. The electrophoresis gel was stained with SYBR® Safe solution (SYBR® Safe DNA gel, 10,000X) (Invitrogen, Germany). Migration was conducted for 20 min at a voltage of 100V.

Finally, the gel was exposed to ultraviolet light from a transilluminator to visualize the DNA bands.

Data analysis

Data analysis was conducted using Epi Info version 6.04 (2007) (Centers for Disease Control and Prevention, Atlanta). Frequencies, means, and standard deviations were calculated. Pearson's Chi-squared test or Fisher's exact test was used to study the relationships between categorical variables. A P-value of 0.05 was considered evidence of a significant statistical difference.

Ethical issue

The study was based entirely on routine clinical and laboratory data. The requirement for informed consent was waived by the medical and scientific management of Angre University Hospital. All data were de-identified from the routine database used. Additionally, these data were protected by a password, with access restricted to the principal investigator and the laboratory manager. The procedures followed complied with the ethical standards of the World Medical Association's Declaration of Helsinki (2008 amendment).

RESULTS

Clinical characteristics of bacterial isolates

Of the 179 strains of GNB identified, 51 (28.49%) were resistant to carbapenem. The study excluded 17 (33.33%) isolates that failed to grow on subculture, resulting in 34 (66.67%) isolates being the subject of a molecular study. Based on the PCR assays, 16 (8.94%) of the GNB isolates tested positive for one or more carbapenemase genes. All carbapenemase-producing (CP) strains were from hospitalized patients. The median age of the patients was 29.03 ± 28.40 years, with a range of 0 to 74 years. The female gender predominated at 62.5%, resulting in a sex ratio of 0.37. The strains were associated with various clinical infections, including bacteremia (50%, 8/16), suppurations (31.25%, 5/16), and urinary tract infections (18.75%, 3/16).

Bacterial species and antimicrobial susceptibility testing

Among the carbapenemase-producing (CP) strains, Enterobacteriaceae predominated at 87.50% (14/16). Carbapenemase-producing *K. pneumoniae* (KPC) constituted the majority species at 50% (8/16), followed by *E. coli* at 25%. *P. aeruginosa* and *A. baumannii* each represented 6.25%. The antibiotic resistance data for CP strains are shown in Table 2. Most CP strains were highly resistant to all tested antibiotics, including amoxicillin-

Table 2. Cross-resistance rate of strains by other antibiotics.

Resistance phenotype	Antibiotics tested [n (%)]								
	AMC	CRO	IMP	ETP	GEN	AMK	LEV	CIP	SXT
CP	16 (100)	16 (100)	3 (18.75)	16 (100)	13 (81.25)	5 (31.25)	11 (68.75)	10(62.50)	16 (100)
No CP*	18 (100)	18 (100)	4(22.23)	17 (94.44)	13 (72.22)	1 (5.56)	12 (66.67)	7 (38.89)	17 (94.44)
ESBL	26 (53.06)	49 (100)	0(0)	1 (2.04)	37 (75.51)	3 (6.12)	39 (79.60)	25 (51.02)	47 (95.92)

p= 0.000000767722 (Fisher exact test), CP: carbapenemase-producing, ESBL: extended-spectrum beta-lactamase, AMC: amoxicillin – clavulanic acid, CRO: ceftriaxone, IMP: imipenem, ETP: ertapenem, GEN: gentamycin, AMK: amikacin, OFL: ofloxacin, LEV: levofloxacin, CIP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole. *Carbapenem-resistant strains by mechanisms other than carbapenemase production.

clavulanic acid (100%), cefoxitin (100%), ceftazidime (100%), ertapenem (100%), ciprofloxacin (62.50%), and cotrimoxazole (100%). The level of antibiotic resistance among CP strains was significantly higher ($p < 0.05$) than that of other strains, including those resistant to carbapenems by non-enzymatic mechanisms and those producing extended-spectrum beta-lactamases (ESBL). Additionally, strains exhibited multidrug resistance (resistant to at least three different classes of antibiotics) in 75% (12/16) of cases.

Prevalence of genes in different carbapenem-resistant isolates

Of the 16 bacterial isolates, one or more carbapenemase genes were detected in 25% of cases. The bla_{OXA-48} gene was the most frequently detected carbapenemase gene, identified in a total of 5 different strains. Four strains (25%) harbored two or three carbapenemase genes. Among the strains producing only one gene, the bla_{OXA-48} gene represented 25% (4/16), followed by NDM at 18.75% (3/16), VIM at 12.50% (2/16), and IMP at 12.50% (2/16), with KPC present in 6.25% (1/16) (Figure 1). Three isolates (18.75%) produced two carbapenemase genes (NDM/OXA-48, IMP/OXA-48, and NDM/KPC), while one strain expressed three genes: VIM, NDM, and OXA-48. Carbapenemase genes were detected most frequently in *K. pneumoniae* (50%), followed by *E. coli* (25%) (Table 3).

DISCUSSION

In recent years, the emergence of carbapenemase-producing GNB has been reported and is now recognized as a significant global problem. In this study, the rate of carbapenemase genes was found to be 8.94% among GNB. Higher rates have been reported in some African studies, such as 58.7% in Sudan (Elbadawi et al., 2021), 13% in Algeria (Aggoune et al., 2018), 28.6% in Uganda (Okoche et al., 2015), and 35.24% in Tanzania (Mushi et al., 2014). In comparison, lower prevalences have been observed, such as 1.6% for clinical samples and 5.26% for fecal carriage in Gabon (Dikoumba et al., 2023), and 0.9 and 2.5% in Burkina Faso (Sanou et al., 2020) and Chad (Mahamat et al., 2019), respectively. The discrepancies observed between African countries can be attributed to varying levels of carbapenem consumption. The higher or lower cost of carbapenems in these countries may reduce the risk of excessive use and, consequently, the emergence of resistance. Moreover, the frequencies of carbapenemase-producing Enterobacteriaceae in Italy, Greece, and Romania were reported to be 7.5, 7.4, and 5.0%, respectively (Sader et al., 2015). These differences between African and developed countries could be explained by the presence of surveillance systems for multidrug-resistant bacteria and the effectiveness of measures to combat their spread. Among carbapenemase-producing Enterobacteriaceae (CPE), *K. pneumoniae* predominated at 50%, a result consistent with many other studies. Carbapenemase genes were found in *K. pneumoniae* in 46.5% of cases in Nigeria (Yusuf et al., 2014) and in 83.93% (n=47) in Algeria (Aggoune et al., 2018). In a multicenter study of CPE in Spain, *K. pneumoniae* predominated in 45% (n=1243) of cases (López-Hernández et al., 2020). However, 28% (13/42) of CPE isolated in Tunisia were represented by *Proteus mirabilis* (Maamar et al., 2019). Carbapenemase-producing species were dominated by *E. coli* in Tanzania (Mushi et al., 2014) and *Enterobacter* species in Minnesota, USA (Kim et al., 2020). In this study, the most prevalent gene was blaOXA-48 at 25%, detected in *K. pneumoniae*, *E. coli*, and *Enterobacter* spp.

Our genotypic findings are also comparable to other studies in Africa. For instance, in Senegal, all the carbapenemase genes described were class D (OXA-48, OXA-23, and OXA-51) and originated from *A. baumannii*, *K. pneumoniae*, *E. coli*, Enterobacter cloacae and *Enterobacter sakazakii* (Sekyere et al., 2016). In Morocco, the OXA-48 gene predominated, primarily found in *K. pneumoniae*, *Klebsiella oxytoca*, and *E. cloacae* (Manenzhe et al., 2015). In Algeria, the OXA-48 type was found in 98.21% of cases (Aggoune et al., 2018). However, in Tanzania, the IMP gene was the most prevalent (21.6%) (Mushi et al., 2014), as were the NDM genes in Tunisia (Maamar et al., 2019), KPC in the

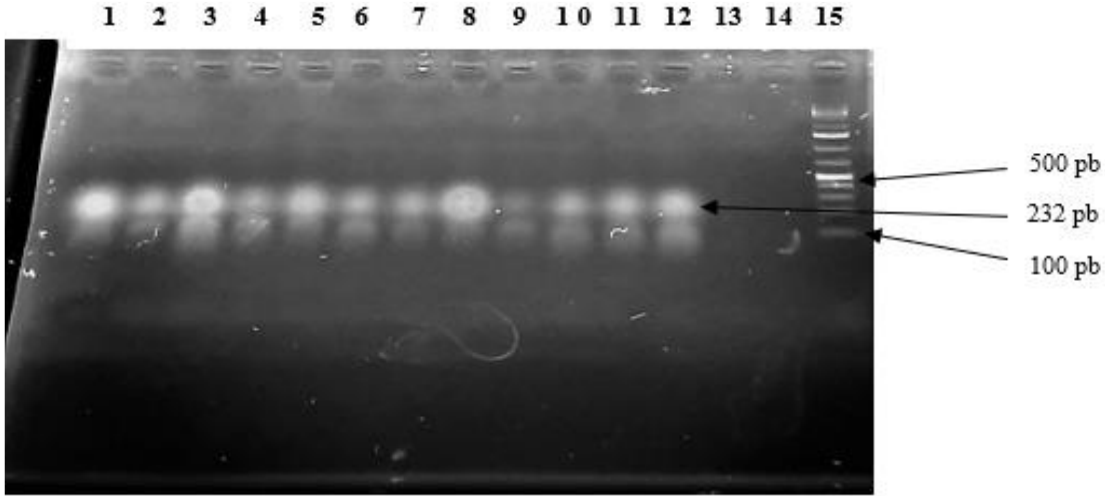

Figure 1. Electrophoretic profile of bla_{IMP} gene amplicons (232 pb).

Table 3. Distribution of carbapenemase encoding genes in different species.

Bacteria species	Genes									
	OXA-48 [n (%)]	NDM [n (%)]	VIM [n (%)]	IMP [n (%)]	KPC [n (%)]	NDM/ OXA-48 [n (%)]	IMP/OXA-48 [n (%)]	NDM/ KPC [n (%)]	OXA-48/ NDM/VIM [n (%)]	Total [n (%)]
K. pneumoniae	2 (25)	1 (12.5)	2 (25)	0	0	1 (12.5)	0	1 (12.5)	1 (12.5)	8 (50)
E. coli	2 (50)	1 (25)	0	1 (25)	0	0	0	0	0	4 (25)
K. oxytoca	0	0	0	0	1 (100)	0	0	0	0	1 (6.25)
Enterobacter spp	0	0	0	0	0	0	1 (100)	0	0	1 (6.25)
P. aeruginosa	0	0	0	1 (100)	0	0	0	0	0	1 (6.25)
A. baumannii	0	1(100)	0	0	0	0	0	0	0	1 (6.25)
Total	4 (25)	3 (18.75)	2 (12.5)	2 (12.5)	1 (6.25)	1 (6.25)	1 (6.25)	1 (6.25)	1 (6.25)	16 (100)

USA (Nora et al., 2015), and in Korea (Kim et al., 2020). These results indicate that the distribution of carbapenemase genes is variable and differs by region, consistent with data in the literature (Nordmann and Poirel, 2022).

All strains of carbapenemase-producing GNB showed a high level of resistance to most of the antibiotics tested, including amoxicillin, ceftriaxone, ertapenem, gentamicin, and ciprofloxacin. They were classified as multidrug-resistant (MDR) bacteria. Similar results have been reported elsewhere; for instance, in Algeria, carbapenemase-producing GNB exhibited resistance to cefotaxime (100%), ertapenem (98.21%), imipenem (62.5%), and amikacin (76.78%) (Aggoune et al., 2018). In Tunisia, CPE strains demonstrated very high levels of resistance to ceftriaxone (97%), ciprofloxacin (83%), norfloxacin (90%), gentamicin (98%), and amikacin (55%), with the most preserved activity observed for tigecycline (43%) and fosfomycin

(33%) (Maamar et al., 2019). The resistance rates in Ethiopia were also notably high, with 100% resistance to ampicillin and amoxicillin-clavulanic acid, and 87.5% to sulfamethoxazole-trimethoprim, 75% to cefepime, and 75% to ciprofloxacin; however, all strains were sensitive to amikacin (Saba et al., 2021). A study conducted in Norway found that all carbapenemase-producing strains were multidrug-resistant, with resistance rates of 41% to meropenem, 39% to imipenem, and 3% to ertapenem. Resistance to ciprofloxacin was 83%, and to tigecycline, it was 58%. Very high resistance was also observed in the aminoglycoside class, with resistance rates of 51% for gentamicin, 63% for amikacin, and 83% for tobramycin (Ørjan et al., 2017).

This study had some limitations. It was retrospective, which restricted the exhaustive collection of all clinical data due to missing information. Consequently, it was impossible to determine the probable risk factors associated with the acquisition of carbapenemase-producing bacteria. Only sixteen strains were included, representing all carbapenemase isolates detected during the study period, which limits the generalizability of our results. Future research should continue as part of an ongoing surveillance program to assess the geographic distribution and spread of these strains. Additionally, other mechanisms of resistance to carbapenems could not be investigated. Finally, the high rate of multidrug-resistant bacteria raises concerns about a possible nosocomial origin, which was not explored in this study.

Conclusion

The study found a relatively low proportion of carbapenemase-producing GNB. Most of the carbapenemase genes were detected in *K. pneumoniae*, with the OXA-48 gene predominating. Carbapenems, which are well tolerated and still active against most bacteria, require preservation of their efficacy. Therefore, continuous surveillance to monitor the prevalence of carbapenemase-producing GNB is essential, particularly in hospitals, to prevent the onset of epidemic outbreaks of infection and to encourage the proper use of antibiotics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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