Microbes and Infectious Diseases 2024; 5(2): 759-769

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Multi resistance to carbapenems by production of Verona integron-encoded metallo-β-lactamase (VIM) -type carbapenemases in Gram-negative bacilli isolated at Centre Hospitalier Universitaire de Tengandogo (CHU-T) and Hôpital Saint Camille de Ouagadougou (HOSCO) in Burkina Faso

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ARTICLE INFO

Article history:
Received 14 January 2024
Received in revised form 14 January 2024
Accepted 5 March 2024

Keywords:
Carbapenem resistance
Carbapenemase
BlaVIM
Gram-negative bacilli
Burkina Faso

ABSTRACT

Background: The production of metallo-beta-lactamases (MBL) such as VIM (Verona integron-encoded metallo-β-lactamase), IMP (Imipenem-resistant *Pseudomonas*) and NDM (New Delhi metallo-β-lactamase) in Gram-negative bacilli (GNB) resistant to carbapenems is a real concern for clinicians, given the therapeutic impasses involved. However, the existence of resistance genes encoding these enzymes is virtually undocumented in Burkina Faso. **Aim:** To genotypically demonstrate carbapenem resistance through the production of VIM-type carbapenemases in GNB strains collected at the Centre Hospitalier Universitaire de Tengandogo (CHU-T) and the Hôpital Saint Camille de Ouagadougou (HOSCO) in Burkina Faso. **Methods:** In this study, the resistance profile of 158 strains of GNB to imipenem, meropenem, ertapenem, doripenem and aztreonam was determined using the disc diffusion method. Resistant strains were analyzed by conventional PCR to detect blaVIM using specific primers. **Results:** Of 158 GNB strains collected, 91 (57.6%) were resistant to at least one of the carbapenems and/or aztreonam. The highest prevalence of resistant strains was observed in *Escherichia coli* (*E. coli*) 45.1% (n=41) and *Klebsiella pneumoniae (K. pneumonia)* 26.5% (n=24), which are the majority species. The blaVIM gene was detected in only 7 resistant strains (7.7%), including 3.3% (n=3/91) of *E. coli,* and 1.1% (n=1/91) of each of the species *Pseudomonas aeruginosa (P. aeruginosa)*, *Klebsiella oxytoca (K. oxytoca)*, *Proteus mirabilis (P. mirabilis)* and *Serratia marcescens (S. marcescens)*. **Conclusion:** This study established the existence of blaVIM gene, which is involved in the resistance of GNB to carbapenems through the production of VIM-type enzymes at CHU-T and HOSCO in Burkina Faso.

DOI: 10.21608/MID.2024.262512.1760

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Introduction

The antimicrobial therapy of modern medicine is threatened by a worldwide increase in resistance, especially in Gram-negative bacilli (GNB) [1]. In this antimicrobial therapy, carbapenems are used as a last resort to treat infections caused by these bacteria that are resistant to other antibiotics. However, the most worrying problem of antimicrobial resistance concerns carbapenem-resistant GNB [2], as the rapid emergence and spread of these pathogens presents a real challenge for infection management [3]. Carbapenem resistance in GNB results from the expression of enzymes called carbapenemases, that inactivate these antibiotics and/or from non-enzymatic mechanisms [4]. The production of MBLs is one of the most common enzymatic mechanisms of carbapenem resistance in clinically important GNBs, posing a serious challenge for antimicrobial therapy [5]. MBLs are Ambler class B carbapenemases [6]. Of the 94 MBL types currently identified, VIM are among enzymes which represent a growing challenge to clinical care and are identified in clinical strains of pathogenic GNB including *Enterobacteriaceae* and *P. aeruginosa* [5]. Like other MBLs, VIM-type carbapenemases hydrolyze all beta-lactams except monobactams [7]. VIM-type enzymes are the most widespread MBL in the world after NDM-type enzymes, with a prevalence between 22 and 39% [5]. The spread of these MBL is emphasis by the intra and interspecies distribution of *VIM* genes (bla_{VIM}) situated within class 1 integrons harboured on broad-host range plasmids [8].

VIM-type carbapenemases have been described in several countries and are widespread in Africa, Europe and the Middle East [9-11]. Several other studies have reported the presence of bla_{VIM} gene in West Africa in Nigeria [12, 13], in North Africa in Egypt [14, 15] and in Morocco [16]. In Burkina Faso, a few studies have also revealed that clinical strains of *Enterobacteriaceae* carried bla_{VIM} gene [17-19]. Despite the threat posed by MBLs, particularly the VIM types, to the therapeutic management of infections caused by MBL-producing germs, these studies do not allow a true estimate to be made of the extent of carbapenem resistance mediated by these enzymes in Burkina Faso. Hence, the interest of this study in reinforcing the inadequate scientific data on the molecular epidemiology of these carbapenemases in our country.

The aim of our study was to genotypically demonstrate carbapenem resistance mediated by the production of VIM-type carbapenemases in GNB strains from two hospitals in Ouagadougou, Burkina Faso.

Methods

Sampling and identification of bacterial strains

Our study was carried out in Ouagadougou, Burkina Faso. It was a retrospective study conducted on 158 bacterial strains. These strains come from previous studies and were collected from September 2018 to October 2018 and from September 2022 to August 2022 in the bacteriology section of the laboratories of the Centre Hospitalier Universitaire de Tengandogo (CHU-T) and Hôpital Saint Camille de Ouagadougou (HOSCO) in Burkina Faso. All of 158 strains were GNB resistant to third-generation cephalosporins and/or aztreonam, including 130 strains collected at HOSCO and 28 strains at CHU-T. The strains were isolated from urine, feces, pus, blood cultures, vaginal and vulvar swabs and peritoneal fluids from hospitalised and non-hospitalised patients. They were identified using API 20E tests (BioMérieux S.A., Marcy l'Etoile, France).

Antibiotic susceptibility testing

The antibiotic susceptibility of the strains was tested by antibiogram using the disc diffusion method on Mueller-Hinton agar medium (Liofilchem, Italy) in accordance with the recommendations of EUCAST/CA-SFM vs 2022 [20]. The following antibiotics: imipenem IPM (10 µg), meropenem MRP (10 µg), ertapenem ETP (10 µg), doripenem DOR (10 µg) and aztreonam ATM (30 µg) were used to identify carbapenem-resistant strains. Susceptible strains were classified as "susceptible" and intermediate-susceptible or resistant strains as "resistant" using the critical limits of inhibition diameters [20].

Molecular detection of the VIM carbapenemase gene

Bacterial DNA extraction

Bacterial DNA was extracted from isolated colonies obtained from previously strains stored in Luria Bertani storage medium, which were awakened by culturing on MH agar for 18-24h at 37°C. Bacterial DNA was extracted using the boiling method of **Dashti *et al.***[21] with a few modifications. Two to three identical colonies were suspended in 200 µl of sterile distilled water in a 1.5

ml Eppendorf tube. The resulting suspension was heated in a water bath (MEMMERT, Rost fret) at 100ºC for 15 min to release the bacterial genetic material. The heated suspension was then centrifuged at 12,000 rpm for 10 minutes (NF 048 centrifuge) to remove the DNA from the cellular debris. The supernatant containing the DNA was recovered and transferred to a new Eppendorf tube. The extracted DNA is quantified and stored at -20 or -80ºC until further use.

Molecular identification of gene encoding VIM carbapenemase

All isolates resistant to at least one carbapenem and/or aztreonam were screened by conventional PCR (Polymerase chain reaction), using forward and reverse *bla*$_{VIM}$ specific oligonucleotide primers (5'-GTTTGGTCGCATATCGCAAC-3' and 3'-AATGCGCAGCACCAGGATAG- 5') respectively with a size of 382 base pair (bp) [22]. Reaction mixture of 20 µL was composed of 4 µL Firepol® Master Mix 5X, 0.5 µL of each primer (Forward and reverse), 14 µL of PCR water and 1 µL of DNA extract. Amplification reactions were performed using the GeneAmp System PCR 9700 thermal cycler (Applied Biosystems, California, USA). The cycling protocol involved initial denaturation for 5 min at 96°C, subsequently denatured at 96°C for 30 seconds, annealed at 61°C for 30 seconds, extension step for 30 seconds at 72°C with 30 cycles, and a final extension time of 7 min at 72°C. The holding temperature was 4°C for infinity (∞). The PCR products (6 to 8 µl) were analyzed by electrophoretic migration on a 1.5% agarose gel at 100 volts for 35 min in TAE 1X buffer supplemented with ethidium bromide (1 µg/ mL). A 100 bp DNA ladder molecular weight marker (Solis Biodyne, Estonia) was used as a reference. The PCR products were viewed under ultraviolet light using the Vilber E-Box transilluminator.

Results

Bacterial strains and antibiotic susceptibility testing

Of 158 GNB isolates used for susceptibility testing tested, 91 (57.6%) were resistant to at least one carbapenem and/or aztreonam as shown in **(figure 1)**. Among the 91 resistant strains, 11 bacterial species were identified: *E. coli, K. pneumonia, P. aeruginosa, P. mirabilis, Enterobacter cloacae* (*E.cloacae*), *Enterobacter aerogenes* (*E. aerogenes*), *Citrobacter freundii* (*C. freundii*), *S. marcescens, Serratia odorifera* (*S.odrifera*), *K. oxytoca*, *Salmonella arizonae* (*S.arizonae*). **Table 1** illustrates the distribution of resistant strains by bacterial species according to area sampling. *E. coli* 45.1% (n=41) and *K. pneumoniae* 26.5% (n=24) were the predominant resistant species followed by *P. aeruginosa* 9.9% (n=9), *P. mirabilis* 4.4% (n=4) and *S. marcescens* 4.4% (n=4) **(Table 3).** Most resistant strains were from cytobacteriological examinations of urines 50.5% (n=46), stools 33% (n=30) and pus 13.2% (n=12) and from hospitalized patients 60.4% (n=55), mostly female 51.6% (n=47) were children and adults from 0 to 4 years and 25 to 64 years respectively (**Table 3**).

The distribution of bacterial species according to antibiotic resistance patterns are shown in **(table 2**). For each imipenem, meropenem and doripenem resistance rate was 22.0% (n=20), and 44.0% (n=40), 94.5% (n=86) for ertapenem and aztreonam respectively (**Table 2**). *Escherichia coli* presented more resistant strains with the highest resistance rates for aztreonam 41.8% (n=38) and ertapenem 20.9% (n=19) followed by *K. pneumoniae* with 25.3 % (n=23) and 11.0% (n=10) respectively.

Molecular detection of gene encoding VIM carbapenemase

Molecular characterization of gene encoding VIM-type carbapenemase by conventional PCR, using specific primers revealed that, out of 91 resistant isolates, 7 strains (7.7%) harboured *bla*$_{VIM}$ gene (**Table 4**) as showing DNA bands appeared approximatively at 382 bp (**Figure 2**).

VIM gene was mainly detected in *E. coli* isolates with a prevalence of 3.3% (n=3/91). Indeed, 2 *E. coli* strains with positive *bla*$_{VIM}$ gene were resistant to ertapenem and one other to all carbapenems tested. Therefore, only 1 (1.1%) strain of *P. aeruginosa*, *K. oxytoca*, *P. mirabilis*, *S. marcescens* was harbored the resistance gene. *P. aeruginosa* strain and *K. oxytoca* strain were resistant respectively to imipenem and to all carbapenems; while *bla*$_{VIM}$ gene-positive strains of *P. mirabilis*, *S. marcescens* were sensitive to all carbapenem. However, *bla*$_{VIM}$ was not found in any strain of *K. pneumoniae* (26.4%; n=24), *E. cloacae* (3.3%; n=3), *E. aerogenes* (1.1%; n=1), *C. freundii* (2.2%; n=2), *S. odorifera* (1.1%; n=1) and *S. arizonae* (1.1%; n=1) (**Table 4**). Bacterial strains carrying *bla*$_{VIM}$ were only isolated from urine

(57.1%; n=4/7), stool (14.3%; n=1/7) and pus (28.6%; n=2/7) specimens, which were mostly collected from women (71.4%; n=5/7) whose were mainly hospitalized patients.

As for correlation between carbapenem resistance and resistance gene detection, prevalence of *bla*$_{VIM}$ gene in carbapenem-resistant strains was for: ertapenem (57.1%; n=4/7), imipenem (42.9%; n=3/7), meropenem (28.6%; n=2/7) and doripenem (28.6%; n=2/7).

Table 1. Frequencies of bacterial resistant strains according to collection sites.

Bacterial species	Area sampling		
	HOSCO n (%)	**CHUT n (%)**	**Total n (%)**
Escherichia coli	31 (34,1)	10 (11,0)	41 (45,1)
Klebsiella pneumoniae	21 (23,1)	3 (3,3)	24 (26,4)
Pseudomonas aeruginosa	9 (9,9)	0	9 (9,9)
Proteus mirabilis	4 (4,4)	0	4 (4,4)
Enterobacter cloacae	2 (2,2)	1 (1,1)	3 (3,3)
Enterobacter aerogenes	1 (1,1)	0	1 (1,1)
Citrobacter freundii	2 (2,2)	0	2 (2,2)
Serratia marcescens	3 (3,3)	1 (1,1)	4 (4,4)
Serratia odorifera	1 (1,1)	0	1 (1,1)
Klebsiella oxytoca	0	1 (1,1)	1 (1,1)
Salmonella arizonae	0	1 (1,1)	1 (1,1)
Total	74 (81,3)	17 (18,7)	91 (100)

n= number of resistant strains of each bacterial species; %= proportion of resistant strains of each bacterial species, HOSCO = Saint Camille Hospital Center in Ouagadougou; CHU-T = Tengandogo University Hospital Center.

Table 2. Distribution of bacterial species according to antibiotic resistance patterns.

Bacterial species	**IPM^{R} (n'/%)**	**MRP^{R} (n'/%)**	**ETP^{R} (n'/%)**	**DOR^{R} (n'/%)**	**ATM^{R} (n'/%)**
Escherichia coli (n=41)	7 (7,7)	7 (7,7)	19 (20,9)	8 (8,8)	38 (41,8)
Klebsiella pneumoniae(n=24)	4 (4,4)	7 (7,7)	10 (11,0)	5 (5,5)	23 (25,3)
Pseudomonas aeruginosa (n=9)	7 (7,7)	2 (2,2)	6 (6,6)	4 (4,4)	9 (9,9)
Proteus mirabilis (n=4)	0	0	1 (1,1)	0	4 (4,4)
Enterobacter cloacae (n=3)	1 (1,1)	1 (1,1)	1 (1,1)	0	2 (2,2)
Enterobacter aerogenes (n=1)	0	0	0	0	1 (1,1)
Citrobacter freundii (n=2)	0	0	0	0	2 (2,2)
Serratia marcescens (n=4)	0	1 (1,1)	1 (1,1)	1 (1,1)	4 (4,4)
Serratia odorifera (n=1)	0	0	0	0	1 (1,1)
Klebsiella oxytoca (n=1)	1 (1,1)	1 (1,1)	1 (1,1)	1 (1,1)	1 (1,1)
Salmonella arizonae (n=1)	0	1 (1,1)	1 (1,1)	1 (1,1)	1 (1,1)
Total	20 (22,0)	20 (22,0)	40 (44,0)	20 (22,0)	86 (94,5)

R= Resistant; n= strain number of each bacterial species; n'= number of resistant strains of each bacterial species to antibiotic overall of the total of strains; %= percent correlated to the total number of resistant strains bacterial; IPM= Imipenem, MRP= Meropenem, ERT= Ertapenem, DOR = Doripenem, ATM = Aztreonam

Table 3. Distribution of resistant bacterial species according to biological samples, sex and hospitalized status of patient.

Characteristics	Bacterial species											Total (n/%)
	E. coli	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Proteus mirabilis***	***Enterobacter cloacae***	***Enterobacter aerogenes***	***Citrobacter freundii***	***Serratia marcescens***	***Serratia odorifera***	***Klebsiella oxytoca***	***Salmonella arizonae***	
Specimen (n/%)												
Urine	21 (23,1)	14 (15,4)	4 (4,4)	1 (1,1)	1 (1,1)	0	1 (1,1)	2 (2,2)	1 (1,1)	1 (1,1)	0	46 (50,6)
Feces	12 (13,2)	8 (8,8)	1 (1,1)	3 (3,3)	2 (2,2)	1 (1,1)	1 (1,1)	2 (2,2)	0	0	0	30 (33,0)
Pus	6 (6,6)	1 (1,1)	4 (4,4)	0	0	0	0	0	0	0	1 (1,1)	12 (13,2)
Blood	1 (1,1)	0	0		0	0	0	0	0	0	0	1 (1,1)
Vaginal swab	1 (1,1)	0	0	0	0	0	0	0	0	0	0	1 (1,1)
Vulvaire swab	0	1 (1,1)	0	0	0	0	0	0	0	0	0	1 (1,1)
Total	41 (45,1)	24 (26,4)	9 (9,9)	4 (4,4)	3 (3,3)	1 (1,1)	2 (2,2)	4 (4,4)	1 (1,1)	1 (1,1)	1 (1,1)	91 (100)
Sex (n/%)												
Female	18 (19,8)	14 (15,4)	5 (5,5)	4 (4,4)	0	0	2 (2,2)	2 (2,2)	0	1 (1,1)	1 (1,1)	47 (51,6)
Male	17 (18,7)	6 (6,6)	4 (4,4)	0	3 (3,3)	0	0	1 (1,1)	1 (1,1)	0	0	32 (35,2)
None	6 (6,6)	4 (4,4)	0	0	0	1 (1,1)	0	1 (1,1)	0	0	0	12 (13,2)
Total	41/45,1	24 (26,4)	9 (9,9)	4 (4,4)	3 (3,3)	1 (1,1)	2 (2,2)	4 (4,4)	1 (1,1)	1 (1,1)	1 (1,1)	91 (100)
Hospitalisation (n/%)												
Yes	25 (27,5)	15 (16,5)	5 (5,5)	4 (4,4)	2 (2,2)	0	1 (1,1)	1 (1,1)	1 (1,1)	1 (1,1)	0	55 (60,4)
No	9 (9,9)	6 (6,6)	4 (4,4)	0	1 (1,1)	1 (1,1)	1 (1,1)	2 (2,2)	0	0	0	24 (26,4)
None	7 (7,7)	3 (3,3)	0	0	0	0	0	1 (1,1)	0	0	1 (1,1)	12 (13,2)
Total (n/%)	41 (45,1)	24 (26,4)	9 (9,9)	4 (4,4)	3 (3,3)	1 (1,1)	2 (2,2)	4 (4,4)	1 (1,1)	1 (1,1)	1 (1,1)	91 (100)

Table 4. Distribution of *VIM* gene according to resistant bacterial species.

Bacterial species	*VIM* gene '+' (n/%)	*VIM* gene '-' (n/%)	Total of resistant strains
Escherichia coli	3/3,3	38/41,8	41/45,1
Klebsiella pneumoniae	0	24/26,4	24/26,4
Pseudomonas aeruginosa	1/1,1	8/8,8	9/9,9
Proteus mirabilis	1/1,1	3/3,3	4/4,4
Enterobacter cloacae	0	3/3,3	3/3,3
Enterobacter aerogenes	0	1/1,1	1/1,1
Citrobacter freundii	0	2/2,2	2/2,2
Serratia marcescens	1/1,1	3/3,3	4/4,4
Serratia odorifera	0	1/1,1	1/1,1
Klebsiella oxytoca	1/1,1	0	1/1,1
Salmonella arizonae	0	1/1,1	1/1,1
Total	7/7,7	84/92,3	91/100

n= number of resistant strains of each bacterial species harbouring or not gene resistance; '+'indicating gene detected; '-'= indicating gene not detected; %= percent correlated to the total number resistant strains bacterial.

Figure 1. Petri dishes showing resistance of strain to antibiotics tested.

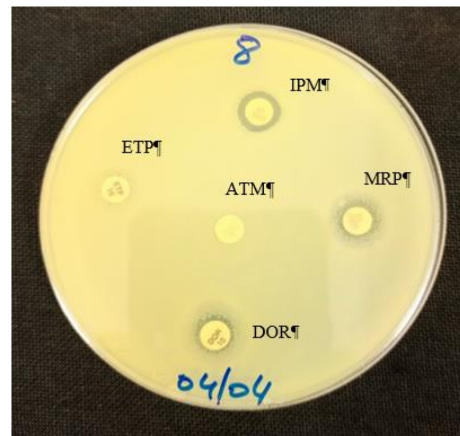
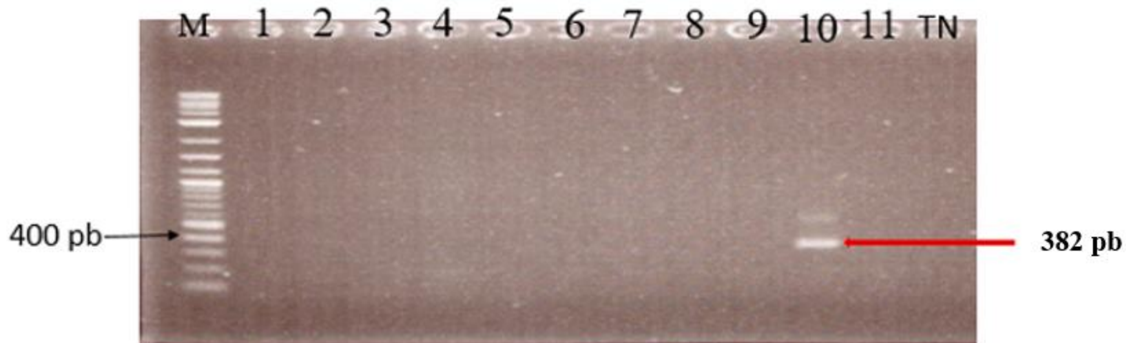


Figure 2. Agarose gel electrophoresis of blaVIM gene amplicons (382 bp). M= molecular weight marker, DNA ladder 100 pb (Solis Biodyne, Estonia); TN= negative control; Lanes 1-11 correspond to strains samples; Lane 10 corresponds to a strain with positive VIM carbapenemase gene.



Discussion

Currently, the proliferation of carbapenemases constitutes the uppermost pressing problem in GNB [23]; as MBL production has become the most common mechanism of carbapenem resistance in clinically relevant GNB and represents a major challenge for antimicrobial therapy [5]. Despite the threat posed by resistance to carbapenems through MBL production in clinically important GNB, data on the epidemiology of genes encoding these carbapenem resistance enzymes are still lacking in Africa and particularly in Burkina Faso. The present study was therefore undertaken to help improve epidemiological data on MBL-type carbapenemase resistance genes, with a view to better monitoring of carbapenem-resistant GNB in our country.

The aim of the study was to genotypically demonstrate carbapenem resistance through the production of VIM-type carbapenemases in GNB from two hospitals, the Centre Hospitalier Universitaire de Tengandogo (CHU-T) and the Hôpital Saint Camille de Ouagadougou (HOSCO) in Burkina Faso. Overall 158 GNB isolates collected, 57.6% (n=91) of them were carbapenem-resistant including *E. coli* (45.1%, n=41), *K. pneumoniae* (26.5%, n=24) which were the predominant species (**Table 1**). Similar results were also reported in Cameroon [24] and Ghana [25], where the most prevalent species were *E. coli* (50% and 46%) and *K. pneumoniae* (37.5% and 17%) respectively. In Burkina Faso, **Kabore *et al.***[26] made the same observation but with a higher rate for *E. coli* (82.69%), which was more resistant to antibiotics including carbapenems, and a lower rate for *K. pneumoniae* (9.62%). Furthermore, resistant strains in our study were mostly isolated from urine (50.5%, n=46), including 23.1% (n=21) of *E. coli*; urine specimens were predominantly originated from female patients (51.6% n=47) and hospitalized

(60.4%, n=55). This higher prevalence of *E. coli* resistant strains could be explained by the predominance of urine specimen and by fact that *E. coli* is known as the bacterial specie most incriminated in urinary tract infections [24]. **Haji *et al.***[23] have also observed that urine was the dominant type of biological samples (62%) and originated from women (60%). However, **Balkhair *et al.***[27] have reported a lowest carbapenem resistance rate for *E. coli* (2.9%) and slightly higher rates at 46.4% and 29.9% for *K. pneumoniae* and *P. aeruginosa* respectively. Furthermore, the prevalence of carbapenem resistance in our study (57.6%) was higher than those (30.9%, 37%, 27.7%) observed respectively by **Haji *et al.***[23], **Armin *et al.***[28] and **Balkhair *et al.***[27].

Concerning the antibiotic susceptibility profiles, our bacterial strains showed slightly high carbapenem resistance level with rates at 44.0% for ertapenem and 22.0% for imipenem, meropenem and doripenem (**Table 2**). Similar resistance rates to ertatpenem (40.0%) and imipenem (23.0%) in *Enterobacterales* were reported in Morocco by **Belouad *et al.***[29]. Moreover, **Djuikoue *et al.***[24] in Cameroon have recorded lower levels resistance in *Enterobacterales* with rates at 14.4%, 13.8% and 7.5% respectively for imipenem, ertapenem and meropenem. However, **Haji *et al***.[23] have observed slightly higher resistance rates for imipenem (41%) and meropenem (40%) and a slightly low resistance level for ertapenem (36%). The differences observed between the resistance rates in our study and those in the other studies could be explained partly by the size of the samples and partly by the method of phenotypic detection of antibiotic resistance. In our study, any strain with reduced sensitivity to at least one of carbapenems was categorized resistant and suspected as a carbapenemase-producing strain in accordance with the CA-SFM vs 2020 [30]. This detection of carbapenem-resistant strains was carried out using manual disc diffusion method [20], which is not always easy used to demonstrate resistance to carbapenems [19]. On the other hand, in the study by **Haji *et al.***[23], the resistance profile of bacterial strains to carbapenems was determined using an automated method [31], making it possible to improve resistance detection.

All bacterial isolates resistant to carbapenem and/or to aztreonam therefore suspicious of carbapenemase production, were screening by molecular approaches to detect *VIM* gene. In our study, bla_{VIM} was found in 7 isolates (7.7%, n=7/91) resistant to carbapenems and/or aztreonam including 3 strains of *E. coli* (3.3%, n=3/91) and 1 strain (1.1%, n=1/91) for each species *P. aeruginosa*, *K. oxytoca*, *P. mirabilis*, *S. marcescens* (**Table 4**). This study is not the first to report the presence of VIM-type carbapenemase resistance genes in Burkina Faso. Some previous studies by **Sanou *et al.***[17], **Dembele *et al.***[18], **Kabore *et al.***[19] and **Ouattara *et al.***[32] in Burkina Faso have also identified bla_{VIM} gene in 1, 2, 2 and 4 strains of *E. coli* resistant to carbapenems, respectively. Whereas, in our study, carbapenemase-VIM encoding gene was identified not only in *E. coli*, but also in other *Enterobacteriaceae* (**Table 4**) and then in *P. aeruginosa*. Likewise, to the best of our knowledge, our study is one of the first in our country to record detection of bla_{VIM} gene in *K. oxytoca*, *P. mirabilis*, *S. marcescens*, *P. aeruginosa* strains resistant to carbapenems at CHU-T and HOSCO in Burkina Faso. Furthermore, our low bla_{VIM} detection rates, in agreement with those of these previous findings, might underlined a weak spread of VIM-like enzymes in carbapenem-resistant GNB in our country. A surveillance study of infections related to carbapenem-resistant Enterobacterales and *P. aeruginosa* in Africa, from the ATLAS program, has also recorded a low detection rate of bla_{VIM} at 7.0% (n=11/156), including 3 strains of *E. coli* [9]. Then, our findings were corroborated by those of **Estabrook *et al.***[11] according to which bla_{VIM} was identified only in 4.7% (n=9/94) of meropenem-nonsusceptible *Enterobacterales* strains from Africa, collected as part of a global surveillance study. However, our carbapenem-resistant strains frequency (57.6%, n=91/158) and bla_{VIM} detection rate (7.7%, n=7/91) were discordant with the findings of **Haji *et al.***[23] in Iraq, who have recorded lower carbapenem resistance rate 30.9% (n=34/110) and higher prevalence of *VIM* gene 49% (n=26/53).

Of interest, *P. mirabilis* (n=1) and *S. marcescens* (n=1) strains with positive bla_{VIM} gene, were sensitive to all carbapenems in our study. Other authors had also reported some cases of GNB strains susceptible to carbapenems, which were positive to bla_{VIM} gene and to other carbapenemase-encoding gene such as bla_{NDM}, bla_{IMP}, bla_{KPC}, bla_{GES}, $bla_{\mathrm{OXA\text{-}48}}$ [33, 34]. Such results could be justified by the fact that resistance genes not expressed in these carbapenem-sensitive strains would not have been detected by phenotypic tests,

thus favouring their silent spread in hospital and community environments [34].

Finally, among carbapenem-resistant strains of *K. pneumoniae* (26.4%; n=24), *E. cloacae* (3.3%; n=3), *E. aerogenes* (1.1%; n=1), *C. freundii* (2.2%; n=2), *S. odorifera* (1.1%; n=1) and *S. arizonae* (1.1%; n=1) (**Table 4**), none strain has harboured VIM-type carbapenemase-encoding gene. The non-detection of bla_{VIM} in carbapenem-resistant strains in our study was also reported in a study by **Belouad *et al.***[29] in Morocco, who have not detected *VIM* gene in carbapenem-resistant *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, *E. cloacae*). In this Moroccan study, none bla_{VIM} gene was not found in these carbapenem-resistant *Enterobacteriaceae*, but bla_{OXA-48} and bla_{NDM} genes were predominantly detected. Likewise, previous studies in our country [17-19, 35] have recorded the presence of NDM, IMP, OXA-48, KPC, GES carbapenemase-encoding genes involved in carbapenem resistance in clinical GNB isolates. Thus, these resistance genes not researched in our study might justified carbapenem resistance in our *VIM* gene-negative isolates. Further, these bacterial isolates with negative bla_{VIM} would exhibited non-enzymatic carbapenem resistance mechanisms such as modification or loss of porins [36, 37] and over-expression of efflux pumps [37], and then being non-susceptible to carbapenems.

Conclusion

In addition to the existence of numerous extended-spectrum beta-lactamases (ESBLs) sufficiently described in multi-resistant enterobacteria in Burkina Faso, the emergence and spread of carbapenemase-producing GNB in hospital and community settings is another real public health problem in our country. Our study revealed the presence of bla_{VIM} gene encoding VIM-type carbapenemases in carbapenem-resistant GNB of clinical interest from HOSCO and CHU-T in Ouagadougou, Burkina Faso. Carbapenemases of the VIM type, as well as enzymes of the NDM, IMP, KPC and OXA-48 types, cause therapeutic impasses that increase healthcare costs and mortality. Consequently, national epidemiological surveillance is needed to prevent and control the spread of these resistance genes in our country. We therefore recommend this will be made possible by setting up platforms for phenotypic detection and genotypic characterization of these carbapenemases in both public and private medical biology laboratories.

Acknowledgements

Our thanks to all managers and patients of the two hospitals whose sampling was done, to the staff of LABIOGENE/CERBA who actively contributed to accomplishment of this work, to Joseph Ki-Zerbo University and University of Dedougou in Burkina Faso, which have authorized this study.

Conflicts of interests

The authors declare having no competing interests.

Financial disclosure

The authors report no funding.

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