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Multi-resistance to carbapenems by the production of Imipenemase (IMP)-types carbapenemases in Gram-negative bacilli in Burkina Faso

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The growing prevalence of Imipenemase (IMP) metallo-beta-lactamases (MBL) producing strains of Gram-negative bacilli (GNB) pathogens is a real concern for clinicians in the light of therapeutic impasses driven. However, resistance genes encoding these enzymes are hardly documented in Burkina Faso. This study aims to show carbapenem-resistance mediated by the production of IMP-type carbapenemase in GNB clinical strains collected in Ouagadougou, Burkina Faso. Strains resistance profile to imipenem, meropenem, ertapenem, doripenem and aztreonam was determined by the disk diffusion method. Classical polymerase chain reaction (PCR) was carried out to detect β-lactamase (*bla*) gene of IMP in resistant strains. Out of 158 GNB collected, 91 (57.6%) were resistant to at least one of the carbapenems and/or to aztreonam. The highest prevalence of resistant strains was observed in *Escherichia coli* (45.1%; n=41) and *Klebsiella pneumoniae* (26.5% n=24). Among 32 resistant strains *bla*$_{IMP}$ gene positive (35.2%), *Escherichia coli* was the predominant species carrying resistance gene (18.7%, n=17/91). The findings strengthen the scarce existing scientific data on antimicrobial resistance mediated by metallo-beta-lactamases (MBL) in Burkina Faso.

Key words: Carbapenem resistance, carbapenemase, blaIMP, Gram-negative bacilli, Burkina Faso.

INTRODUCTION

Antibiotic therapy aims to kill pathogen bacteria. Unfortunately, these bacteria to protect themselves develop resistance to antibiotics used to treat them. Bacteria resistant to several types of antibiotics are sometime referred to as multi-resistant. Increased and inappropriate use of antimicrobial drugs promotes the emergence of antimicrobial resistant bacterial strains and related infections (Masoud et al., 2021; Kim et al., 2023). Some of those bacteria have become resistant to several antibiotics, including carbapenems and third-generation cephalosporins (Palacios-Baena et al., 2021; Arumugham et al., 2022). The emergence and spread of multidrug-resistant Gram-negative bacilli (MDR) has become a major public health concern worldwide (Dembele et al., 2020). The spread of MDR Gram-negative bacilli is increasingly reported in both hospital and community

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settings worldwide (Manenzhe et al., 2015). In the last decade, an alarming increase in the prevalence of carbapenemase-producing Gram-negative bacilli of serious nosocomial infections has been shown worldwide (Bourafa et al., 2018; Haji et al., 2021). Indeed, carbapenems were considered as last line drugs for controlling multidrug-resistant Gram-negative pathogens including extended-spectrum beta lactamases (ESBL)-producing Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* (Haji et al., 2021; Ibrahim et al., 2021; Dikoumba et al., 2023). However, over-prescribing of carbapenems has led to emergence and global dissemination of carbapenem-aresistant organisms, including carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *A. baumanii* (Aruhomukama et al., 2019; Haji et al., 2021) which are on World Health Organization (WHO) list of critical priority 1 pathogens of urgent need for new antimicrobial development (Tacconelli et al., 2018). Carbapenemase enzymes are the most common mechanism by which resistance to carbapenems occurs in these GNB (Shaker et al., 2018; Aruhomukama et al., 2019; Dziri et al., 2020; Haji et al., 2021). Several types of carbapenemases have been described. However, KPC (*K. pneumonia* carbapenemase, class A carbapenemase), class B metallo-beta-lactamases (New Dehli metallo-beta-lactamase-NDM, Verona Integron-encoded Metallo-beta-lactamase-VIM, Imipenemase-IMP) and OXA-48 and its variants (class D carbapenemase) are the most predominant carbapenemases in carbapenem-resistant GNB (Halat and Moubareck, 2020; Owusu et al., 2023). Imipenemases (IMPs), Verona Integron-encoded Metallo-β-lactamases (VIMs) and New Delhi Metallo-β-lactamase (NDMs) are the three MBLs types with an increasing challenge for clinical care and were identified in many human pathogens including Enterobacteriaceae and *P. aeruginosa* (Yang et al., 2023). The genes encoding these MBLs are genetically located within a variety of integrons, where they have been incorporated as gene cassettes (Halat and Moubareck, 2020), and these integrons associated with plasmids or transposons facilitated their spread by horizontal transfert between bacteria (Diene and Rolain, 2014). IMPs enzymes, subject of this study, are able to hydrolyse nearly all beta-lactams, specifically the carbapenems (Li et al., 2023). Many IMPs variants are involved in carbapenem resistance. The first genetically transferable IMP-1 was identified in *P. aeruginosa* and *Serratia marcescens* in Japan in 1990 (Watanabe et al., 1991). Currently, at least 91 IMP variants have been discovered worldwide (Li et al., 2023) including IMP-68 in Japan (Kubota et al., 2019) and IMP-89, IMP-91 and IMP-96 recently identified in Chinese clinical isolates (Li et al., 2023). Thus, South East Asian region remains the largest reservoir of IMP-type MBL (Halat and Moubareck, 2020; Li et al., 2023). However, these MBL have been reported in various other countries: Italy and Portugal (Cornaglia et al., 1999; Riccio et al., 2000), USA (Limbago et al., 2011), Australia (McCarthy et al., 2017) and Lebanon (Halat et al., 2017). In Africa, IMP enzymes had been in Egypt (Masoud et al., 2021; Benmahmod et al., 2019; Abbas et al., 2019), Morocco (Barguigua et al., 2013), Tunisia (Chouchani et al., 2011; Dziri et al., 2020), Tanzania (Mushi et al., 2014) and Sudan (Adam and Elhag, 2018). In Burkina Faso, most of the studies were much focused on ESBL-producing Enterobacteriaceae which occurs resistant to beta-lactam (Metuor Dabire et al., 2013; Metuor et al., 2019a; Metuor Dabire et al., 2019b; Tiemtore et al., 2022) at the expense of carbapenemases, mainly MBL despite the threat they represent for heath care. Nevertheless, previous studies (Sanou et al., 2020; Dembele et al., 2021; Kabore et al., 2022, 2023) have reported resistance to carbapenems in GNB by the production of carbapenemases. Although, bla_{IMP-2} gene was detected in *E. coli* clinical isolate from rural settings by Dembele et al. (2021); no other study, to the researchers knowledge, have reported IMP-type carbapenemase gene in urban settings, especially in hospital settings. Thus, this study aimed to report carbapenems multi-resistance mediated by IMP-type carbapenemase producing in GNB from urban hospitals in Burkina Faso.

METHODS

Sample and bacterial isolates identification

This study was conducted in Ouagadougou, Burkina Faso. This was a cross-sectional study with retrospective data collection of 158 bacterial strains which were collected from September 2018 to October 2018 and from September 2022 to August 2022 in Bacteriology Department of the Laboratories of University Hospital Center of Tengandogo (CHU-T) and of Saint Camille Hospital of Ouagadougou (HOSCO) in Burkina Faso. These bacterial samples were GNB strains resistant to at least one third generation cephalosporin and/or aztreonam, including 130 isolates from HOSCO and 28 others from CHU-T. They were isolated in the following specimens: Urine, stool, pus, blood cultures, vaginal and vulvar swabs and peritoneal fluids collected from outpatients and hospitalized patients. API 20E gallery tests (BioMerieux S.A., Marcy Etoile, French) were used for bacterial species identification.

Antibiotic susceptibility testing

Disk diffusion method on Mueller-Hinton (MH) agar (Liofilchem, Italia) was used to perform the antibiotic susceptibility test of the strains, while following the recommendations the Antibiogram Committee of the French Microbiology Society/European Committee for Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) versus 2022 (EUCAST/CASFM, 2022). The following antibiotics, purchased from Liofilchem (Italia) were used to carry out carbapenem-resistant strains: Imipenem IPM (10 µg), Meropenem MRP (10 µg), Ertapenem ETP (10 µg), Doripenem DOR (10 µg) and Aztreonam ATM (30 µg). Susceptible strains were categorized «Sensitive, S» and strains with intermediate susceptibility or resistant were classified «Resistant, R», using critical limits of inhibition diameters according to CA-SFM vs 2022 guidelines (EUCAST/CASFM, 2022).

Table 1. Primers used for *bla*$_{IMP}$ gene detection.

Researched gene	Primers	Sequence (5'- 3')	Size (pb)	Reference
bla$_{IMP}$	IMP-F	CATGGTTTGGTGGTTCTTGT	488	Huang et al. (2012)
	IMP-R	ATAATTTGGCGGACTTTGGC		

Molecular detection of gene encoding IMP-type carbapenemase

Extraction of bacterial DNA

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 to 24 h at 37°C. Bacterial DNA extraction was done by the boiling method (Dashti et al., 2009) with few modifications. A total of 2 to 3 identical colonies were suspended in 200 µL of distilled sterile water in 1.5 mL labeled Eppendorf tube. The resulting suspension was boiled at 100°C for 15min in water bath (MEMMERT, Rost fret) to release bacterial genetic material and then centrifuged (NF 048 centrifuge) at 12000 rpm for 10 min to remove genetic material from others debris. The supernatant containing DNA was transferred to a new Eppendorf tube. DNA extract was assayed for quantity and purity, using NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, United States) and then stored at -20°C for further usage.

Molecular identification of gene encoding IMP carbapenemase

All isolates resistant to at least one carbapenem and/or aztreonam were screened by conventional PCR (Polymerase Chain Reaction), using specific oligonucleotide primers (Table 1) to gene encoding beta-lactamase of *bla*$_{IMP}$ family. 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 GeneAmp System PCR 9700 Thermal Cycler (Applied Biosystems, California, USA) according to PCR program shown in Table 2.

After amplification, 6 to 8 µL of each PCR product were separated by 1.5% agarose gel electrophoresis at 100 volts for 35 min in TAE 1X buffer mixed to ethidium bromide (1 µg/mL) in migration tank. A molecular weight marker, DNA ladder (Solis Biodyne, Estonia) 100 pb was used as a reference. Amplified DNA bands were visualized using a UV transilluminator (E-Box Vilber).

RESULTS

Bacterial strains and antibiotic susceptibility testing

In this study, 57.6% (91/158) GNB strains resistant to at least one carbapenem and/or aztreonam were detected (Figure 1). Among the 91 resistant strains, 11 bacterial species were identified and the majority of strains 81.3% (n=74) were from Saint Camille Hospital of Ouagadougou (Table 3). *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.

The distribution of bacterial species according to antibiotic resistance patterns are shown in Table 4. The resistance rates observed were: 94.5% (n=86) for aztreonam, 44.0% (n=40) for ertapenem and 22.0% (n=20) for each imipenem, meropenem and doripenem. For ertapenem, the carbapenem with the highest resistance, *E. coli* strains recorded the highest level of resistance (20.9%, n=19/91) followed by *K. pneumoniae* (11%, n=10/91) and *P. aeruginosa* (6.6%, n=6/91). On the other hand, the susceptibility of the isolates to antibiotics revealed that almost all strains were highly resistant to aztreonam.

Molecular detection of gene encoding IMP carbapenemase

Molecular characterization of gene encoding IMP-type carbapenemase performed by conventional PCR, using specific primers (Table 1) revealed that, out of 91 resistant isolates, 32 strains (35.2%) harbored *bla*$_{IMP}$ gene as showing DNA bands appeared approximatively at 488 pb (Figure 2).

More than half of the bacterial strains carrying bla$_{IMP}$ gene were *E. coli* (18.7%, n=17/91) as indicated in Table 5 showing resistance gene distribution according to resistant bacterial species. Also, most of resistant isolates positive to carbapenemase-IMP encoding gene were recovered from urines samples (46.9%, n=15/32) collected from patients predominantly hospitalized (71.9%, n=23/32) and female (50%, n= 16/32). As regard correlation between carbapenem resistance profile and bla$_{IMP}$ gene detection, ertapenem non-susceptible strains exhibited the highest prevalence of *IMP* gene (68.8%, n=22/32), compared to gene detection rates in isolates resistant to other carbapenems: 25.0% (n=8/32) for doripenem and 31.3% (n=10/32) each for imipenem and meropenem.

DISCUSSION

Carbapenems over-prescribing has led to emergence and global increased dissemination of carbapenem-resistant bacteria which reduced the effectiveness of these antibiotics in the treatment of related infections

Table 2. PCR program for *bla*$_{IMP}$ gene detection.

Amplification step	Temperature/duration
Initial denaturation	96°C / 5 min
Denaturation	96°C / 30 s
Hybridization	54°C / 30 s
Elongation	72°C / 30 s
Final elongation	72°C / 7 min
Number of cycles	30

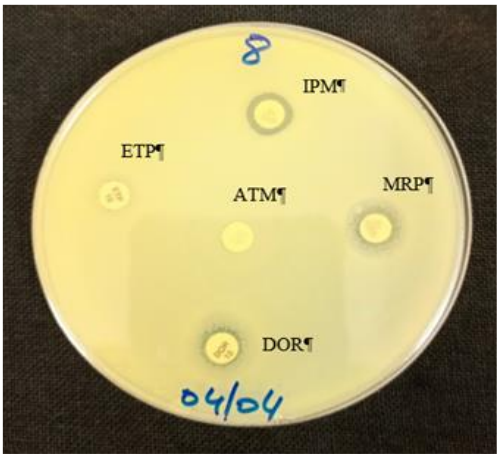

Figure 1. Petri dishes showing resistance of strain to antibiotics tested.

Table 3. Frequencies of bacterial resistant strains according to area sampling.

Bacterial species	Area sampling		Total (n/%)
	HOSCO (n/%)	CHUT (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.

(Aruhomukama et al., 2019; Haji et al., 2021; Das, 2023). An alarming increase in the prevalence of carbapenemase-producing Gram-negative bacilli of serious nosocomial infections has been reported worldwide (Bourafa et al., 2018; Haji et al., 2021).

This current study was undertaken to show

Table 4. 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'/%)
E. coli (n=41)	7 (7.7)	7 (7.7)	19 (20.9)	8 (8.8)	38 (41.8)
K. pneumoniae (n=24)	4 (4.4)	7 (7.7)	10 (11.0)	5 (5.5)	23 (25.3)
P. aeruginosa (n=9)	7 (7.7)	2 (2.2)	6 (6.6)	4 (4.4)	9 (9.9)
P. mirabilis (n=4)	0	0	1 (1.1)	0	4 (4.4)
E. cloacae (n=3)	1 (1.1)	1 (1.1)	1 (1.1)	0	2 (2.2)
E. aerogenes (n=1)	0	0	0	0	1 (1.1)
C. freundii (n=2)	0	0	0	0	2 (2.2)
S. marcescens (n=4)	0	1 (1.1)	1 (1.1)	1 (1.1)	4 (4.4)
S. odorifera (n=1)	0	0	0	0	1 (1.1)
K. oxytoca (n=1)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)
S. 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.

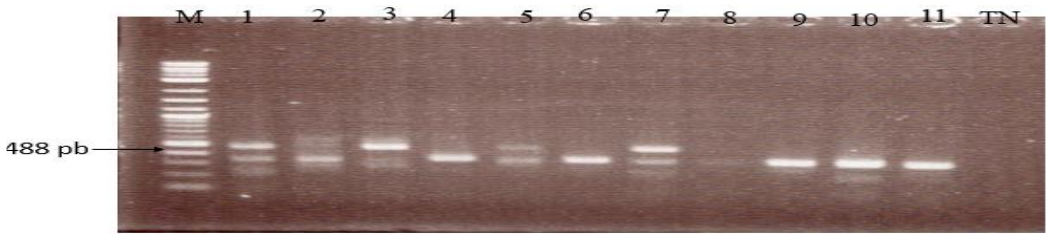

Figure 2. Agarose gel electrophoresis of bla$_{IMP}$ gene amplicons (488 bp). M= molecular weight marker, DNA ladder 100 pb (Solis Biodyne, Estonia); TN = negative control; Lanes 1-11 correspond to strains with positive IMP carbapenemase gene.

carbapenem resistance in GNB by production of IMP-type carbapenemase from urban hospitals centers in Ouagadougou, Burkina Faso. Overall 158 GNB strains collected in HOSCO and in CHU-T, 57.6% (n=91) of them were carbapenem-resistant including *E. coli* (45.1%, n=41) and *K. pneumoniae* (26.5%, n=24) which were the predominant resistant species followed by *P. aeruginosa* (9.9%, n=9). In Ghana, Owusu et al. (2023) reported these similar findings, where *E. coli* and *K. pneumoniae* were the predominant GNB species at 46% (n=83) and 17% (n=30), respectively. Furthermore, studies conducted in other countries have reported both same species with the most carbapenem-resistant strains (Haji et al., 2021; Armin et al., 2023). The results are in agreement with the study in Burkina Faso by Kabore et al. (2022) who reported that GNB is mainly more resistant to antibiotics including carbapenems were *E. coli* and *K. pneumoniae*, but at higher rate (82.69%) and lower rate (9.62%) respectively. In contrast, Balkhair et al. (2023) found a lowest resistance rate to carbapenems for *E. coli* (2.9%) and a higher carbapenem level resistance at 46.4 and 29.9% respectively for *K. pneumoniae* and *P. aeruginosa*. One the other hand, this study carbapenem-resistant strains prevalence (57.6%, n=91/158) was higher than those (30.9, 37 and 27.7%) observed respectively by Haji et al. (2021), Armin et al. (2023) and Balkhair et al. (2023). Indeed, any strain exhibiting reduced sensitivity to at least one of carbapenems and/or aztreonam was categorized resistant and therefore was suspicious of carbapenemase production. This fact and sample size would explain the prevalence of carbapenem-resistant strains. The majority of the *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates were originated from urine (50.5%) collected mainly from female patients (51.6%). Also, Haji et al. (2021) observed urine samples were the dominant type (62%) from which these species were isolated.

Regarding antibiotic resistance profile, the strains showed slightly high resistance level to carbapenems (Table 4). Thus, imipenem with 22.0% as resistance rate, is currently one the most effective carbapenem as reported by Masoud et al. (2021). However, Haji et al. (2021) have recorded slightly higher resistance rates for imipenem (41%) and meropenem (40%) and a slightly low resistance level for ertapenem (36%). In this study,

Table 5. Distribution of *IMP* gene according to resistant bacterial species.

Bacterial species	*IMP* gene '+' (n/%)	*IMP* gene '-' (n/%)	Total
E. coli	17 (18.7)	24 (26.4)	41 (45.1)
K. pneumonia	6(6.6)	18 (19.8)	24 (26.4)
P. aeruginosa	3 (3.3)	6 (6.6)	9 (9.9)
P. mirabilis	1 (1.1)	3 (3.3)	4 (4.4)
E. cloacae	1 (1.1)	2 (2.2)	3 (3.3)
E. aerogenes	1 (1.1)	0	1 (1.1)
C. freundii	0	2 (2.2)	2 (2.2)
S. marcescens	2 (2.2)	2 (2.2)	4 (4.4)
S. odorifera	0	1 (1.1)	1 (1.1)
K. oxytoca	1 (1.1)	0	1 (1.1)
S. arizonae	0	1 (1.1)	1 (1.1)
Total	32 (35.2)	59 (64.8)	91 (100)

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

carbapenem resistance profile was carried out by an automated method (Hu et al., 2019) which improve resistance detection. Whereas, in this study phenotypic detection of carbapenem-resistant strains has been performed using manual disk diffusion method (EUCAST/CASFM, 2022), by which it is difficult to demonstrate carbapenem resistance (Kabore et al., 2023).

All bacterial isolates resistant to carbapenem and/or to aztreonam therefore suspicious of carbapenemase production were screening by molecular approaches to detect *IMP* gene. In this current study, *bla*$_{IMP}$ gene was found in 35.2% (n=32/91) of carbapenem-resistant strains, while in 64.8% (n=59/91) other strains none resistance *IMP* gene was found (Table 5). A previous study of Dembele et al. (2021) in Burkina Faso has demonstrated the presence of *bla*$_{IMP-2}$ gene in Imipenem-resistant *E. coli* strains isolated from children with diarrhea in rural settings. Whereas, in this study, carbapenemase-IMP encoding gene was identified not only in *E. coli*, but also in other enterobacteria (Table 5) and then in *P. aeruginosa* recovered from children or adult patients in urban health centers in Ouagadougou, Burkina Faso.

IMP gene was recorded at a slightly high prevalence (35.2%, n=32/91) and more than half of gene positive strains (18.7%, n=17) were *Escherichia coli*. These findings are comparable to those reported in Iraq, where detection rate of *bla*$_{IMP}$ gene was 43% (n=23/53) in clinical GNB strains, and this gene most prevalent in *Escherichia coli* (50%) (Haji et al., 2021). Our carbapenemase-*IMP* gene frequency (35.2%, n=32/91) was highest than those recorded in previous studies in Sudan (26.4%) (Adam et Elhag, 2018), in Egypt (11.8%) (Abbas et al., 2019) and in Iran (13%) (Armin et al., 2023). This increasing detection rate of *bla*$_{IMP}$ comparing to frequencies of these previous studies would suggested a currently worrying spread of carbapenem-resistant GNB producing IMP-type carbapenemase (Li et al., 2023). In addition, to the best of our knowledge, our study is one of the first in our country to record detection of *bla*$_{IMP}$ gene in *Pseudomonas aeruginosa* carbapenem resistant clinical strains with a prevalence of 3.3% (Table 5) in health centers of Ouagadougou. Until then, Kabore et al., in their study, had been limited to the phenotypic detection of MBL in Imipenem-resistant *Pseudomonas aeruginosa* clinical strains . This detection rate of *bla*$_{IMP}$ gene in *Pseudomonas aeruginosa* is certainly underestimated given the relatively small sample size of our study. Thus, our study firstly reporting the circulation of *Pseudomonas aeruginosa* producing IMP-type carbapenemases in health centers of Ouagadougou, Burkina Faso.

In contrast, out of 64.8% (n=59/91) resistant strains, none strain has harbored *bla*$_{IMP}$ gene encoding IMP-type carbapenemase although some of them (12.1%, n=11/91) were resistant to all carbapenems and aztreonam tested. This fact could be explained by the existence of other resistance mechanisms in our isolates, such as the production of ESBLs or other carbapenemases and defects in cell wall permeability. Indeed, in a previous study conducted in 2022 by Tiemtore et al. (2022) in Burkina Faso, *bla*$_{GES}$ gene encoding Guiana Extended-Spectrum (GES) carbapenemase has been detected in Imipenem-resistant enterobacteriaceae strains included in this study. Thus, eventual presence of GES-2 variant, exhibiting an increased hydrolysis of carbapenems (Poirel et al., 2001), in our bacterial strains, might justified carbapenem resistance of *IMP* gene-negative isolates. Interestingly, the encoding-carbapenemase genes, such as *bla*$_{NDM}$, *bla*$_{VIM}$ and *bla* $_{KPC}$ were found in GNB clinical isolates as previously reported in other studies in this country (Dembele et al., 2021; Kabore et al., 2023; Ouattara et

al., 2023; Bambara et al., 2023). These carbapenem resistance genes not researched in this study could also explained the finding. Further, non-enzymatic resistance mechanisms to carbapenems such as defecting in wall cell permeability through modification or loss of porins (Zango et al., 2019; Eichenberger and Thaden, 2019; Çekin et al., 2021; Onishi et al., 2023) and over-expression of efflux pumps (Lee et al., 2021; Onishi et al., 2023) would explained the result.

Finally, *IMP* gene was detected in 9.9% (n=9/91) strains which were susceptible to all carbapenems except aztreonam. This presence of MBL gene encoding IMP enzyme in GNB isolates sensitive to carbapenems finding in this study was reported in previous studies in other countries with various frequencies including NDM and VIM (Anoar et al., 2014; Adam and Elhag, 2018). These results corroborate hypothesis that, ordinarily, MBL genes presence among carbapenems sensitive strains indicate that there might be hidden MBL genes not detected by phenotypic tests, leading to the silent spread of these genes in the hospitals and the community (Adam and Elhag, 2018).

Conclusion

Carbapenem-resistant Gram-negative bacilli are a growing threat to public health worldwide. The study aimed to determine carbapenem multi-resistance mediated by IMP carbapenemase production in GNB at Saint Camille Hospital of Ouagadougou (HOSCO) and University Hospital Center of Tengandogo (CHU-T) in Ouagadougou, Burkina Faso. The GNB strains exhibited a high resistance level to antibiotics tested with a prevalence of 57.6%. This study revealed a slightly high prevalence of *bla*$_{IMP}$, gene encoding IMP-type metallo-beta-lactamase in carbapenem-resistant strains. These results confirm the presence of GNB resistant to antibiotics producing IMP-type carbapenemases at HOSCO and CHU-T in Ouagadougou, Burkina Faso. The findings of this study strengthen the scarce existing scientific data on antimicrobial resistance mediated by metallo-beta-lactamases in Burkina Faso. The study recommended an urgent implementation of an antibiotic resistance surveillance system combining clinical aspects of infections related to bacteria producing MBL and other carbapenemases, in order to prevent, monitor and control the spread of antimicrobial resistance gene in our country. Further studies on sequence analysis of *IMP* gene amplicons are also needed to know different IMP variant profile in carbapenem-resistant GNB in Burkina Faso.

Ethics approval

The institutional ethic committee of CERBA/LABIOGENE reviewed and approved the study protocol.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

AUTHORS' CONTRIBUTIONS

Damis Yves Patrik Bouniounou, Amana Metuor Dabire and Yasmine Rahimatou Wend-Kouni Tiemtore and Serge Sougue designed the study, analyzed and interpreted data; Damis Yves Patrik Bouniounou performed the experiments and drafting the manuscript; Yasmine Rahimatou Wend-Kouni Tiemtore and Pegdwende Rose Bonkoungou collected samples and carried out the laboratory investigations under following the recommendations of Amana Metuor Dabire; Jacques Simpore was general supervisor of the study. All authors were involved in critically reviewing the manuscript and have read and approved the final published version of the manuscript.

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REFERENCES

Abbas HA, Kadry AA, Shaker GH, Goda RM (2019). Impact of specific inhibitors on metallo-β-carbapenemases detected in *Escherichia coli* and *Klebsiella pneumoniae* isolates. Microbial Pathogenesis 132:266-274.

Adam MA, Elhag WI (2018). Prevalence of metallo-β-lactamase acquired genes among carbapenems susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. BMC Infectious Diseases 18:668. https://doi.org/10.1186/s12879-018-3581-z

Anoar KA, Ali F, Omer SA (2014). Detection of Metallo β-lactamase enzyme in some Gram-negative bacteria isolated from burn patients in Sulaimani city, Iraq. European Scientific Journal 10(3):485-496.

Armin S, Azimi L, Shariatpanahi G, Shirvani A, Almasian N (2023). The Prevalence of Colonization with Carbapenem-resistant Enterobacteriaceae, *E. coli*, Klebsiella and Enterobacter, and Related Risk Factors in Children. Archives of Pediatric Infectious Diseases 11(2):e134518. https://doi.org/10.5812/pedinfect-134518

Aruhomukama D, Najjuka CF, Kajumbula H, Okee M, Mboowa G, Sserwadda I, Mayanja R, Joloba ML, Kateete DP (2019). blaVIM-and blaOXA-mediated carbapenem resistance among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates from the Mulago hospital intensive care unit in Kampala, Uganda. BMC Infectious Diseases 19:853. https://doi.org/10.1186/s12879-019-4510-5

Arumugham VB, Gujarath R, Cascella M (2022). Third Generation Cephalosporins. StatPearls Publishing, Treasure Island (FL). http://europepmc.org/abstract/MED/31751071

Balkhair A, Al Saadi K, Al Adawi B (2023). Epidemiology and mortality outcome of carbapenem- and colistin-resistant *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* bloodstream infections. IJID Regions 7:1-5. https://doi.org/10.1016/j.ijregi.2023.01.002

Bambara LEB, Metuor Dabire A, Traore O, Tiemtore Y, Zohoncon T, Simpore J (2023). Genetic and Kinetic Profiles of Bacterial Resistance Enzymes to IMP and NDM Type Antibiotics in Gram-negative Pathogenic Bacilli. International Journal of Biochemistry Research & Review 32(6):7-21.

Barguigua A, El Otmani F, Talmi M, Zerouali K, Timinouni M (2013). Prevalence and types of extended spectrum beta-lactamases among urinary *Escherichia coli* isolates in Moroccan community. Microbial Pathogenesis 61(62):16-22. https://doi.org/10.1016/j.micpath.2013.04.010

Benmahmod AB, Said HS, Ibrahim RH (2019). Prevalence and mechanisms of carbapenem resistance among *acinetobacter baumannii* clinical isolates in Egypt. Microbial Drug Resistance 25(4):480-488. https://doi.org/10.1089/mdr.2018.0141

Bourafa N, Chaalal W, Bakour S, Lalaoui R, Boutefnouchet N, Diene SM, Rolain JM (2018). Molecular characterization of carbapenem-resistant Gram-negative bacilli clinical isolates in Algeria. Infection and Drug Resistance 11:735-742. https://doi.org/10.2147/IDR.S150005

Çekin ZK, Dabos L, Malkoçoğlu G, Fortineau N, Bayraktar B, Iorga BI, Naas T, Aktaş E (2021). Carbapenemase -producing *Pseudomonas aeruginosa* isolates from Turkey: first report of *P. aeruginosa* high-risk clones with VIM-5– and IMP-7–type carbapenemases in a tertiary hospital. Diagnostic Microbiology and Infectious Disease 99(1):115174. https://doi.org/10.1016/j.diagmicrobio.2020.115174

Chouchani C, Marrakchi RIM, Ferchichi L, Salabi AEL, Wals TR (2011). VIM and IMP metallo- beta -lactamases and other extended-spectrum beta -lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from environmental samples in a Tunisian hospital. APMIS 119:725-732. https://doi.org/10.1111/j.1600-0463.2011.02793.x

Cornaglia G, Riccio ML, Mazzariol A, Lauretti L, Fontana R, Rossolini GM (1999). Appearance of IMP-1 metallo-β-lactamase in Europe. Lancet London England 353:899-900. https://doi.org/10.1016/S0140-6736(98)05954-6

Das S (2023). The crisis of carbapenemase-mediated carbapenem resistance across the human–animal–environmental interface in India. Infectious Diseases Now 53(1):104628. https://doi.org/10.1016/j.idnow.2022.09.023

Dasht AA, Jadaon MM, Abdulsamad AM, Dashti HM (2009). Heat Treatment of Bacteria: A Simple Method of DNA Extraction for Molecular Technics. Kuwait Medical Journal 41(2):117-122. https://www.researchgate.net/publication/266888615

Dembele R, Konate A, Traore O, Kabore WAD, Soulama I, Kagambega A, Traor AS, Guessennd NK, Aidara-Kane A, Gassama-Sow A, Barro N (2020). Extended spectrum beta-lactamase and fluoroquinolone resistance genes among *Escherichia c*oli and Salmonella isolates from children with diarrhea, Burkina Faso. BMC Pediatrics 20:459. https://doi.org/10.1186/s12887-020-02342-z

Dembele R, Soulama I, Aime W, Kabore D, Konate A, Golo DCN, Traore O, Seck A, Traore AS, Guessennd N, Gassama-sow A, Barro N (2021). Molecular Characterization of Carbapenemase - Producing Enterobacterales in Children with Diarrhea in Rural Burkina Faso. Journal of Drug Delivery and Therapeutics 11(1):84-92.

Dien SM, Rolain JM (2014). Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae, Pseudomonas* and *Acinetobacter species*. Clinical Microbiology and Infection 20(9):831-838. https://doi.org/10.1111/1469-0691.12655

Dikoumba AC, Onanga R, Jean-Pierre H, Didelot MN, Dumont Y, Ouedraogo AS, Ngoungou EB, Godreuil S (2023). Prevalence and Phenotypic and Molecular Characterization of Carbapenemase-Producing Gram-Negative Bacteria in Gabon. American Journal of Tropical Medicine and Hygiene 108(2):268-274.

Dziri O, Dziri R, El Salabi AA, Chouchani C (2020). Carbapenemase producing gram-negative bacteria in Tunisia: History of thirteen years of challenge. Infection and Drug Resistance 13:4177-4191.

Eichenberger EM, Thaden JT (2019). Epidemiology and mechanisms of resistance of extensively drug resistant gram-negative bacteria. Antibiotics 8(2):37. https://doi.org/10.3390/antibiotics8020037

EUCAST/CASFM (2022). Comité de l 'antibiogramme de la Société Française de Microbiologie, Recommendations 2022. In Société Française de Microbiologie.

Haji SH, Aka STH, Ali FA (2021). Prevalence and characterisation of carbapenemase encoding genes in multidrug- resistant Gram-negative bacilli. PLoS ONE 30(11):e0259005. https://doi.org/10.1371/journal.pone.0259005

Halat DH, Moubareck CA (2020). The current burden of carbapenemases: Review of significant properties and dissemination among gram-negative bacteria. Antibiotics 9(4):186. https://doi.org/10.3390/antibiotics9040186

Halat DH, Moubareck CA, Sarkis DK (2017). Heterogeneity of Carbapenem Resistance Mechanisms among Gram-Negative Pathogens in Lebanon: Results of the First Cross-Sectional Countrywide Study. Microbial Drug Resistance 23(6):733-743. https://doi.org/10.1089/mdr.2016.0077

Hu W, Li M, Lu W, Guo S, Li J (2019). Evaluation of MASTDISCS combi Carba plus for the identification of metallo-β-lactamases, KPC and OXA-48 carbapenemase genes in Enterobacteriaceae clinical isolates. Letters in Applied Microbiology 70(1):42-47.

Huang S, Dai W, Sun S, Zhang X, Zhang L (2012). Prevalence of Plasmid-Mediated Quinolone Resistance and Aminoglycoside Resistance Determinants among Carbapeneme Non-Susceptible Enterobacter cloacae. PLoS ONE 7(10):e47636.

Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN (2021). Multidrug-resistant *Acinetobacter baumannii* as an emerging concern in hospitals. Molecular Biology Reports 48:6987-6998.

Kabore B, Cisse H, Zongo KJ, Sanou I, Traore Y, Savadogo A (2022). Phenotypic detection of metallo- β -lactamase in imipenem- resistant *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* at Schiphra Hospital of Ouagadougou in Burkina Faso. Microbes and Infectious Diseases 3(1):128-134.

Kabore B, Ouedraogo HS, Zongo O, Ouedraogo GA, Tapsoba F, Bougma S, Zongo KJ, Zeba B, Traore Y, Sanou I, Savadogo A (2023). Emergence of New Delhi Metallo- β -Lactamase (NDM) Genes Detected from Clinical Strains of *Escherichia coli* Isolated in Ouagadougou, Burkina Faso. International Journal of Microbiology 2023:4813225.

Kim EJ, Lee J, Yoon Y, Lee D, Baek Y, Takano C, Sakai J, Iijima T, Kanamori D, Gardner H, McLaughlin RE, Kilgore PE, Nakamura A, Ogihara T, Hayakawa S, Hoshino T, Kim DW, Seki M (2023). Development of a novel loop-mediated isothermal amplification assay for ß-lactamase gene identification using clinical isolates of Gram-negative bacteria. Frontiers in Cellular and Infection Microbiology 12:1000445. https://doi.org/10.3389/fcimb.2022.1000445

Kubota H, Suzuki Y, Okuno R, Uchitani Y, Ariyoshi T, Takemura N, Mihara F, Mezaki K, Ohmagari N, Matsui M, Suzuki S, Sekizuka T, Kuroda M, Yokoyama K, Sadamasu K (2019). IMP-68, a Novel IMP-Type Metallo-beta-Lactamase in Imipenem-Susceptible *Klebsiella pneumoniae.* MSphere 4:e00736-19. https://doi.org/10.1128/mSphere.00736-19

Lee YJ, Huang CH, Ilsan NA, Lee IH, Huang TW (2021). Molecular epidemiology and characterization of carbapenem-resistant *klebsiella pneumoniae* isolated from urine at a teaching hospital in Taiwan. Microorganisms 9:271. https://doi.org/10.3390/microorganisms9020271

Li X, Mu X, Chen F, Lu X, He J, Zheng Y, Zhou D, Yin Z, Wang P (2023). Characterization of Three Novel IMP Metallo-β-Lactamases, IMP-89, IMP-91, and IMP-96, and Diverse blaIMP-Carrying Accessory Genetic Elements from Chinese Clinical Isolates. Microbiology Spectrum 11(3):e04986-22. https://doi.org/10.1128/spectrum.04986-22

Limbago BM, Rasheed JK, Anderson KF, Zhu W, Kitchel B, Watz N, Munro S, Gans H, Banaei N, Kallen AJ (2011). IMP-producing carbapenem-resistant *Klebsiella pneumoniae* in the United States. Journal of Clinical Microbiology 49(12):4239-4245.

Manenzhe RI, Zar HJ, Nicol MP, Kaba M (2015). The spread of carbapenemase-producing bacteria in Africa: A systematic review. Journal of Antimicrobial Chemotherapy 70(1):23-40.

Masoud SM, Abd El-Baky RM, Aly SA, Ibrahem RA (2021). Co-existence of certain ESBLs, MBLs and plasmid mediated quinolone resistance genes among MDR *E. coli* isolated from different clinical specimens in Egypt. Antibiotics 10:835. https://doi.org/10.3390/antibiotics10070835

McCarthy KL, Jennison A, Wailan AM, Paterson DL (2017). Draft

Genome Sequence of an IMP-7- Producing *Pseudomonas aeruginosa* Bloodstream Infection Isolate from Australia. Genome Announcements 5(27):e00596-17. https://doi.org/10.1128/genomeA.00596-17.

Metuor DA, Tiemtore RYWK, Bangre YA, Zohoncon, TM, Sougue S, Zongo JK, Simpore J (2019a). Detection of multidrug-resistant enterobacteria simultaneously producing extended-spectrum -lactamases of the PER and GES types isolated at Saint Camille Hospital Center, Ouagadougou, Burkina Faso. African Journal of Microbiology Research 13(26):414-420. https://doi.org/10.5897/ajmr2019.9147

Metuor Dabire A, Sougue S, Tiemtore RYWK, Zohoncon TM, Bangre YA, Ouedraogo P, Kabre E, Simpore J (2019b). Coexistence between (TOHO-type and BES-type) extended-spectrum -lactamase genes of identified enterobacteria at Saint Camille Hospital, Ouagadougou, West Africa. International Journal of Genetics and Molecular Biology 11(2):34-40.

Metuor Dabire A, Zongo KJ, Zeba B, Moussawi J, Baucher M, El Jaziri M (2013). Resistances to the oxyimino-cephalosporins by CTX-M-15 producing Klebsiella isolated from the urine samples of patients in the University Hospital Complex Paediatric Charles De Gaulle (CHUP-CDG) of Ouagadougou in Burkina Faso. Journal of Asian Scientific Research 9(3):882-890.

Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F (2014). Carbapenemase genes among multidrug resistant gram-negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. BioMed Research International 2014:303104. https://doi.org/10.1155/2014/303104

Onishi R, Shigemura K, Osawa K, Yang YM, Maeda K, Tanimoto H, Kado M, Fang SB, Fujisawa M (2023). Molecular characteristics and genetic analysis of the genes related to carbapenemase, efflux pump, and outer membrane proteins in carbapenem-resistant *Klebsiella pneumoniae*. Letters in Applied Microbiology 76:1-7. https://doi.org/10.1093/lambio/ovac069

Ouattara AK, Ouedraogo B, Tiemtore RYW, Metuor Dabire A, Sougue S, Simpore J (2023). Detection of blaVIM Gene in β -Lactam Resistant *E. coli* Isolated from Clinical Samples in Ouagadougou, Burkina Faso. Open Access Library Journal 10:e10534. https://doi.org/10.4236/oalib.1110534

Owusu FA, Obeng-Nkrumah N, Gyinae E, Kodom S, Tagoe R, Tabi BKA, Dayie NTKD, Opintan JA, Egyir B (2023). Occurrence of Carbapenemases, Extended-Spectrum Beta-Lactamases and AmpCs among Beta-Lactamase-Producing Gram-Negative Bacteria from Clinical Sources in Accra, Ghana. Antibiotics 12(6):1016. https://doi.org/10.3390/antibiotics12061016

Palacios-Baena ZR, Giannella M, Manissero D, Rodríguez-Baño J, Viale P, Lopes S, Wilson K, McCool R, Longshaw C (2021). Risk factors for carbapenem-resistant Gram-negative bacterial infections: a systematic review. Clinical Microbiology and Infection 27(2):228-235.

Poirel L, Weldhagen GF, Naas T, De Champs C, Dove MG, Nordmann P (2001). GES-2, a class A β-lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. Antimicrobial Agents and Chemotherapy 45(9):2598-2603.

Riccio ML, Franceschini N, Boschi L, Caravelli B, Cornaglia G, Fontana R, Amicosante G, Rossolini GM (2000). Characterization of the metallo-β-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla(IMP) allelic variants carried by gene cassettes of different phylogeny. Antimicrobial Agents and Chemotherapy 44(5):1229-1235.

Sanou S, Ouedraogo AS, Aberkane S, Vendrell J, Ouchar O, Bouzimbi N, Hema A, Poda A, Zoungrana J, Ouedraogo GA, Carrière C, Jean-Pierre H, Ouedraogo/Traore R, Godreui, S (2020). Prevalence and Molecular Characterization of Extended Spectrum b-Lactamase, Plasmid-Mediated Quinolone Resistance, and Carbapenemase-Producing Gram-Negative Bacilli in Burkina Faso. Microbial Drug Resistance pp. 1-7.

Shaker OA, Gomaa HE, Elmasry SA, Abdel Halim RM, Abdelrahman AH, Kamal JS (2018). Evaluation of combined use of temocillin disk and mastdisks inhibitor combination set against polymerase chain reaction for detection of carbapenem-resistant enterobacteriaceae. Open Access Macedonian Journal of Medical Sciences 6(2):242-247.

Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outterson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Magrini N (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases 18:318-327.

Tiemtore RYW, Dabire, AM, Ouermi D, Sougue S, Benao S, Simpore J (2022). Isolation and Identification of *Escherichia coli* and *Klebsiella pneumoniae* Strains Resistant to the Oxyimino-Cephalosporins and the Monobactam by Production of GES Type Extended Spectrum Bêta-Lactamase (ESBL) at Saint Camille Hospital Center in Ouagadougou. Infection and Drug Resistance 15:3191-3204. https://doi.org/10.2147/IDR.S360945

Watanabe M, Iyobe S, Inoue M, Mitsuhashi S (1991). Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 35(1):147-151.

Yang Y, Yan YH, Schofield CJ, McNally A, Zong Z, Li GB (2023). Metallo-β-lactamase-mediated antimicrobial resistance and progress in inhibitor discovery. Trends in Microbiology 31(7):735-748.

Zango UU, Ibrahim M, Shawai SA, Abubakar Shamsuddin IM (2019). A review on β -lactam antibiotic drug resistance. MOJ Drug Design Development and Therapy 3(2):52-58. https://doi.org/10.15406/mojddt.2019.03.