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Genetic and Kinetic Profiles of Bacterial Resistance Enzymes to IMP and NDM Type Antibiotics in Gram-negative Pathogenic Bacilli

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Enterobacteria are among the most frequent isolated strains in hospitalized patients. Currently, carbapenems have a broad spectrum of activity and are considered to be the last resort for the treatment of nosocomial infections. However, overuse of these valuable compounds has led to a rapid increase in antimicrobial resistance. The aim was to study the genetic and kinetic profiles of IMP and NDM bacterial resistance enzymes in Gram-negative pathogenic bacilli.
Methodology: The carbapenems used for strain susceptibility testing consisted of Ertapenem, Meropenem, Imipenem and Doripenem. Detection of the IMP and NDM resistance genes encoding carbapenemases was carried out by conventional PCR at LABIOGENE, and hydrolysis of the enzymatic crude extracts was performed.
Results: Strains showed high levels of resistance to Ertapenem (64%), Meropenem (41%), Imipenem (53%) and Doripenem (73%). PCR amplification of carbapenem resistance genes showed that 32% of strains carried *bla$_{IMP}$*, 16% of strains carried *bla$_{NDM}$*. Both bla$_{IMP}$ & *bla$_{NDM}$* were identified with a prevalence of 6.76%. *Escherichia coli* was the majority species harboring these genes. The kinetic constants of NDM carried by *E. coli* are Km=333µM and Vmax=0.5 A/min. Those of IMP carried by Klebsiella sp are Km=500µM and Vmax=0.6 A/min.
Conclusion: This study enabled us to determine the rate of carbapenem resistance in Gram-negative bacteria. We noticed that Meropenem was the most active molecule. The study also enabled us to determine the affinity of two different bacterial enzymes to nitrocefin.

Keywords: BGN; carbapenem; carbapenemases; bla$_{VIM}$; bla$_{NDM}$.

1. INTRODUCTION

Enterobacteria are often responsible for urinary tract infections, pulmonary infections, septicemia and other intra-abdominal infections [1]. Escherichia coli is a frequent cause of community-acquired urinary tract infections [2]. Pseudomonas aeruginosa and Acinetobacter baumannii are opportunistic pathogens, often responsible for severe pulmonary infections and bacteremia, particularly in intensive care units [3].

The introduction of antibiotics into clinical use was arguably the greatest medical breakthrough of the twentieth century [4]. As well as treating infectious diseases, antibiotics have made many modern medical procedures possible, including cancer treatment, organ transplants and open-heart surgery. The beta-lactam antibiotics are the most important family of antibiotics, both in terms of the number and diversity of the molecules they can be used for, and their indications in the treatment and prophylaxis of bacterial infections. This family includes penicillins, cephalosporins, carbapenems and monobactams (Cavallo, 2004). Currently, carbapenems, such as Imipenem, Ertapenem, Meropenem and Doripenem, have a broad spectrum of activity and are crucial for the treatment of nosocomial infections [5]. However, the overuse of these valuable compounds has led to a rapid increase in antimicrobial resistance [6]. Indeed, Betalactamases are produced by Gram-negative bacilli, and constitute the main mechanism of resistance to betalactamins. Four classes have been identified according to Ambler's classification. Classes A, C and D are active serine enzymes, while class B groups metallo-beta-lactamases (MBLs) requiring Zn++ ions for their activity [7]. The O'Neill report commissioned by the UK government predicted that without urgent action, 10 million people a year will die from drug-resistant infections by 2050 [8]. The dangers of a post-antibiotic era have prompted policymakers to recognize this threat to human health and promise further subsidies, gradually sparking renewed interest in antibiotic discovery and development [9]. To ensure stability against beta-lactamases, one of the most spectacular additions to the beta-lactam family was the discovery of beta-lactamase inhibitors. With their low intrinsic activity, beta-lactamase inhibitors have to be combined with other beta-lactamins with good antibacterial activity but hydrolyzable by beta-lactamases [10]. This new approach makes it possible to restore the activity of certain betalactamines that have become ineffective against betalactamase-producing bacteria [11]. In view of this information, we were interested in Gram-negative bacilli coding for the IMP and NDM genes, and confirmed that resistance in these bacteria is linked to enzyme production.

2. MATERIALS AND METHODS

This was a cross-sectional study with retrospective data collection. Our study took

place at the Laboratory of Molecular Biology and Genetics (LABIOGENE) and the Pietro Annigoni Biomolecular Research Center (CERBA) from October 2022 to December 2022. Bacterial awakening, antibiotic susceptibility testing and extraction, DNA purity verification, amplification by conventional PCR and migration by electrophoresis were carried out at LABIOGENE. Extraction and hydrolysis of enzymatic crude extracts were carried out at CERBA.

2.1 Sampling

The biological material consisted of 74 bacterial strains of human origin responsible for infections. The 74 strains were isolated between 2009 and 2022, at the Pediatric University Hospital Charles De Gaulle (CHUP-CDG) in Ouagadougou, Burkina Faso, at the Hospital Saint-Camille and at CERBA. These bacterial strains were composed of 6 species, including 39 strains of E. coli, 2 strains of Enterobacter sp., 21 strains of *Klebsiella sp.*, 3 strains of *Proteus sp.*, 7 strains of *Pseudomonas sp.* and 2 strains of *Salmonella sp.* These strains were isolated in a previous study from various biological samples such as urine (n = 36), pus (n = 17), stool (n = 20) and blood (n = 1) and stored at -80°C in LB supplemented with 30% glycerol at LABIOGENE's CERBA facility.

2.2 Antibiotic Susceptibility Testing

The antibiotic susceptibility testing of strains was carried out using the disk diffusion method on Mueller-Hinton (MH) agar, taking into account the recommendations of the Antibiogram Committee of the French Microbiology Society (EUCAST / CA-SFM, 2021). After incubation at 37°C for 18 - 24 hours, the different diameters of the zones of inhibition obtained around the antibiotic discs were measured and interpreted as Sensitive (S) or Resistant (R). The following antibiotics were tested: Ertapenem (ERT), Meropenem (MRP), Imipenem (IMP) and Doripenem (DOR).

2.3 DNA Extraction

DNA extraction was performed by the boiling method. An isolated colony was removed from MH petri dishes and suspended in 200 µl of distilled water in labelled Eppendorf tubes. The tube was then soaked in a 100°C water bath for 15 minutes to release the bacterial genetic material. After centrifugation for 10 min at 12,000 rpm, the supernatant containing the released DNA was transferred to a new Eppendorf tube. After quantification and verification of DNA purity with the nanodrop a portion of the supernatant was used for amplification and the remainder was stored at -80°C.PCRThe PCR was performed using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, California, USA) in a 20 µL reaction mixture. This reaction mix was prepared using 4 µL GREEN PCR Master Mix + 0.5 µL of sense primer + 0.5 µL of antisense primer + 14 µL of PCR water + 1 µL of bacterial DNA from each strain.

The PCR program used is shown in Table 1. The primer sequences for the various carbapenemase genes are shown in Table 2.

Table 1. PCR program according to the type of gene used

	Condition/duration	
Genoa Settings	**bla_{IMP}**	**bla_{NDM}**
Initial denaturation	96°C/5 min	96°C/5 min
Denaturation	96°C/30s	96°C/30s
Hybridization	54°C/30s	62°C /30s
Elongation	72°C /30s	72°C /30s
Final elongation	72°C /7min	72°C /7 min
Number of cycles	30	30

Table 2. Sequences and sizes of the primer fragments

Researched genes	Sequences (5'-3')	Size (pb)	References
bla_{IMP}	For: 5`CATGGTTTGGTGCTTGT3` Rev : 5`ATAATTTGGCGGACTTTGGC3`	500	**(Khosravi and Mihani, 2008)**
bla_{NDM}	For :5`CCATGCGGGCCGTATGAGTGATT3` Rev :5`AAGCTGAGCACCGCATTAGCCG3`	500	**(Khosravi and Mihani, 2008)**

2.4 DNA Electrophoresis on Agarose Gel

The desired DNA fragment was visualized after agarose gel electrophoresis. Electrophoresis is a technique that separates charged molecules (DNA, RNA) according to their size under the effect of an electric field. This separation takes place through the agarose gel matrix. This matrix enables smaller DNA fragments to migrate faster and further than larger ones [12]. PCR-amplified DNA fragments were separated by electrophoresis on agarose gel (1.5%) prepared in a 1X tris base - borate - EDTA solution and containing 8 µL of ethidium bromide. A volume of 8 µL of amplicons was deposited in the different wells of the gel, starting with the second well. The first well was filled with 8 µL of the 100 bp molecular weight marker. The electrophoresis tank was covered with TBE buffer and migration was carried out for 25 minutes at 100V. The migration products obtained were visualized under UV light with the GENE FLASH apparatus and the photos were recorded.

2.5 Enzymatic Activity

BLSE extraction in gram-negative bacteria, β-lactamases are normally secreted and maintained in the periplasm. In practice, crude extracts for analytical purposes (detection and measurement of β-lactamase activity) have always been performed on 4 mL of cell suspension at 108 CFU/mL. The bacterial pellet obtained after centrifugation at 3000 rpm for 30 minutes at 4°C in a centrifuge (BR4i) was suspended in 300µl of 100mM pH 7 phosphate buffer. The contents of the periplasmic space were released by the physical method of freeze/thaw cycles [13,14]. The principle of the method is to prepare a cold bath by cooling acetone with dry ice. This produces a liquid medium at a temperature ranging from -65°C to -75°C. The cells are immersed in this medium for 8 minutes, resulting in instant freezing. They are then transferred to a 37°C water bath for instant thawing. The samples are then centrifuged at 8,000 rpm for 20 min on the MSE microcentrifuge. Cell debris is then removed and supernatants recovered and tested for β-lactamase activity. Hydrolysis of β-lactamines by ESBL crude extracts the various bacterial extracts obtained were tested for β-lactamase activity with nitrocefin. This chromogenic β-lactamine is an all-purpose substrate for virtually all β-lactamases. A reaction medium consisting essentially of 955 µL of 50mM pH7 phosphate buffer, 20 µL of enzyme, 25 µL of nitrocefin in a final concentration of 100µM. The reaction mixture produced is 1 mL in a quartz cuvette. Extracts with β-lactamase activity were tested only with nitrocefin to determine their hydrolytic profile. Enzymatic activities were monitored on a spectrophotometer. The kinetic curves for hydrolysis of β-lactam nuclei by the different extracts were recorded at a wavelength of 482 nm. All experiments were performed at 30°C in 50mM phosphate buffer pH 7. The initial rate (Vo) of hydrolysis of each compound (expressed in absorbance units per minute) was evaluated according to the relationship $Vo = \Delta A\lambda / \Delta t$ in which $\Delta A\lambda / \Delta t$ represents the slope to the origin of the curve of change in absorbance at wavelength λ as a function of time.

2.6 Statistical Analysis

The data collected were entered into Excel 2013. Data processing and analysis was carried out using Stata SPSS 22.

3. RESULTS

3.1 Profile of Bacterial Strain Resistance

The histogram below illustrates the percentage of susceptible and resistant strains according to the antibiotic used. The majority of bacterial strains are resistant to three carbapenems: 64% to Ertapenem, 53% to Imipenem and 73% to Doripenem. On the other hand, the sensitivity rate is high with Meropenem, at 59%.

3.2 Resistance Profile of Bacterial Strains to Ertapenem

Through the data already collected, the determination of the prevalence of bacterial species resistant to Ertapenem is presented in Fig. 2. Thus, *pseudomonas* were the most sensitive species with a prevalence of 57%.

3.3 Resistance Profile of Bacterial Strains to Meropenem

Through this diagram, we noticed a high resistance to *Enterrobacter sp.* and *salmonella sp.* with respective rates of 50% and 100%.

3.4 Resistance profile of bacterial strains to Imipenem

We noticed a strong bacterial sensitivity in *Enterrobacter sp., Proteus sp.* and *Salmonella sp.* although these species are rare in sampling.

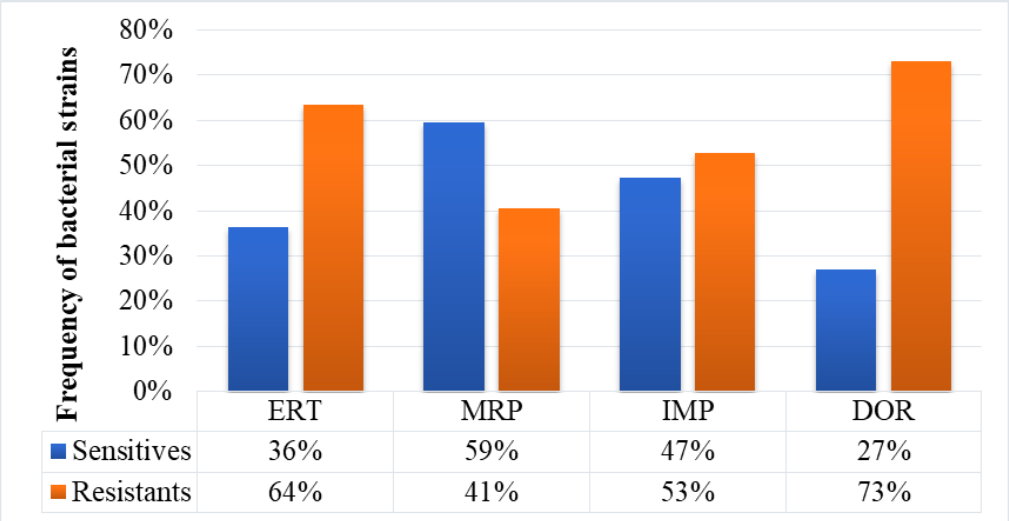

Fig. 1. Profile of bacterial resistance to antibiotics

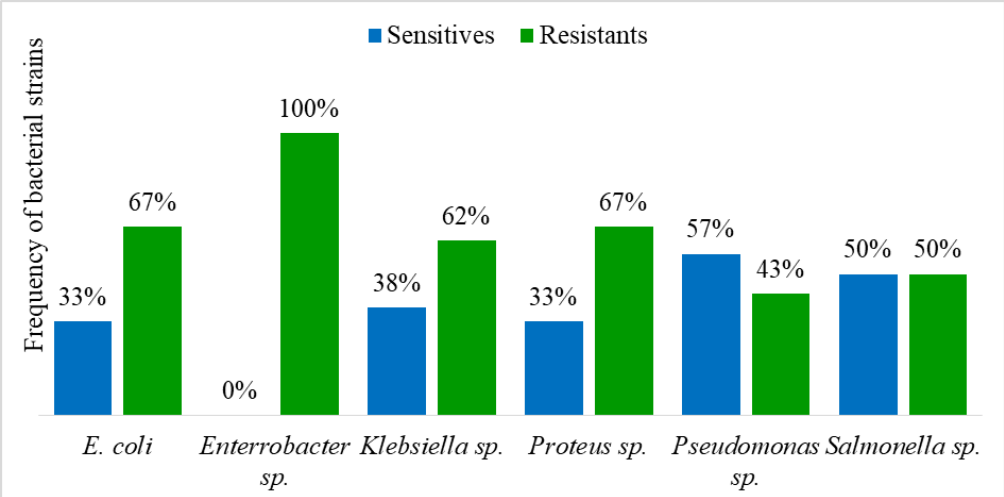

Fig. 2. Resistance profile of bacterial species to Ertapenem

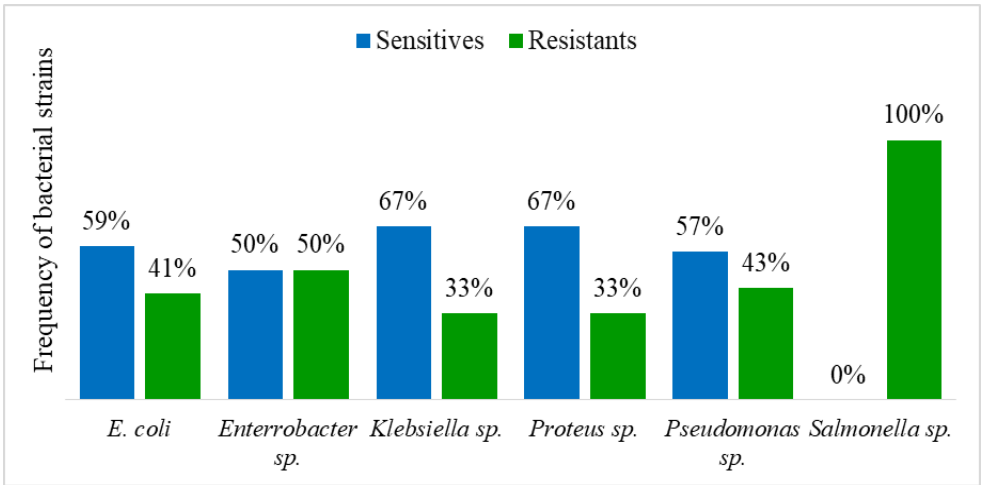

Fig. 3. Resistance profile of bacterial species to Meropenem

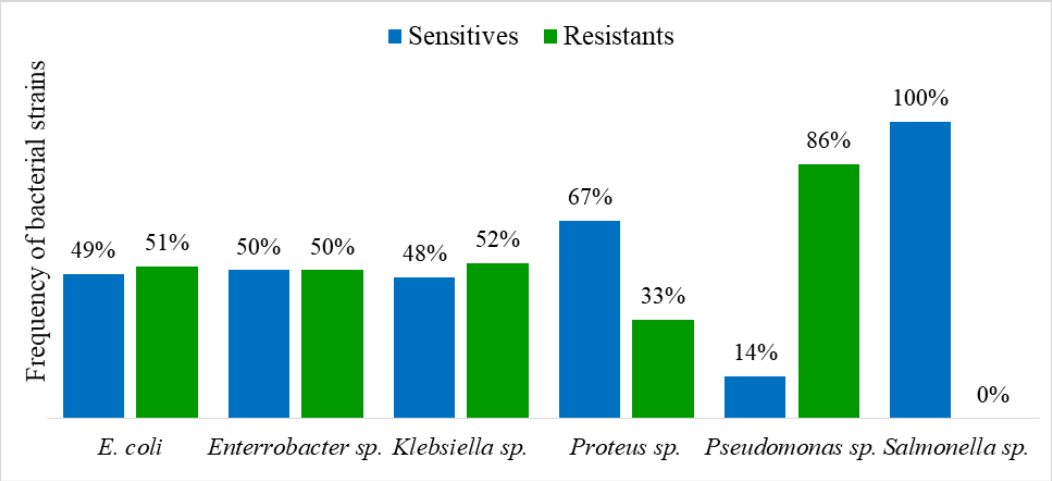

Fig. 4. Resistance profile of bacterial species to Imipenem

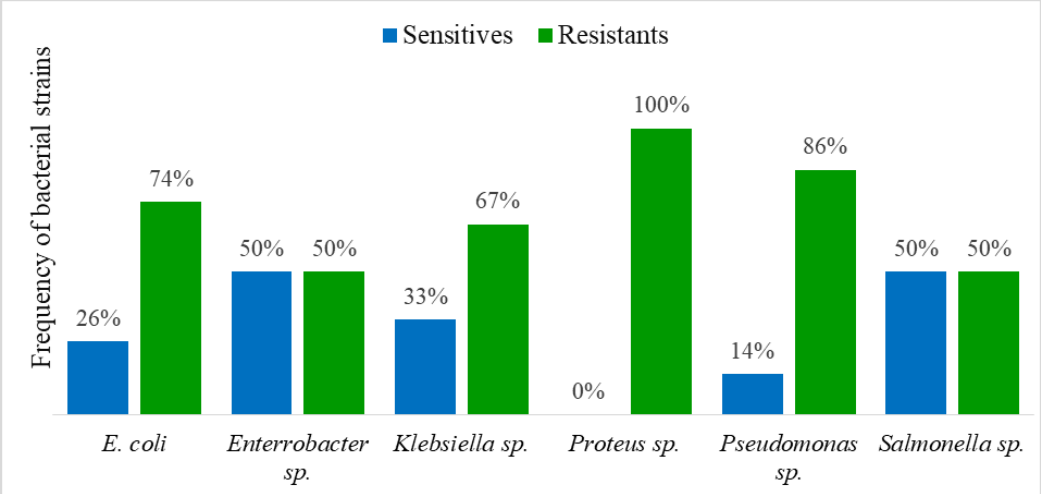

Fig. 5. Resistance profile of bacterial species to Doripenem

3.5 Resistance Profile of Bacterial Strains to Doripenem

By this histogram, we noticed that all the strains are resistant to Doripenem.

3.6 Characterization of Genes Encoding Carbapenemases

The analysis of the PCR products by agarose gel electrophoresis revealed that of the total 74 strains, 32% revealed the presence of *bla*$_{IMP}$ and 16% carried *bla*$_{NDM}$.

3.7 Distribution of Genes According to Bacterial Species

The electrophoretic profile of the *bla*$_{IMP}$ *amplicons* reveal that the size of the DNA fragments which correspond to the size of 500 bp indicate the presence of the IMP. The molecular weight marker used is that of DNA Ladder of 100 bp. Thus the fragments of the F12 samples correspond to the desired size of 500 bp.

The DNA extracts of the strains which have undergone migration are thus mentioned in Fig. 6, (F10 to G3) correspond to: F10=Saddles 69; F11=Saddles 70; F12=Saddles 71; G1=Saddles 72; G2=Urine 73; G3=Saddles 74.

Electrophoretic profiling of NDM gene amplicons reveals that the size of DNA fragments that correspond to the size of 500 bp indicate the presence of the NDM gene**.** Thus the amplicons of samples C1, C4, and C8 have a size corresponding to 500 bp.

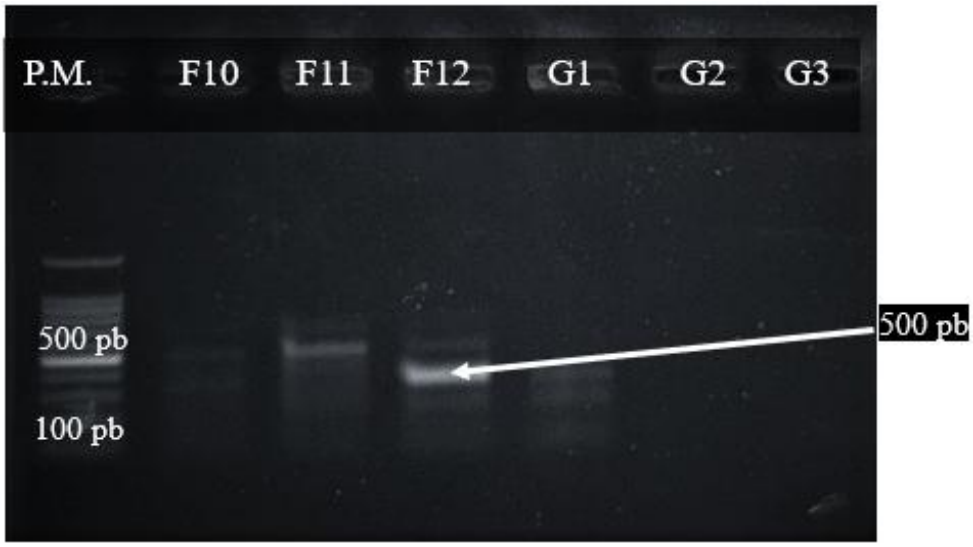

Fig. 6. Revelation of bla_{IMP} amplicons after electrophoresis

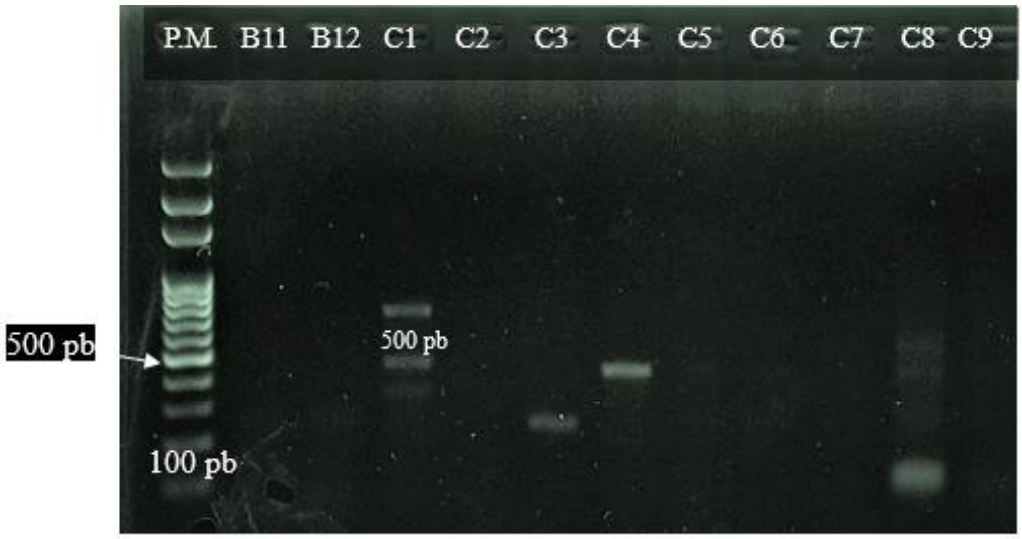

Fig. 7. Revelation of bla_{NDM} amplicons after electrophoresis

The DNA extracts of the strains which have undergone migration are thus mentioned in Fig. 7, (B11 to C9) correspond to: B11=Pus 22; B12 = Pus 23; C1 = Urine 24; C2 = Urine 25; C4 = Urine 26; C5 = Urine 27; C6 = Urine 28; C7 = Urine 29; C8 = Urine 30; C9 = Urine 31.

3.8 Distribution of bla_{NDM} and bla_{IMP} According to Pathological Products and Bacterial Species

The most represented pathological product is that of urine, of which the most represented bacterial species is that of *E. coli*. Among the 21 strains of *E. coli* present in the urine, 6 carried the NDM gene, i.e. 28%. In the stools, he had one (1) strain of *E. coli* carrying the NDM gene among 10 strains or 10%. In the other pathological products (pus and blood), no bacterial species carried this gene.

The most represented pathological product is that of urine, of which the most represented bacterial species is that of *Klebsiella sp.* (46%) followed by *E. coli* (23%). In the stool, *E. coli* and *Klebsiella sp.* presented the bla_{IMP} at respective rates of 50% each. In pus, 28% of *E. coli* carried the IMP gene. In the blood, no strain carried this gene.

Table 3. Distribution of *bla$_{NDM}$* and *bla$_{IMP}$* according to the bacterial species and the pathological product

Origin	Microorganisms	bla$_{NDM}$			bla$_{IMP}$		
		negative	Positive	Total	negative	Positive	Total
Urine	*E. coli*	15	6	**21**	16	5	**21**
	Kliebsiella sp.	8	5	**13**	7	6	**13**
	Salmonella sp.	0	0	**0**	0	0	**0**
	Enterrobacter sp.	0	0	**0**	0	0	**0**
	Pseudomonas sp.	2	0	**2**	2	0	**2**
	Proteus sp.	0	0	**0**	0	0	**0**
saddles	*E. coli*	9	1	**10**	5	5	**10**
	Kliebsiella sp.	4	0	**4**	2	2	**4**
	Salmonella sp.	1	0	**1**	1	0	**1**
	Enterobacter sp.	0	0	**0**	0	0	**0**
	Pseudomonas sp.	2	0	**2**	0	2	**2**
	Proteus sp.	3	0	**3**	3	0	**3**
Pus	*E. coli*	7	0	**7**	5	2	**7**
	Kliebsiella sp.	4	0	**4**	4	0	**4**
	Salmonella sp.	1	0	**1**	1	0	**1**
	Enterrobacter sp.	2	0	**2**	2	0	**2**
	Pseudomonas sp.	1	0	**1**	1	0	**1**
	Proteus sp.	0	0	**0**	0	0	**0**
blood	*E. coli*	1	0	**1**	1	0	**1**
	Kliebsiella sp.	0	0	**0**	0	0	**0**
	Salmonella sp.	0	0	**0**	0	0	**0**
	Enterrobacter sp.	0	0	**0**	0	0	**0**
	Pseudomonas sp.	0	0	**0**	0	0	**0**
	Proteus sp.	0	0	**0**	0	0	**0**

3.9 bla_{NDM} and bla_{IMP} Genes According to Bacterial Species

Among the 74 bacterial strains, there are 2 strains *of E. coli* that carry both genes and 3 strains of *Klebsiella sp* that carry both genes (IMP and NDM).

3.10 Enzymatic Kinetics of Two Samples Each Carrying the bla_{NDM} or bla_{IMP} Genes

In order to determine the kinetic parameters, the absorbances of the two bacterial species were determined as a function of time (in minutes). Two curves are thus obtained as shown in Fig. 10 and Fig. 11. The enzyme secreted by *Klebsiella sp*. is IMP and that secreted by *E. coli* is NDM.

Compared to the IMP enzyme carried by *Klebsiella sp.*, the NDM enzyme carried by *E. coli* hydrolyzes nitrocefin at 0.1 A at the initial time (t = 0 min).

The hydrolysis curves of nitrocefin by the IMP and NDM enzymes were determined with a concentration of 50 µM. Thus, the Vo was calculated through the slope of each curve at respective concentrations of 50µM, 75µM and 100µM. We thus obtain the Table 4, used to determine the Michaelis-Menten curve.

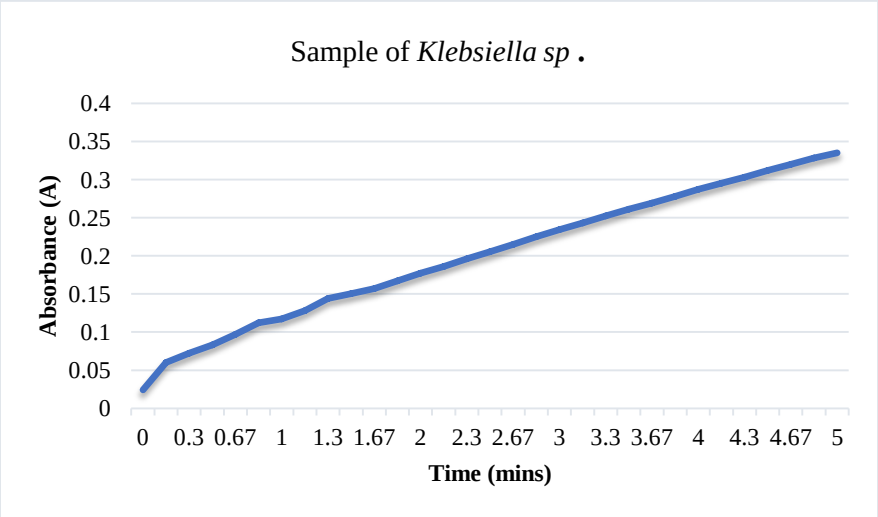

Fig. 8. Curve of the hydrolysis of nitrocefin by the IMP enzyme carried by *Klebsiella sp.*

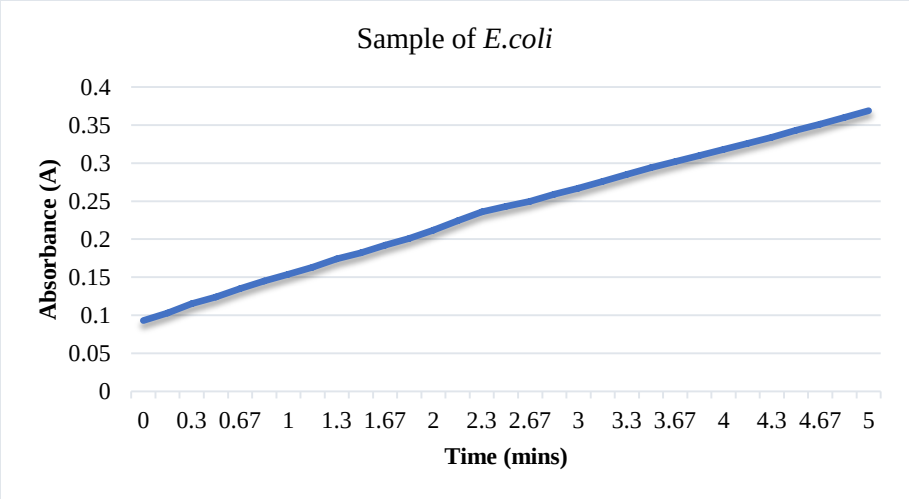

Fig. 9. Curve of the hydrolysis of nitrocefin by the NDM enzyme carried by *E. coli*

Table 4. Kinetics of $_{NDM}$ and $_{IMP}$ enzymes according to the concentration of nitrocefin

Sample (Klebsiella sp. (IMP) – E. coli (NDM))		
C (mM)	***Vo of $_{IMP}$ (A/min)***	***Vo of $_{NDM}$ (A/min)***
50	*0.034*	*0.046*
75	*0.052*	*0.076*
100	*0.065*	*0.092*

This table allowed us to obtain the Michaelis-Menten curve of the IMP enzyme carried by a *Klebsiella sp.* We find that the velocities increase exponentially with increasing concentrations as shown in Fig. 10.

Through Table 5, we were able to perform the Michaelis-Menten curve of the NDM enzyme carried by *E. coli* as shown in Fig. 11.

From the data of these curves, the inverse of Vo and C is calculated, as shown in Table 5.

This table is thus used to determine the Lineweaver and Burk plot of the two enzymes IMP and NDM carried respectively by *Klebsiella sp.* and *E. coli* after hydrolyzing nitrocefin.

The reciprocals of the concentration and the absorbance of the NDM enzyme gave respective values of -0.002 Mm $^{-1}$ and 0.6 A/min.

3.11 Determination of the Kinetic Constants of NDM Carried by *E. coli*

$\frac{-1}{Km}$ = - 0.003 $\frac{1}{Vmax}$ = 2

Km = 333 µM or 0.3 mM Vmax = 0.5 A/min

In contrast to the plot shown in Fig. 12, the reciprocals of the concentration and absorbance of the IMP enzyme yielded values of -0.002 Mm $^{-1}$ and 0.6 A/min, respectively.

3.12 Determination of the Kinetic Constants of IMP Carried by *Klebsiella sp.*

$\frac{-1}{Km}$ = - 0.002 $\frac{1}{Vmax}$ = 1.5

Km = 500 µM or 0.5 mM Vmax = 0.6 A/min

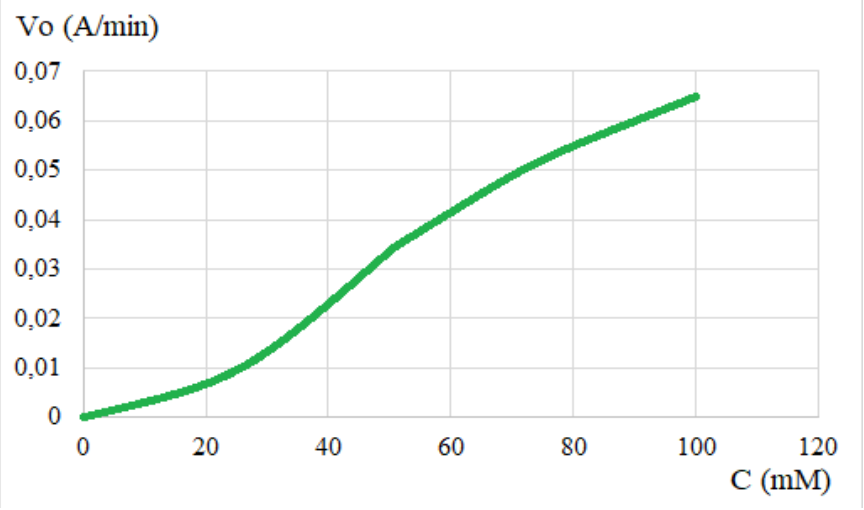

Fig. 10. Michaelis-Menten curve of the IMP enzyme

Table 5. Double inverse of *bla$_{IMP}$* carried by *Klebsiella sp.* and *bla$_{NDM}$* carried by *E. coli*

Samples of *Klebsiella sp.* (IMP) and *E. coli* (NDM)		
1/C (mM $^{-1}$)	**1/Vo de $_{IMP}$ (min/A)**	**1/Vo de $_{NDM}$ (min/A)**
0.02	29.41	21.74
0.013	19,23	13,16
0.01	15.38	10.87

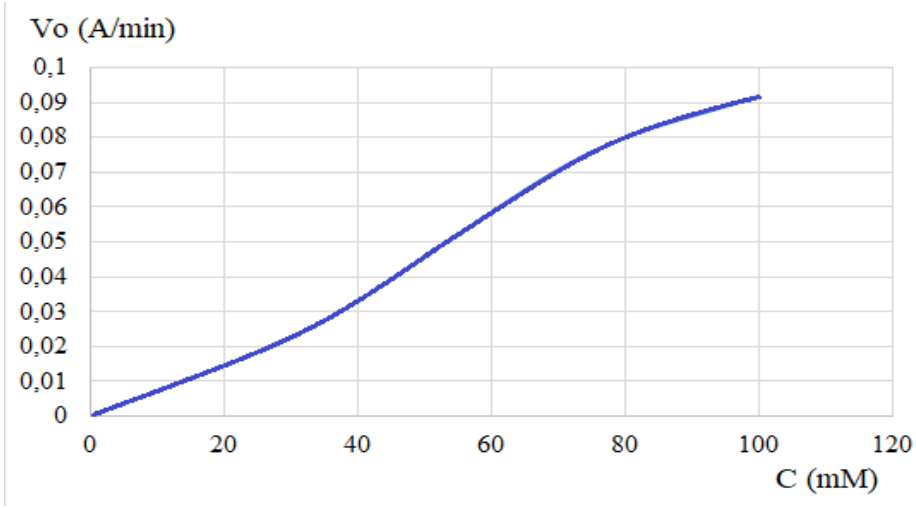

Fig. 11. Michaelis-Menten curve of the NDM enzyme

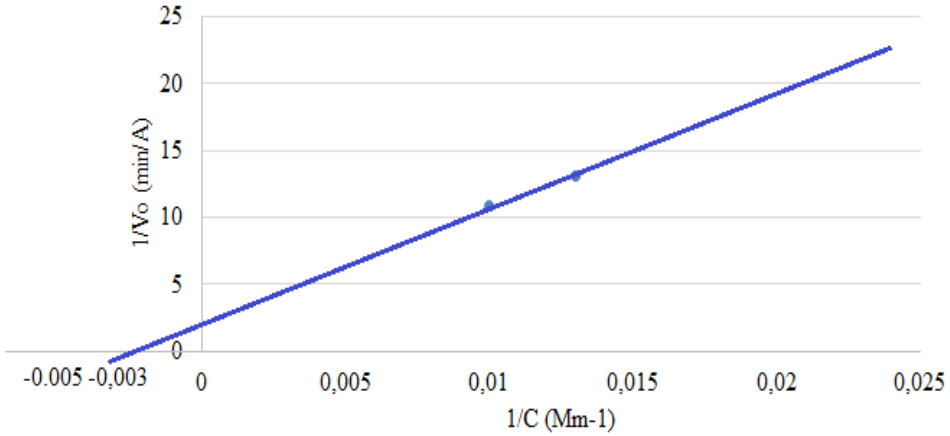

Fig. 12. Plot of the double inverse of the NDM enzyme carried by *E. coli*

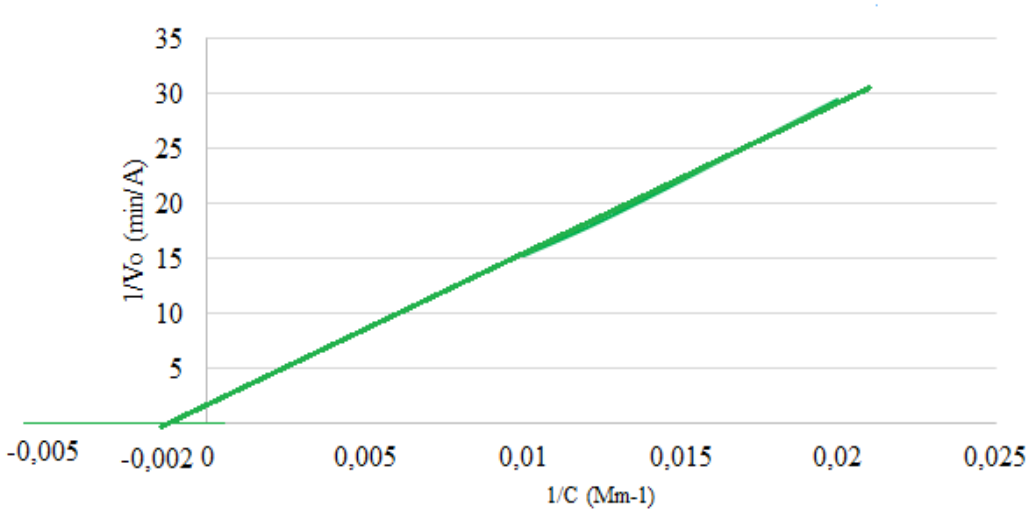

Fig. 13. Plot of the double inverse of the IMP enzyme carried by *Klebsiella sp.*

4. DISCUSSION

Gram-negative bacilli are responsible for the most common human infections. Bacillus resistance to antibiotics is a real public health problem. This resistance affects both developed and developing countries, where self-medication and the uncontrolled sale of medicines outside legal structures coexist [15]. Bacillus resistance to carbapenems can result from a variety of mechanisms. Carbapenemase production is the most powerful mechanism of resistance to carbapenems in Gram-negative bacilli. We have characterized the *bla$_{IMP}$* and *bla$_{NDM}$* genes encoding carbapenemases in gram-negative bacteria isolated from sick children at the Pediatric University Hospital Charles De Gaulle Center (CHUP-CDG), CERBA and Hospital Saint Camille in Ouagadougou, and the results of antibiotic susceptibility testing showed high resistance to Doripenem and slightly high susceptibility to Meropenem. Multiple resistance was also observed for Ertapenem, Meropenem, Imipenem and Doripenem in Gram-negative bacilli, at 64%, 41%, 53% and 73% respectively. In depth, we observed that *E. coli*, *Enterrobacter sp.*, *Klebsiella sp.*, *Proteus sp.* and *Salmonella sp.* have high resistance to Ertapenem, with rates of 67%, 100%, 62%, 67% and 50% respectively. On the other hand, sensitivity is high in Pseudomonas, with a prevalence of 57%. With Meropenem, *E. coli*, *Enterrobacter sp.*, *Klebsiella sp.*, *Proteus sp.* and *Pseudomonas sp.* had high sensitivities at 59%, 50%, 67%, 67% and 57% respectively. On the other hand, no sensitivity was observed in *Salmonella sp.* With Imipenem, resistance was high in *E. coli*, *Enterrobacter sp.*, *Klebsiella sp.*, and *Pseudomonas sp.*, with rates of 51%, 50%, 52% and 86% respectively. With Doripenem, all bacterial species were resistant. Our results are also comparable with those of Bouguessa and Amara (2018), who found that 50% of strains were resistant to Imipenem, 28.75% were intermediate and 21.25% were susceptible. For Ertapenem, only 42 strains were tested, of which, 45.24% of strains were resistant, 47.62% were susceptible and 7.14% were intermediate. These results are very similar to those reported in 2016 by Nahed et al., whose resistance to Ertapenem was 42.5%, and 45% for Imipenem [16]. El Kazzaz and Abou El khier also found in 2013 that 47% of Gram-negative pathogen clinical isolates obtained from patients admitted to Mansoura University Hospital, were resistant to Imipenem. The rate of bacterial resistance during our study, could be explained by the continued use of carbapenems in treatment due to the high prevalence of ESBL-producing strains [17]. The presence of MBL genes among carbapenem-susceptible strains indicates that phenotypic methods based on antibiotic susceptibility testing of bacterial strains do not detect all carbapenemases, leading to the silent spread of these genes in hospitals and the community. The sensitivity of phenotypic methods depends on the quantity of carbapenemases produced by the bacteria.

Analysis of PCR products by agarose gel electrophoresis revealed that of the 74 strains in total, 32% carried the IMP gene, 16% revealed the presence of the NDM gene. Our results are comparable to those found in a similar study in Tanzania, i.e. 12% of strains encoding *bla$_{IMP}$* and 4% encoding *bla$_{NDM}$* [18]. In Sudan 26.4% of strains coded for *bla$_{IMP}$* and 1.5% for bla$_{NDM}$ [19]. However, in Iraq the *bla$_{IMP}$* gene was the most represented (18.6%) followed by *bla$_{NDM}$* (1.12%) [20]. MBLs hydrolyze almost all betalactams except monobactams by a mechanism that depends on the presence of zinc ions; As a result, MBLs are inhibited by the zinc chelator EDTA [20]. Thus, the presence of zinc ions in pathological products such as urine and feces could affect the activation of these genes via an epigenetic mechanism. Indeed, strains containing inactivated *bla$_{IMP}$*, and *bla$_{NDM}$* genes, may not be detected by PCR. Metallo-β-lactamase genes have also been reported in several countries, at variable rates, such as Iran [21], the Middle East [22], India [23], Nepal [24], Sudan [19], Tunisia [25] with rates of 53.3%, 52%, 21.9%, 80.00%, 36.1% and 59% respectively. It is important to note, however, that the prevalence of carbapenemases varies from region to region, country to country, city to city, hospital to hospital and department to department [26]. These variations would depend on several factors such as surveillance measures for carbapenemase-producing bacteria, long hospital stays and antibiotic misuse. Among carbapenemase gene-positive isolates, *E. coli* species harbored more genes with a rate of 52% for *bla$_{IMP}$* and 52% for *bla$_{NDM}$*. Our results are comparable to those reported in Sudan, where *E. coli* was the species hosting more IMP and NDM carbapenemase genes at a rate of 36.1% [19]. In contrast, in Uganda, *K. pneumoniae* was the species harboring more metallo-β-lactamase genes at a rate of 52.2% [27]. Indeed, *E. coli* and *K. pneumoniae* being the majority species, it is highly likely that the number of *bla$_{IMP}$*, or *bla$_{NDM}$* genes is in the majority. The coexpression of

carbapenem resistance genes in bacilli showed that 5 strains exhibited bla_{IMP} and bla_{NDM}, i.e. 2 *E. coli* strains and 3 *Klebsiella sp.* strains. In Sudan, a 2018 study by Adam and Elhag [19] found that 2 *E. coli* and 1 *P. aeruginosa* harbored both bla_{VIM} and bla_{IMP} genes, 1 *K. pneumoniae*, 1 *E. coli* and 2 *P. aeruginosa* harbored bla_{IMP} and bla_{NDM}, 1 *K. pneumoniae*, 1 *E. coli*, 1 *P. aeruginosa* and 1 *P. vulgaris* harbored bla_{VIM}, bla_{IMP}, and bla_{NDM} at the same time. The coexpression of carbapenemase genes could be explained by the fact that these genes are carried by plasmids, which can harbor at least one resistance gene. In addition to characterizing our bacterial genes (IMP and NDM), it is also crucial to have an idea of enzyme production. Thus, Michaelis constants were determined after hydrolysis of nitrocefin by enzymatic crude extracts. In our study, the Km of the NDM enzyme carried by *E. coli* (333 µM) is lower than that of the IMP enzyme carried by *Klebsiella sp.* (500 µM). The lower the Km, the higher the affinity for the enzyme. Thus, NDM carried by *E. coli* has greater affinity than IMP carried by *Klebsiella sp.* This suggests that NDM hydrolyzes antibiotics more rapidly than IMP. Consequently, bacteria producing NDM are more formidable than those producing IMP. The kinetics of these two enzymes allow us to confirm that the majority of our strains use an enzymatic resistance mechanism [28-31].

5. CONCLUSION

Bacterial resistance to antibiotics is a public health problem in Burkina Faso and worldwide. This study enabled us to determine the rate of carbapenem resistance in Gram-negative bacteria in three laboratories in Ouagadougou, namely CERBA, Saint-Camille and Charles de Gaulle Pediatrics. This study showed that Meropenem was the most active molecule. Analysis of PCR products by agarose gel electrophoresis revealed the presence of carbapenemase-encoding resistance in certain strains. We were thus able to detect the bla_{NDM} and bla_{IMP} genes mainly in E. coli, but we were unable to detect the bla_{VIM} genes. We were also able to identify both bla_{NDM} and bla_{IMP} in bacterial strains. It also enabled us to determine the affinity of two different bacterial enzymes to an antibiotic. In our study, the antibiotic used as substrate is nitrocefin. This affinity could make it possible to reduce the time taken to administer the drugs, or even the dosage of the treatment.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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