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Research Article

Emergence of New Delhi Metallo-β-Lactamase (NDM) Genes Detected from Clinical Strains of *Escherichia coli* Isolated in Ouagadougou, Burkina Faso

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The emergence and spread of carbapenem resistance in Gram-negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* through the production of carbapenemases is a global phenomenon. It threatens patient care and leads to therapeutic impasses. This study aims to genotypically determine the prevalence of the most frequent carbapenemase genes among multidrug-resistant *E. coli* strains isolated from patients at a biomedical analysis laboratory. A total of fifty-three unduplicated *E. coli* strains isolated from patient samples with a multidrug-resistant (MDR) profile were subjected to polymerase chain reaction (PCR) testing for carbapenem resistance genes. This study allowed us to identify fifteen strains carrying resistance genes among the fifty-three *E. coli* strains. All fifteen strains produced the metallo-β-lactamase enzymes; this represents a rate of 28.30% of study strains. Among these strains, ten carried the NDM resistance gene, NDM and VIM genes were detected in three strains and VIM was identified in two strains of *E. coli*. However, carbapenemases A (KPC and IMI), D (OXA-48), and IMP were not detected in the strains studied. Thus, NDM and VIM are the main carbapenemases detected in the strains in our study.

1. Introduction

Escherichia coli (*E. coli*) is a versatile microorganism; it is a well-known commensal of the normal gut microbiome that can sometimes also be a very virulent and often deadly pathogen [1]. *E. coli* is the most frequent cause of urinary tract infections (UTI) in Burkina and is also involved in other infections such as bloodstream infections and infections of surgical wounds [2–4]. The uncontrolled use of antibiotics in recent years to fight human bacterial infection has led to selection pressure in pathogen and commensal bacteria [5]. Thus, in *E. coli,*multidrug-resistant (MDR) strains have emerged. Indeed, many antibiotic resistance genes have been described in *E. coli* such as beta-lactam resistance genes TEM, SHV, CTX-M, and quinolone resistance genes (qnr) [6–8]. The most clinically important resistance is that linked to carbapenems, which are beta-lactams of last resort used for the treatment of serious

bacterial infections [9]. Several carbapenem resistance genes have been described in *E. coli* [10]; NDM-1 (New Delhi metallo-beta-lactamase-1) is the most recently discovered transferable molecular class B. It was first described in *Klebsiella pneumoniae* and *E. coli* isolated in Sweden in 2008 from a patient transferred from a New Delhi hospital [11].

Enterobacteriaceae are responsible for a large proportion of nosocomial infections and are associated with significant morbidity and mortality; the emergence and dissemination of resistance to carbapenems pose a major public health problem. So, information of carbapenemase-producing bacteria study is limited in Burkina Faso. Knowledge of the types of resistance genes will guide the choice of appropriate antibiotic therapy. It will make it possible to formulate recommendations on the use of antibiotics as well as the implementation of infection control strategies. This study aimed to determine genotypically the prevalence of genes encoding resistance to carbapenems in *E. coli* species. This involved looking for carbapenemases: *Klebsiella pneumoniae* carbapenemase (KPC), Imipenem-hydrolyzing-β-lactamase (IMI), active on imipenem (IMP), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded for metallo-β-lactamase (VIM), and oxacillinase (OXA-48) in *E. coli* multidrug-resistant isolated in Ouagadougou, Burkina Faso.

To carry out the study, strains presenting a multidrug-resistance profile were collected and carbapenem sensitivity test was conducted on Mueller–Hinton agar (MHA) using disc method. Then, bacterial DNA extracts were made for the PCR reaction on strains showing resistance or reduced sensitivity to carbapenems.

2. Materials and Methods

2.1. Bacterial Isolates. This was a retrospective study of clinical strains collected after routine analysis. A total of 53 unduplicated strains of *E. coli* multidrug-resistant (MDR) were collected from cultured urine and pus specimens at Schiphra Hospital Medical Analysis Laboratory, from inpatient and outpatient specimens (Figure 1), from February to September 2020. Most of the strains collected came from urine culture and only four strains were isolated from pus (*E. coli* P80, *E. coli* P66, *E. coli* P46, *E. coli* P49) culture. Clinical strains were identified by standard bacteriological methods using Gram stain and biochemical method using API 20E kits (bioMérieux, Marcy-l'Étoile, France) gallery. Pure strains were inoculated into Luria Bertani broth supplemented with 20% glycerol and stored at −20°C for subsequent tests and analyses.

2.2. Antimicrobial Susceptibility Testing. Antibiotic sensitivity was performed on Mueller–Hinton agar medium (MH) according to the CA-SFM/EUCAST guidelines [12]. A bacterial suspension corresponding to 0.5 McFarland was inoculated by swab on MH medium, then the antibiotic discs were deposited. After 18–24 hours of incubation at 37°C, the reading was carried out and the strains were classified susceptible, intermediate, or resistant with respect to the critical diameter according to CA-SFM/EUCAST guidelines. Following antibiotics were tested on the strains in routine examination: Fosfomycin, Norfloxacin, Gentamicin, Cotrimoxazole, Ciprofloxacin, Levofloxacin, Ceftriaxone, Cefixime, Chloramphenicol, Cefuroxime, Imipenem, Amikacin, Ticarcillin-Clavulanate, Ceftazidime, Meropenem, Cefepime, Ticarcillin, Aztreonam, Netilmicin, Nitrofuran, and Amoxicillin-Clavulanate. The susceptibility profile to the different antibiotics of all the strains is presented by Kaboré et al. [4]. These antibiotics were used in our study because they are the first-line antibiotics used for bacterial infections. However, carbapenems were used in the study to determine resistance to these so-called last-resort antibiotics for Gram-negative infections. After the collection of the bacterial strains, the antibiogram was performed on the strains with carbapenems, imipenem, meropenem, and ertapenem (company Liofilchem, Italy). When resistance to carbapenems has been found, the Imipenem-EDTA and Imipenem-boric acid test were performed for observation of the inhibition diameter, this for phenotypic detection of metallo-beta-lactamase and carbapenemase class A [13].

2.3. Molecular Characterization for Detection of Carbapenemase-Encoding Genes

2.3.1. DNA Extraction. A total of 2-3 identical colonies of *E. coli* were resuspended in 250 μL of distilled sterile water in 1.5 mL Eppendorf tube. This was boiled at 100°C for 10 min and immediately frozen at −20°C for 10 min, centrifuged at 13000 rpm for 5 min, and the supernatant containing DNA was stored at −20°C for further using [14–17]. The presence of DNA was assessed by qualitative analysis in agarose gel electrophoresis.

2.3.2. Molecular Identification of Carbapenemase Genes. All strains of *E. coli* were tested for PCR using oligonucleotide primers specific for the carbapenem resistance genes listed in Table 1. Genes were amplified by polymerase chain reaction (PCR) on a Mastercycler nexus gradient (Eppendorf, flexlid). Reaction mixture consisted of a total volume of 25 μL; composed of 4 μL of Master Mix (Inqaba Biotec), 2 μL of primers (Forward and Reverse), 17 μL of nuclease-free water, and 2 μL of DNA extract. PCR reagents were provided by Inqaba Biotec West Africa, Nigeria.

Amplification reactions were performed in a thermal cycler (Mastercycler nexus gradient) according to the following program: initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 45 seconds, annealing at specific primer temperature (Table 1), extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes for 35 cycles. *Pseudomonas aeruginosa ATCC 27853* was used in the PCR cycle and used as a negative control for the carbapenemase genes sought.

After PCR amplification, 4 μL of each reaction were separated by 1.2% agarose gel electrophoresis for 120 min at 80 V in TAE 1X buffer in the migration tank (ENDURO™ GEL XL). DNA was stained with ethidium bromide (1 μg/mL) and amplified DNA bands were visualized using a UV transilluminator (UVP PhotoDoc-lt Imaging System).

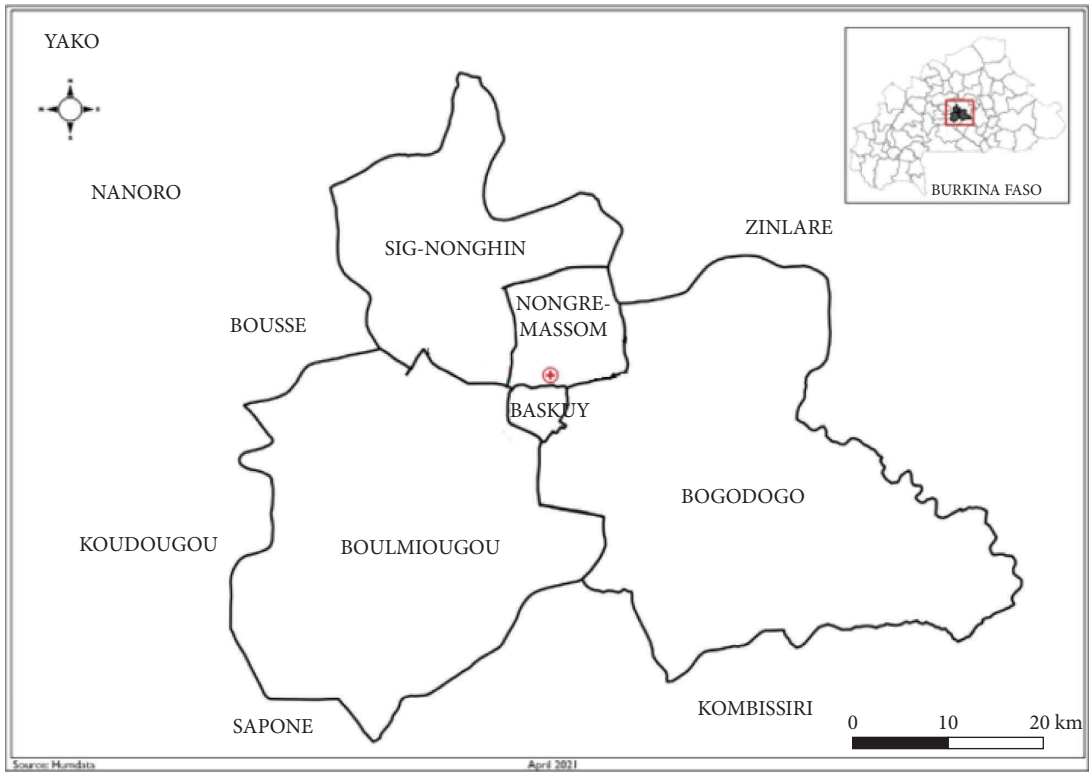

Figure 1: Health map of the city of Ouagadougou showing the sampling site at the biomedical laboratory of the Schiphra hospital. The city is divided into five health districts, and our sample collection site is in the Nongre-Massom health district.

Table 1: Primers used for detection of carbapenemases genes in *E. coli*.

Target	Sequence (5′ ⟶ 3′)	Annealing temp. °C	Amplicon size (bp)	References
IMI	F: CTACGCTTTAGACACTGGC R: AGGTTTCCTTTTCACGCTCA	57	481	[16]
KPC	F: CTGTCTTGTCTCTCATGGCC R: CCTCGCTGTRCTTGTCATCC	60	796	[18–20]
OXA-48	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTTGTGATGGC	58	744	[18]
IMP	F: GTTTATGTTCATACWTCG R: GGTTTAAYAAAACAACCAC	45	432	[21, 22]
NDM	F: TGGCAGCACACTTCCTATC R: AGATTGCCGAGCGACTTG	58	488	[23]
VIM	F: AGTGGTGAGTATCCGACAG R: TCAATCTCCGCGAGAAG	52	212	[23]

3. Results and Discussion

3.1. Antibiotic Testing. All the strains were tested on several classes of antibiotics including beta-lactams and proved to be multiresistant (MDR) [4]. The phenotypic profile of carbapenemase-producing strains detected by polymerase chain reaction is shown in Table 2.

Figure 2 shows the phenotypic profile of metallo-beta-lactamase production observed when these strains are carbapenemases-producing type MBL.

Inhibition zone ≥7 mm with the Imipenem-EDTA disk was compared to the Imipenem disk alone and was considered as MBL positive [24, 25].

It is difficult to demonstrate resistance to carbapenems by the disc diffusion method. In our study, only two (*E. coli* 1318 and *E. coli* 919) strains showed resistance by disc diffusion method, while the PCR technique revealed 15 strains showing the presence of carbapenemase genes. In fact, according to the CA-SFM [12], any strain with reduced sensitivity to at least one of the carbapenems must therefore

Table 2: Phenotypic outcome for carbapenem antibiotics from carbapenemase-producing strains.

Bacterial strains	Inhibition diameter (mm)			Patient's age (years)/sex
	Imipenem (10 μg)	Meropenem (10 μg)	Ertapenem (10 μg)	
E. coli 471	24	24	23	70/M
E. coli 919	15	00	00	62/M
E. coli 1318	10	00	00	11/F
E. coli 1473	28	30	30	79/M
E. coli 1476	27	30	29	29/F
E. coli 403	25	28	25	79/M
E. coli 405	26	27	26	85/M
E. coli 513	27	28	26	Not specified
E. coli 514	29	24	15	42/M
E. coli 583	29	29	27	77/M
E. coli 596	24	29	27	25/F
E. coli 1321	28	28	30	72/F
E. coli 1831	25	26	21	60/M
E. coli 469	26	26	24	69/F
E. coli 543	26	26	25	83/M
CA-SFM acceptable limit for *E. coli* ATCC 25922	26–32	28–35	29–36	
Target for *E. coli* ATCC 25922	29	31-32	32-33	

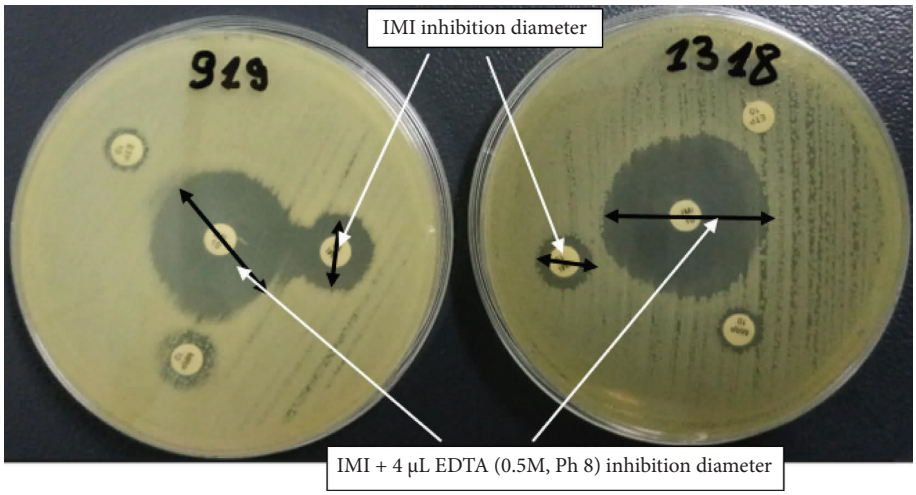

Figure 2: Phenotypic detection of metallo-beta-lactamases by combined disk test for *E. coli* 919 and *E. coli* 1318.

be considered suspicious of carbapenem production. Furthermore, the CA-SFM recalls that Ertapenem is the carbapenem with the best sensitivity for the detection of carbapenem-producing strains. Thus, any strain showing reduced sensitivity to Ertapenem (MIC >0.5 mg/L or an inhibition diameter (10 μg/ml disk) <25 mm) by the agar diffusion test can be subjected to the screening algorithm for carbapenemase-producing strains. Given the results of sensitivity to carbapenems, it should not be assumed that the strains (*E. coli* 1473, *E. coli* 1476, and *E. coli* 1321) can be detected as carbapenemase producers because these strains present a phenotypic sensitivity to carbapenems and to Ertapenem considered carbapenem of choice for the detection of carbapenemase-producing strains. The average age of the patients is 60.21 years with extremes of 11 years and 85 years. Male sex is predominant at 64.28% ($n = 9$) with extreme ages 60 years and over except one male patient who is 42 years old. At these ages, infections are more frequent with recurrent antibiotic treatments resulting in selection pressure for resistant strains.

3.2. Carbapenemase Genes. Among the strains of *E. coli*, including 49 from urinary infections and 4 from pus, we found ten strains whose PCR reaction products showed bands corresponding to the bands of the NDM genes at 488 bp, which represents a rate of 18.87% and PCR products from two strains showed bands at 212 bp corresponding to VIM. However, we found that the PCR products of three strains showed two bands at both 488 bp and 212 bp (NDM and VIM); these are *E. coli* 1318, *E. coli 514,* and *E. coli 583* strains (Table 3). Indeed, the PCR did not reveal the presence of any bands for the genes corresponding to base pairs 481 (IMI), 796 (KPC), 744 (OXA-48 likes), and 432 (IMP). No bands corresponding to a carbapenemase gene were observed with the PCR products of the DNA extracts of the

Table 3: Antibiotic resistance genes screened in *E. coli* strains.

Species	Carbapenemase A		Carbapenemase B		Carbapenemase D	
	bla_{KPC}	bla_{IMI}	bla_{NDM}	bla_{IMP}	bla_{VIM}	bla_{OXA-48}
E. coli 471	–	–	–	–	+	–
E. coli 919	–	–	+	–	–	–
E. coli 1318	–	–	+	–	+	–
E. coli 1473	–	–	+	–	–	–
E. coli 1476	–	–	+	–	–	–
E. coli 403	–	–	+	–	–	–
E. coli 405	–	–	+	–	–	–
E. coli 513	–	–	-	–	+	–
E. coli 514	–	–	+	–	+	–
E. coli 583	–	–	+	–	+	–
E. coli 596	–	–	+	–	–	–
E. coli 1321	–	–	+	–	–	–
E. coli 1831	–	–	+	–	–	–
E. coli 469	–	–	+	–	–	–
E. coli 543	–	–	+	–	–	–

(+): resistance gene detected; (–): resistance gene not detected.

strains from pus samples, namely: *E. coli* P80, *E. coli* P66, *E. coli* P46, and *E. coli* P49. Figure 3 shows the band profile of the NDM resistance genes visualized with the UVP PhotoDoc-lt imaging system after migration, and Figure 4 shows the band profile of the VIM resistance genes.

Table 3 shows the result of the genotypic investigation of the resistance genes sought, with (–) indicating that the gene sought was not detected and (+) indicating that the gene sought was detected.

The presence of carbapenemase-producing bacteria in hospitals poses a serious challenge. The problem with carbapenemases is that most of the resistance genes are located on mobile elements and are therefore easily transferred from one bacterial species to another [26–28]. Indeed, out of 53 multidrug-resistant *E. coli* strains, we identified 15 strains producing metallo-beta-lactamase genes, of which ten harboured the NDM gene, two strains harboured the VIM gene, and three harboured both genes at the same time (NDM and VIM). All these strains came mainly from urinary infections (49 strains were of urinary origin and 4 came from pus). The rate of carbapenemase-producing *E. coli* among the multidrug-resistant strains was 28.30%, which confirms the multidrug-resistant nature of these strains. In our study, only carbapenemase B (NDM and VIM) were detected in *E. coli* strains, which could explain their rapid diffusion within the bacterial strains. Other resistance genes were not researched and the strains could harbour these genes, hence their multidrug-resistance; since these genes have already been described in *E. coli* in Burkina Faso (TEM, SHV, CTX-M,. . .), so genes for resistance to other families of antibiotics (quinolones, aminoglycosides, etc.) were not sought. However, previous studies had revealed a high rate of extended-spectrum beta-lactamases among clinical strains in Burkina Faso [6, 7, 29]. In West Africa, the prevalence of ESBL varies from one country to another; thus, rates of 49.4% and 63.4% to 96% have been reported respectively in Ghana and Mali in hospitals and in the community [30]. Our study revealed the presence of DNA bands at 488 bp for NDM and 212 bp for VIM of the PCR

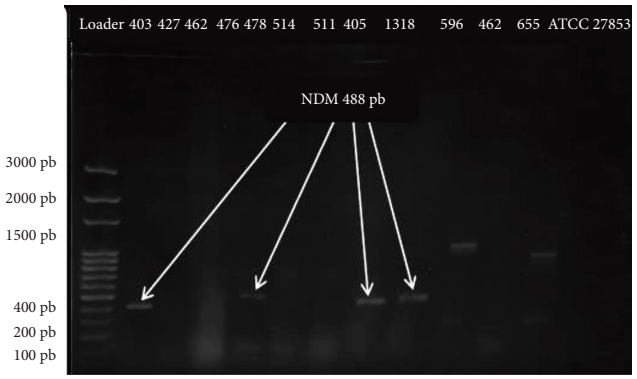

Figure 3: Agarose gel electrophoresis of NDM gene amplicons (488 bp).

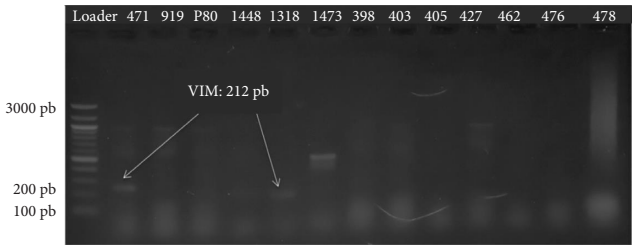

Figure 4: Agarose gel electrophoresis of VIM gene amplicons (212 bp).

reaction products of *E. coli* strains. Indeed, the presence of carbapenemase genes of the NDM, VIM, and OXA type had been detected in strains of *E. coli* and other Gram-negative bacilli [29]. An OXA-181-like carbapenemase was detected in four species of *E. coli* for the first time in Burkina Faso [31]. These authors have been able to demonstrate the plasmids on which these genes are located, namely IncX3 for the OXA-181 type genes and *Inc*F for the NDM-1 genes. In West Africa, few studies have been reported on the resistance of carbapenemase-producing Gram-negative bacilli. However, some case studies in Togo, Mali, and Nigeria have

reported the presence of OXA and NDM genes [32–34]. NDM, OXA, and VIM genes have been reported in most African countries [35]. The movement of populations for economic and health reasons could explain the rapid spread of the NDM gene around the world; indeed, travelers to endemic areas are at risk of exposure to resistant pathogens and are likely to return colonized [36, 37]. Described in *Klebsiella pneumoniae* isolated from a Swedish patient of Indian origin with a urinary tract infection in New Delhi, India, and designated NDM-1 [12], more than twenty NDM variants have been described to date [38]. The genetic variability of the NDM gene could explain its worldwide distribution. Asian continent serves as the major reservoir of NDM producers, with around 58.15% abundance of NDM-1 variant distributed mostly in China and India [39].

3.2.1. Limit of the Study. We did not have the opportunity to confirm the expression of carbapenemase genes by DNA sequencing technique.

4. Conclusion

Our study highlighted the presence of resistance genes NDM and VIM among the clinical strains of *E. coli* in Burkina. NDM and VIM enzymes hinder the action of beta-lactams and other antibiotics, resulting in therapeutic impasses. Therefore, increased surveillance should prevent the spread of these resistance genes, which are a major concern worldwide. Finally, a structural study of these enzymes could make it possible to develop new molecules in association with beta-lactams to combat these resistances.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Investigation, analysis, and original draft preparation are realized by KB, HSO, AGO, and SB. Paper is revised by OZ, FT, KJZ, and BZ. The validation of results from investigation and review of paper are supervised by YT, IS, and AS. All authors have read and approved the final manuscript.

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References

[1] P. Cools, "The role of *Escherichia coli* in reproductive health: state of the art," *Research in Microbiology*, vol. 168, no. 9-10, pp. 892–901, 2017.

[2] F. Kaboré, T. Kambou, B. Zango et al., "Épidémiologie d'une Cohorte de 450 Lithiases Urinaires Au CHU Yalgado Ouédraogo de Ouagadougou (Burkina Faso)," *Progrès en Urologie: journal de l'Association francaise d'urologie et de la Societe francaise d'urologie*, vol. 23, no. 12, pp. 971–976, 2013.

[3] A. S. Ouédraogo, D. Somé, P. W. H. Dakouré et al., "Profil Bactériologique des Infections du site opératoire au Centre Hospitalier Universitaire Souro Sanou de Bobo Dioulasso," *Medecine Tropicale*, vol. 71, no. 1, pp. 49–52, 2011.

[4] B. Kaboré, G. A. Ouédraogo, H. Cissé et al., "PCR fingerprinting of multi - drug resistant Escherichia coli bacteria isolates from hospital in Ouagadougou, Burkina Faso," *BMC Microbiology*, vol. 22, no. 1, pp. 118–8, 2022.

[5] A. Szmolka and B. Nagy, "Multidrug resistant commensal Escherichia coli in animals and its impact for public health," *Frontiers in Microbiology*, vol. 4, pp. 258–13, 2013.

[6] K. J. Zongo, A. Metuor Dabire, L. G. Compaore et al., "First detection of bla TEM, SHV and CTX-M among gram negative bacilli exhibiting extended spectrum -lactamase phenotype isolated at university hospital center, yalgado ouedraogo, Ouagadougou, Burkina Faso," *African Journal of Biotechnology*, vol. 14, no. 14, pp. 1174–1180, 2015.

[7] D. S. Kpoda, A. Ajayi, M. Somda et al., "Distribution of resistance genes encoding ESBLs in enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso," *BMC Research Notes*, vol. 11, no. 1, pp. 471–479, 2018.

[8] F. D. Salah, S. T. Soubeiga, A. K. Ouattara et al., "Distribution of quinolone resistance gene (qnr) in ESBL- producing *Escherichia coli* and Klebsiella spp. in lomé, Togo," *Antimicrobial Resistance and Infection Control*, vol. 8, pp. 104–108, 2019.

[9] J. D. Lutgring and B. M. Limbago, "The problem of carbapenemase-producing-carbapenem-resistant- enterobacteriaceae detection," *Journal of Clinical Microbiology*, vol. 54, no. 3, pp. 529–534, 2016.

[10] H. Grundmann, C. Glasner, B. Albiger et al., "Occurrence of carbapenemase-producing Klebsiella pneumoniae and *Escherichia coli* in the European survey of carbapenemase-producing enterobacteriaceae (EuSCAPE): a prospective, multinational study," *The Lancet Infectious Diseases*, vol. 17, no. 2, pp. 153–163, 2017.

[11] D. Yong, M. A. Toleman, C. G. Giske et al., "Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India," *Antimicrobial Agents and Chemotherapy*, vol. 53, no. 12, pp. 5046–5054, 2009.

[12] F. Jehl, J. P. Bru, F. Caron et al., "Comite de l 'antibiogramme de la Societe Francaise de Microbiologie," *Societe Francaise Microbiol*, vol. 1, no. 1, p. 181, 2020.

[13] J. Cohen Stuart and M. A. Leverstein-Van Hall, "Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae," *International Journal of Antimicrobial Agents*, vol. 36, no. 3, pp. 205–210, 2010.

[14] M. Purohit, D. K. Mendiratta, V. S. Deotale, M. Madhan, A. Manoharan, and P. Narang, "Detection of metallo-β-lactamases producing acinetobacter baumannii using microbiological assay, disc synergy test and PCR," *Indian Journal of Medical Microbiology*, vol. 30, no. 4, pp. 456–461, 2012.

[15] V. Cattoir, L. Poirel, V. Rotimi, C. J. Soussy, and P. Nordmann, "Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-

producing enterobacterial isolates," *Journal of Antimicrobial Chemotherapy*, vol. 60, no. 2, pp. 394–397, 2007.

[16] P. Mlynarcik, M. Roderova, and M. Kolar, "Primer evaluation for PCR and its application for detection of carbapenemases in enterobacteriaceae," *Jundishapur Journal of Microbiology*, vol. 9, no. 1, pp. e29314–e29316, 2016.

[17] S. Khalid, N. Ahmad, S. M. Ali, and A. U. Khan, "Outbreak of efficiently transferred carbapenem-resistant blaNDM-producing gram-negative bacilli isolated from neonatal intensive care unit of an Indian hospital," *Microbial Drug Resistance*, vol. 26, no. 3, pp. 284–289, 2020.

[18] L. Poirel, C. Héritier, V. Tolün, and P. Nordmann, "Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 1, pp. 15–22, 2004.

[19] G. Cuzon, T. Naas, H. V. Truong et al., "Worldwide diversity of *Klebsiella pneumoniae* that produce β-lactamase blaKPC-2 gene," *Emerging Infectious Diseases*, vol. 16, no. 9, pp. 1349–1356, 2010.

[20] B. E. Lixandru, A. I. Cotar, M. Straut et al., "Carbapenemase-producing Klebsiella pneumoniae in Romania: a six-month survey," *PLoS One*, vol. 10, no. 11, p. e0143214, 2015.

[21] K. M. Hujer, A. M. Hujer, E. A. Hulten et al., "Analysis of antibiotic resistance genes in multidrug-resistant acinetobacter sp . Isolates from military and civilian patients treated at the walter reed army medical center," *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 12, pp. 4114–4123, 2006.

[22] M. S. Amudhan, U. Sekar, A. Kamalanathan, and S. Balaraman, "Bla IMP and bla VIM mediated carbapenem resistance in Pseudomonas and acinetobacter species in India," *J. Infect. Dev. Ctries.*vol. 6, no. 11, pp. 757–762, 2012.

[23] T. Kirtikliene, N. Donatas, S. Ana et al., "Evaluation of the inter- and intrahospital spread of multidrug resistant gram-negative bacteria in Lithuanian hospitals," *Microbial Drug Resistance*, vol. 25, no. 3, pp. 1–10, 2018.

[24] M. Anwar, H. Ejaz, A. Zafar, and H. Hamid, "Phenotypic detection of metallo-beta-lactamases in carbapenem resistant Acinetobacter baumannii isolated from pediatric patients in Pakistan," *Journal of pathogens*, vol. 2016, Article ID 8603964, 6 pages, 2016.

[25] Z. Moulana, A. Babazadeh, Z. Eslamdost, M. Shokri, and S. Ebrahimpour, "Phenotypic and genotypic detection of metallo-beta-lactamases in Carbapenem resistant Acinetobacter baumannii," *Caspian journal of internal medicine*, vol. 11, no. 2, pp. 171–176, 2020.

[26] S. R. Partridge, S. M. Kwong, N. Firth, and S. O. Jensen, "Mobile genetic elements associated with antimicrobial resistance," *Clinical Microbiology Reviews*, vol. 31, no. 4, p. e00088-17, 2018.

[27] N. P. Marathe, F. Berglund, M. Razavi et al., "Sewage effluent from an Indian hospital harbors novel carbapenemases and integron-borne antibiotic resistance genes," *Microbiome*, vol. 7, no. 1, p. 97, 2019.

[28] N. Kieffer, S. Ebmeyer, and D. J. Larsson, "Evidence for Pseudoxanthomonas mexicana as the recent origin of the blaAIM-1 carbapenemase gene," *International Journal of Antimicrobial Agents*, vol. 59, no. 4, Article ID 106571, 2022.

[29] S. Sanou, A. S. Ouedraogo, S. Aberkane et al., "Prevalence and molecular characterization of extended spectrum β-lactamase, plasmid-mediated quinolone resistance, and carbapenemase-producing gram-negative bacilli in Burkina Faso," *Microbial Drug Resistance*, vol. 27, no. 1, pp. 18–24, 2021.

[30] V. Storberg, "ESBL-producing Enterobacteriaceae in Africa–a non-systematic literature review of research published 2008–2012," *Infection Ecology and Epidemiology*, vol. 4, no. 1, Article ID 20342, 2014.

[31] A. S. Ouédraogo, F. Compain, M. Sanou et al., "First description of IncX3 plasmids carrying blaOXA-181 in Escherichia coli clinical isolates in Burkina Faso," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 5, pp. 3240–3242, 2016.

[32] S. Dossim, A. B. Rémy, S. Mounerou, and T. Kpatcha, "Occurence of carbapenemase-producing enterobacteriaceae in Togo," *International Journal of Antimicrobial Agents*, vol. 53, no. 4, pp. 530–532, 2018.

[33] A. Muggeo, A. Maiga, I. Maiga et al., "First description of IncX3 NDM-5-producing plasmid within *Escherichia coli* ST448 in Mali," *Journal of Medical Microbiology*, vol. 69, no. 5, pp. 685–688, 2020.

[34] A. Olowo-okere, Y. K. E. Ibrahim, B. O. Olayinka et al., "Phenotypic and genotypic characterization of clinical carbapenem- resistant enterobacteriaceae isolates from sokoto, northwest Nigeria," *New Microbes and New Infections*, vol. 37, Article ID 100727, 2020.

[35] R. I. Manenzhe, H. J. Zar, M. P. Nicol, and M. Kaba, "The spread of carbapenemase-producing bacteria in Africa: a systematic review," *Journal of Antimicrobial Chemotherapy*, vol. 70, no. 1, pp. 23–40, 2015.

[36] Z. Jian, L. Zeng, T. Xu et al., "Antibiotic resistance genes in bacteria: occurrence, spread, and control," *Journal of Basic Microbiology*, vol. 61, no. 12, pp. 1049–1070, 2021.

[37] I. Frost, T. P. Van Boeckel, J. Pires, J. Craig, and R. Laxminarayan, "Global geographic trends in antimicrobial resistance: the role of international travel," *Journal of Travel Medicine*, vol. 26, no. 8, p. taz036, 2019.

[38] Z. Liu, J. Li, X. Wang et al., "Novel variant of New Delhi metallo-β-lactamase, NDM-20, in *Escherichia coli*," *Frontiers in Microbiology*, vol. 9, p. 248, 2018.

[39] A. U. Khan, L. Maryam, and R. Zarrilli, "Structure, genetics and worldwide spread of New Delhi metallo-β-lactamase (NDM): a threat to public health," *BMC Microbiology*, vol. 17, pp. 101–112, 2017.