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Detection of *bla*$_{VIM}$ Gene in β-Lactam Resistant *E. coli* Isolated from Clinical Samples in Ouagadougou, Burkina Faso

Abdoul Karim Ouattara[1,2*#], Blandine Ouédraogo[1#], Rahimatou Yasmine Wendkuni Tiemtoré[1], Amana Mètuor Dabiré[1,3], Serge Sougué[1], Jacques Simporé[1,4]

[1]Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE), UFR-SVT, Université Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[2]Centre Universitaire de Manga, Université Norbert Zongo, , Koudougou, Burkina Faso
[3]Département de Biochimie-Microbiologie, Université de Dédougou, Dédougou, Burkina Faso
[4]Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso
Email: *ak.ouattara02@gmail.com

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Abstract

Escherichia coli, a Gram-negative bacterium, is commonly associated community and hospital-acquired infections, with significant antibiotic resistance implications for public health. This study aimed to detect the *bla*$_{VIM}$ gene in *E. coli* strains isolated from the Centre Hospitalier Universitaire Pédiatrique Charles De Gaulle (CHUP-CDG) in Ouagadougou, Burkina Faso. *E. coli* strains were isolated from various biological samples (urine, pus, blood, stool, and cerebrospinal fluid) from 2009 to 2013 at CHUP-CDG. Antibiotic susceptibility testing for cefotaxime, ceftazidime, and imipenem was performed using the disc diffusion method on Mueller-Hinton agar. Classical PCR was used to identify the *bla*$_{VIM}$ gene. The susceptibility test showed high resistance of strains to third generation cephalosporins. The resistance rate was 82.86% (29/35), 80.00% (28/35) and 11.42% (4/35) for cefotaxime, ceftriaxone, and imipenem, respectively. Analysis of PCR products revealed that 11.42% (4/35) of strains harbored the *bla*$_{VIM}$ gene. Significantly, 75.00% (3/4) of strains with the *bla*$_{VIM}$ gene were isolated from urine samples. The present study demonstrated the presence of the *bla*$_{VIM}$ gene in *E. coli* resistant strains to β-lactams at CHUP-CDG. Our results suggest the presence of other resistance genes in view of the low rate of *bla*$_{VIM}$ found in resistant strains. Surveillance measures are necessary to prevent the spread of these resistant strains.

*Corresponding author.
#These authors contributed equally to this work.

Subject Areas

Microbiology

Keywords

E. coli, *Bla*$_{VIM}$, Cephalosporins, Multidrug Resistance, Burkina Faso

1. Introduction

Escherichia coli is a bacterial species in the genus Escherichia and belonging to the *Enterobacteriaceae* family [1]. Most strains of *E. coli* are non-pathogenic agents in the intestinal flora of humans and animals. However, some strains have acquired virulence factors that allow them to cause significant intestinal and extraintestinal pathology [2]. Intestinal and extraintestinal infections caused by pathogenic *E. coli* include urinary tract infections [3], neonatal meningitis [4], sepsis [5], skin infections and colisepticemia [6]. *E. coli* can survive and adapt in a wide range of external conditions; and it can spread between humans and animals through various routes and cause disease, hence the use of appropriate antibiotics to inhibit or destroy this pathogen [2].

Antibiotics are still needed for the treatment of bacterial diseases worldwide, but antibiotic resistance due to irrational use has become a serious public health problem [7]. *E. coli* resistance to antibiotics may be due to the production of metallo-β-lactamases (MBLs). Metallo-β-lactamases are a group of enzymes that hydrolyze β-lactams, including carbapenems [8]. The first MBL-like VIM enzyme was discovered in *P. aeruginosa* in 1997, and since then, more than 69 variants have been reported [9]. MBLs can spread horizontally through *Enterobacteriaceae* and other clinically important gram-negative bacteria via mobile genetic elements [10]. Furthermore, research has revealed that MBLs are widely distributed in various geographical locations. It is considered a serious concern as they lead to therapeutic impasses [8]. Beta-lactamases with carbapenemase activity are the most potent mechanisms of carbapenem resistance. However, the distribution of carbapenemase genes is poorly documented in Burkina Faso.

The main objective of this study was to identify the VIM-type carbapenemase gene carried by *E. coli* strains at the Centre Hospitalier Universitaire Pédiatrique Charles De Gaulle (CHUP-CDG) of Ouagadougou, Burkina Faso.

2. Materials and Methods

2.1. Type and Period of Study

This was a retrospective collection descriptive study of bacterial samples responsible for human infections [9] [10]. The study was conducted at the Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA)/Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE) from June to September 2021.

2.2. Sampling

The biological material consisted of 35 *Escherichia coli* isolates collected between 2009 and 2013 from sick children at the CHUP-CDG of Ouagadougou in Burkina Faso [11] [12]. These strains were isolated from various biological samples such as urine, pus, cerebrospinal fluid (CSF), stool and blood. The strains were stored at −80˚C in Luria Bertani (LB) supplemented with 30% glycerol at CERBA.

2.3. Strain Sensitivity Testing

The disk diffusion method was used to perform the antibiotic susceptibility test of the strains on Mueller-Hinton agar (MH), while following the guidelines provided by the Antibiogram Committee of the French Microbiology Society [13]. The interpretation of the results was based on the categorization of the strains into either Susceptible (S) or Resistant (R) to the tested antibiotics, which included ceftriaxone (CRO), cefotaxime (CTX), and imipenem (IMP).

2.4. DNA Extraction

DNA extraction was carried out using the boiling method [14]. An isolated colony was taken from the MH petri dishes and suspended in 200 µL of distilled water in previously labeled Eppendorf tubes. The tube was then placed in a water bath at 100˚C for 15 minutes to release the bacterial genetic material. The supernatant, which contained the released DNA, was transferred to a new Eppendorf tube after a 10-minute centrifugation at 12,000 rpm. The quantity and purity of the DNA extract were assessed using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, United States) and the DNA extract was stored at −20˚C until molecular analysis.

2.5. Gene Amplification

Conventional PCR was performed using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, California, USA) in a 20 µL reaction mixture. This reaction mixture 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 DNA extract from each strain. PCR amplification of the bla_{VIM} gene was performed with the following specific primers: VIM-F 5’ GTTTGGTCGCATATCGCAAC 3’ and VIM-R 5’ AATGCGCAGCACCAGGATAG 3’ with an expected amplicon of 382 bp (Shams, *et al*., 2018). The PCR program used in this study consisted of an initial denaturation step at 96˚C for 5 minutes, followed by 30 cycles of denaturation at 96˚C for 30 seconds, hybridization at 61˚C for 30 seconds, and elongation at 72˚C for 30 seconds. A final elongation step was performed at 72˚C for 7 minutes after the completion of the 30 cycles.

2.6. Agarose Gel Electrophoresis

The amplified DNA fragments obtained through PCR were separated by agarose

gel electrophoresis, which consisted of a 1.5% gel prepared in a 1X tris base-borate-EDTA solution with 0.5 µg/mL of ethidium bromide. A volume of 8 µL of the amplicons was added to each well of the gel, starting from the second well, while the first well was filled with 8 µL of the 100-bp molecular weight marker. Electrophoresis was carried out for 25 minutes at 100 V. After the migration, the gels were visualized using the GeneFlash apparatus (Syngene, Cambridge, UK) under UV light at 365 nm.

2.7. Ethics Approval

The protocol of the current study was reviewed and approved by the institutional ethics committee of CERBA/LABIOGENE.

2.8. Statistical Analyses

The collected data were entered into Microsoft Excel 2019 and then analyzed with statistics and data (STATA) software (Stata Corporation, College Station, TX, USA). The results for categorical variables were presented in terms of frequency and percentage (%).

3. Results

Sensitivity testing on 35 strains of *E. coli*, showed high resistance to third generation cephalosporins. The resistance rate was 82.85% (29/35) for cefotaxime and 80.00% (28/35) for ceftriaxone with 11.42% (4/35) of resistance rate to imipenem. Table 1 shows the distribution of resistance by biological sample.

PCR product examination via agarose gel electrophoresis enabled the detection of 4 strains (11.42%) harboring the bla_{VIM} gene. Figure 1 shows the electrophoretic profile of the bla_{VIM} gene at 382 bp. The majority (75% or 3/4) of the bla_{VIM} gene-carrying strains were from urine samples (Table 2).

4. Discussions

Antimicrobial resistance is a serious threat to human and animal health [15]. Carbapenems, particularly imipenem, were once considered first-line drugs in the treatment of severe bacterial infections [10]. However, in recent years, there

Table 1. Distribution of resistance by biological samples.

Biological samples	Antibiotics		
	CTX	CAZ	IMP
Urine	16 (45.71%)	15 (42.85%)	3 (8.57%)
Pus	10 (28.57%)	10 (28.57%)	1 (2.85%)
Blood	1 (2.85%)	1 (2.85%)	0 (00%)
Stool	1 (2.85%)	1 (2.85%)	0 (00%)
Cerebrospinal fluid	1 (2.85%)	1 (2.85%)	0 (00%)
Total	29 (82.85%)	28 (80.00%)	4 (11.42%)

Table 2. Distribution of the *bla*$_{VIM}$ gene by biological samples.

Biological samples	*Bla*$_{VIM}$ gene
Urine	3 (8.57%)
Pus	1 (2.85%)
Blood	0 (00%)
Stool	0 (00%)
Cerebrospinal fluid	0 (00%)
Total	4 (11.42%)

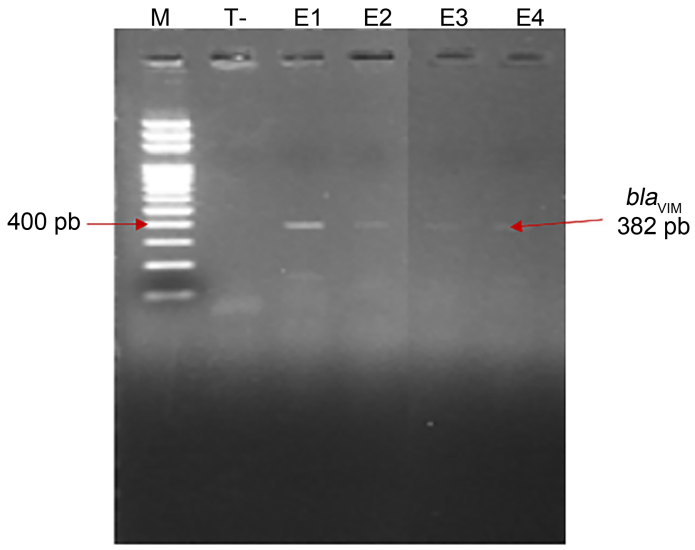

Figure 1. Electrophoretic profile of the *bla*$_{VIM}$ gene at 382 bp. M: Molecular weight marker (100 bp DNA Ladder). T-: Negative control. Samples are labeled E1-E4. The direction of electrophoresis migration is from top to bottom.

has been a significant growth in antibiotic resistance, which may limit treatment options [16]. The objective of this study was to characterize the *bla*$_{VIM}$ gene within *E. coli* strains isolated from sick children from 2009 to 2013 at the CHUP-CDG of Ouagadougou, Burkina Faso.

In the present study, the results of antibiotic susceptibility testing of strains showed high resistance to cefotaxime (82.85%) and ceftriaxone (80.00%). A recent study conducted in the cities of Boromo and Gourcy in Burkina Faso, showed high rates of resistance of *E. coli* and Salmonella isolated from diarrheic children to common antibiotics [17]. High resistance of *E. coli* to ceftriaxone (64.3%; 9/14) was also reported in febrile children under 5 years old in Nanoro, Burkina Faso [18]. In addition, resistance to third generation cephalosporins has also been reported in clinical isolates of *E. coli* in Egypt [19], Togo [20] and Libya [21]. This high level of cephalosporin resistance could be due to the acquisition of antibiotic resistance factors [14] [22]. Several bacterial isolates that were found to produce extended-spectrum β-lactamases and exhibit resistance to

multiple antibiotics were previously reported by our research team [11] [20] [21] [22].

The strains showed the highest susceptibility to imipenem among all β-lactam molecules tested, which confirms its status as the first-line treatment for severe infections caused by multidrug-resistant bacteria [23]. The resistant strains identified in this study were primarily isolated from urine samples as previously reported in studies conducted in Burkina Faso [22] [24] [25], Niger [26] and Togo [27] [28].

PCR detection of carbapenem resistance genes showed that 11.42% (4/35) of strains carried the bla_{VIM} gene. Carbapenems are the treatment of choice for ESBL-producing *Enterobacteriaceae*. Nonetheless, the public health concern is increasing due to the emergence and spread of carbapenemase-producing *Enterobacteriaceae*, which limits the available therapeutic options. The bla_{VIM} gene was reported in two imipenem-resistant *E. coli* isolates in a previous study in Burkina Faso [17]. A study in Nigeria reported the bla_{VIM} gene in four carbapenem-resistant, metallo-β-lactamase (MBL)-producing strains of Pseudomonas aeruginosa [29]. Metallo-β-lactamases are a particular concern due to their increasing prevalence worldwide, especially in Asia, and their ability to resist most recently licensed β-lactam-β-lactamase inhibitors.

The VIM enzymes, which are of this type, are typically encoded by gene cassettes located in either class 1 or class 3 integrons [30]. Studies conducted in Pakistan have reported the presence of VIM-type metallo-β-lactamases in *E. coli*. One of these studies found that out of 145 *E. coli* isolates, 50 isolates (34.48%) were MBL producers and that the bla_{VIM} gene was carried by 8 strains [31]. Another study reported that 15.1% of MBL-producing *E. coli* strains harbored the bla_{VIM} gene [11]. Given the expansion of these MBL-producing clinical isolates, it is important to sustain surveillance efforts and develop new therapeutic solutions, particularly in developing countries such as Burkina Faso.

5. Conclusion

The present study has revealed a significant resistance among clinical strains of *E. coli* to third generation cephalosporins, despite a weak presence of the bla_{VIM} gene. Notably, cefotaxime and ceftriaxone exhibited high resistance rates, while imipenem showed lower resistance rates. The low prevalence of the bla_{VIM} gene suggests the involvement of other resistance genes, necessitating enhanced surveillance to effectively prevent and control the dissemination of metallo-β-lactamase-producing bacteria.

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Conflicts of Interest

The authors declare no conflicts of interest.

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