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Detection of *aac*(3)*IIc*, *aac*(6)*Ib*, *armA* Genes Coding for *Escherichia coli* Resistance to Aminoglycosides in Burkina Faso

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Abstract

Background and Prupose: Antibiotic resistance is a major global health concern. In addition to the existing data on the prevalence of bacterial resistance to antibiotics, there are patchy data on bacterial resistance to aminoglycosides in Burkina Faso. In this study, we determined the prevalence of aminoglycoside resistance genes in *E. coli*, including *aac*(3)-*IIc*, *aac*(6)-*Ib* and *armA* in Ouagadougou, and determined which antibiotics in this class are most affected by resistance. **Material and Methods:** This study was conducted on 216 *E. coli* strains collected from the biomedical analysis laboratories of Saint Camille and Schiphra hospitals. *E. coli* strains were isolated from pus and urine samples collected between September 2018 and January 2019. Antibiotic susceptibility testing was performed using aminoglycosides, β-lactams, fluoroquinolones, and sulfonamides. Aminoglycoside resistance genes were detected in strains with at least one aminoglycoside resistance gene using conventional/multiplex PCR. **Results:** Aminoglycoside resistance was observed in 46.8% (101/216) of strains. The resistance rates were respectively 45.37% for Tobramycin, 32.40% for Gentamicin, 14.81% for Kanamycin, 2.31% for Netilmicin, 1.84% for Neomycin, and 0.46% for Amikacin. PCR showed that 86 strains (85.15%) possessed the *aac*(3)-*IIc* gene, 71 strains or 70.30%) possessed the *aac*(6')-*Ib* gene, and nine strains (8.91%) possessed the *armA* gene. **Conclusion:** Aminoglycoside resistance in pathogenic *E. coli* strains is mainly

due to the presence of the *aac*(3')-*IIc* and *aac*(6')-*Ib* genes. The presence of *armA* was first reported in Burkina Faso. Netilmicin, Neomycin and Amikacin are good therapeutic options for treating urinary tract and pus-forming infections.

Keywords

E. coli, Aminoglycoside Resistance, *acc*(3')-*IIc*, *aac*(6')-*Ib*, *armA*, Burkina Faso

1. Introduction

E. coli is the most common bacterial species in human pathology. It is responsible for more than 80% of tract infections [1] [2]. It can cause several types of infections, including intestinal infections in the form of enteritis, urinary tract infections (uropathogenic strains are responsible for 60% - 80% of urinary tract infections), and other infections (septicemia, neonatal meningitis, and suppurations) [3] [4] [5]. Aminoglycosides are a family of antibiotics used to treat infections caused by gram-negative bacteria, including *E. coli* [6] [7]. They inhibit protein synthesis by binding with high affinity to the A site of the 16S ribosomal RNA of the 30S ribosome [8]. Bacterial resistance to aminoglycosides can result from both chromosomal mutations and the acquisition of mobile genetic elements (plasmids, integrons, and transposons) [9] [10]. This can be caused by one or more of the following mechanisms: aminoglycoside-modifying enzymes, decreased membrane permeability, limited accessibility of aminoglycoside into the cell, structural alteration in the target delaying the attachment of the drug to its site of action, and extrusion of the drug from the cell by efflux pumps [11].

The aminoglycoside-modifying enzyme (AME) is the most important mechanism for resistance to aminoglycosides [12] [13]. These enzymes inactivate aminoglycosides by transferring a functional group to the aminoglycoside structure, rendering aminoglycosides unable to effectively interact with the ribosome. Three types of enzymes transfer functional groups to the aminoglycoside structures. Aminoglycoside nucleotidyltransferases (ANT's) transfer a nucleotide triphosphate to the hydroxyl group, aminoglycoside acetyltransferases (AAC's) transfer the acetyl group of acetyl-CoA to the amine group, and aminoglycoside phosphotransferase (APH) transfers the phosphoryl group of ATP to the hydroxyl group [14] [15]. In Burkina Faso, *E. coli* resistance to aminoglycosides was estimated to be 16.8% in 2017 according to a study conducted at Hospital Saint Camille in Ouagadougou [16]. The objectives of this study were to determine the presence of the aminoglycoside resistance genes aac(3)-IIc, aac(6)-Ib and armA in Burkina Faso among *E. coli* strains, and to determine which antibiotics in this family are most affected by resistance.

2. Materials and Methods

2.1. Ethical Considerations

This study was approved by the institutional ethics committee of Hôpital Saint Camille de Ouagadougou (HOSCO) under its reference N˚ 2018-09-016.

2.2. Bacterial Strains and Antibiotic Susceptibility Testing

The present study was performed on 216 *E. coli* strains collected from September 2018 to February 2019 in the biomedical analysis laboratories of Saint Camille and Schiphra hospitals in the city of Ouagadougou. *E. coli* strains were obtained from urine (206/216) and pus (10/216) cultures. After collection of the strains, the plating was performed on Muller Hinton (MH) agar medium at 37˚C for 24 h to obtain pure strains. Antibiotic susceptibility testing was then performed using the disc diffusion method on Mueller Hinton (MH) agar medium following the 2018 recommendations of the Antibiogram Committee of the French Society of Microbiology [17]; using β-lactam antibiotics (Amoxicillin AML 30 µg, Amoxicillin + Clavulanic Acid AUG 20 µg/10µg, Ceftriaxone CRO 30 µg, Cefixime CFM 5 µg and Imipenem IMI 10 µg), sulfonamides (Trimetroprime/Sulfamethoxazole SXT 1.25 µg/23.75µg), fluoroquinolones (Ciprofloxacin CIP 5 µg and Ofloxacin OFX 5 µg), and aminoglycosides (Amikacin AK 30 µg, Neomycin NEO 30 µg, Netilmicin NET 10 µg, Kanamycin K 30 µg, Tobramycin TOB 10 µg, Gentamicine CN 10 µg). *E. coli* 25,922 was used as a control strain for susceptibility testing and as a negative control strain for aminoglycoside resistance genes.

2.3. Determination of ESBL Strains

A double synergy test was performed to identify the ESBL-producing bacteria. The presence of strains presenting the ESBL phenotype was determined using the agar disk diffusion method in the presence of a synergistic image (champagne cork) between the AUG and CRO or CFM disks. Strains resistant to Amoxicillin + Clavulanic Acid, Ceftriaxone and Cefixime, but which did not show an ESBL phenotype in susceptibility testing, were transferred to an MH medium supplemented with cloxacillin at a concentration of 250 mg/L to inhibit cephalosporinase production and to show synergy when the bacterium produces ESBL.

2.4. Extraction of Genomic DNA

A few colonies of *E. coli* with similar morphology were picked from MH Petri dishes and mixed with sterile distilled water (0.5 mL) in an Eppendorf tube. Bacterial DNA was extracted by thermolysis by heating the Eppendorf tubes in a water bath at 100˚C for 10 min. After cooling to room temperature, samples were centrifuged at 13,000 rpm for 5 min. Supernatants containing DNA were collected and frozen at −20˚C for further use.

2.5. PCR Amplification

The aminoglycoside resistance genes, *aac*(3)-*IIc* and *aac*(6)-*Ib*, tested in this study, were detected by chain reaction using a ready-to-use master mix (5× HOT FIREPol@ Blend Master Mix with 10 mM $MgCl_2$, Solisbiodyne), and the *armA* gene was searched using Taq Maximo with the GeneAmp System PCR 9700 thermal cycler (Applied Biosystems, California, USA). The primers used and their sequences and amplicon sizes are listed in Table 1.

2.6. Statistical Analyses

Statistical analyses were performed using Microsoft Excel 2019 and Epi Info 7.2.2.16. Descriptive analyses were performed, and the results are presented as frequencies and percentages.

3. Results

3.1. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing showed that all the families of antibiotics tested in this study were affected by resistance.

Among the aminopenicillin classes, 94.91% (205/216) of *E. coli* strains were resistant to amoxicillin and 50.93% (100/216) were resistant to Amoxicillin + Clavulanic acid. *E. coli* strains were active against the cephalosporins Ceftriaxone and Cefixime at 46.30% (100/216) and 44.91% (97/216), respectively. Imipenem was the most active antibiotic, although 3.24% (7/216) of strains were susceptible to high-dose (intermediate) imipenem. Among the fluoroquinolones, 72.69% were resistant to ciprofloxacin, and 73.61% were resistant to ofloxacin. The strains exhibited 84.72% resistance to trimethoprim/sulfamethoxazole.

Among the aminoglycoside classes, *E. coli* strains were more resistant to tobramycin (45.37%) and gentamicin (32.40%), whereas amikacin remained the most active antibiotic and was therefore the least affected by resistance (0.46%). The kanamycin, Netilmicin and Neomycin resistance rates were 14.81% (32/216), 2.31% (5/216), and 1.84% (4/216), respectively. Strains with at least one resistance to the aminoglycosides tested numbered 101 (46.75%) and the cumulative frequency of resistance of *E. coli* strains was 46.8%.

Table 1. Sequence of primers used.

Primer	Sequence (5'→3')	Reference	Size (bp)
aac(3)-*IIc*	F: ATATCGCGATGCATACGCGG R: GACGGCCTCTAACCGGAAGG	[18]	877
aac(6')-*Ib*	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	[19]	472
armA	F: ATT CTG CCT ATC CTA ATT GG R: ACC TAT ACT TTA TCG TCG TC	[20]	315

3.2. ESBL Detection and Sensitivity Testing

Antibiotic susceptibility testing of *E. coli* strains revealed 92 ESBL-producing strains (42.59%). Among β-lactamase-producing strains, the percentages of resistance observed for tobramycin were 33.33% and 22.68% for gentamicin, 11.57% for kanamycin, 1.84% for netilmicin, 0.46% for neomycin, and 0.00% for amikacin. Seventy-seven (77) strains among the 92 ESBL-producing strains (83.69%) showed at least one resistance to aminoglycosides and fifteen strains were susceptible to the aminoglycosides tested. Among the 124 non-β-lactamase producing *E. coli* strains (57.41%), the resistance rates were 12.03% for Tobramycin, 9.72% for Gentamicin, 1.38% for Neomycin, 0.46% for Netilmicin and 0.46% for Amikacin, respectively. The overall results of the antibiotic susceptibility tests are summarized in Table 2.

3.3. Resistance Genes

Polymerase chain reaction PCR for the identification of aac(3)-IIc and aac(6)-Ib (Figure 1) allowed us to determine the presence of 86 strains possessing only the aac(3)-IIc gene (85.15%), 71 strains possessing only the aac(6)-Ib gene (70.30%), and 62 strains possessing both the aac(3)-IIc and aac(6)-Ib genes (61.38%). Of the strains tested for *armA*, 12.7% (8/63) were positive (Figure 2). Table 3, summarizes the frequency of resistant *E. coli* strains with aminoglycoside resistance genes.

4. Discussion

The frequencies of resistance of the 216 *E. coli* strains to aminoglycosides were 45.37% (98/216) for tobramycin, 32.40% (70/216) for gentamicin, 14.81% (32/216) for kanamycin, 2.31% (5/216) for netilmicin, 1.84% (4/216) for neomycin, and 0.46% (1/216) for amikacin. These results show that in Burkina Faso, resistance to aminoglycosides was mainly due to Tobramycin and Gentamicin.

Table 2. Summary table of antibiotic sensitivity tests.

	β-Lactams					Aminoglycosides						Sulf.	FQ	
	AML n (%)	AMC n (%)	IMI n (%)	CRO n (%)	CFM n (%)	TOB n (%)	K n (%)	CN n (%)	N n (%)	NET n (%)	AK n (%)	SXT n (%)	CIP n (%)	OFX n (%)
S	11 (5.09)	90 (41.67)	209 (96.76)	116 (53.70)	119 (55.09)	103 (47.69)	180 (83.33)	180 (66.67)	212 (98.15)	206 (95.37)	208 (96.30)	32 (14.81)	57 (26.39)	52 (24.07)
I	-	16 (7.41)	7 (3.24)	1 (0.46)	-	15 (6.94)	4 (1.85)	2 (0.93)	-	5 (2.31)	7 (3.24)	1 (0.46)	2 (0.93)	5 (2.31)
R	205 (94.91)	110 (50.93)	-	99 (45.83)	97 (44.91)	98 (45.69)	32 (14.81)	70 (32.41)	4 (1.85)	5 (2.31)	1 (0.46)	183 (84.72)	157 (72.69)	159 (73.61)

Legend: S, susceptible; I, intermediate; R, resistant; Sulf, sulfonamides; FQ, fluoroquinolones; Amoxicillin AMC, amoxicillin + clavulanic acid; IMI, imipenem; CRO, ceftriaxone; CFM, cefxime; TOB, tobramycin; K, kanamycin; CN, gentamicin; N, neomycin; NET, netilmicin; AK, amikacin; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; OFX, ofloxacin.

Table 3. Frequency of resistant *E. coli* strains with aminoglycoside resistance genes.

	Genes of resistance													
	aac3iic		*aac6ib*		*Arma*		*aac3Xaac6*		*aac3Xarma*		*aac6Xarma*		*aac3Xaac6 Xarma*	
	n	%	n	%	N	%	n	%	n	%	n	%	n	%
Amikacin	2	**1.09***	3	1.85	0	0.00	2	**1.48***	0	0	0	0	0	0
Gentamicin	61	**33.33***	56	**34.57***	9	**40.91***	45	**33.33***	4	**26.67***	5	**46.67***	3	20.00
Tobramycin	75	**40.98***	63	**38.89***	9	**40.91***	52	**38.52***	7	**46.67***	7	**46.67***	3	20.00
Kanamycin	35	19.13	30	18.52	4	18.18	27	20.00	4	26.67	3	20.00	3	20.00
Netilmicin	7	3.83	7	4.32	0.00	0.00	6	4.44	0	0.00	0	0.00	0	0.00
Neomycin	3	1.64	3	1.85	0.00	0.00	3	2.22	0	0.00	0	0	0	0
TOTAL	2	1.09	3	1.85	0	0.00	2	1.48	0	0	0	0	0	0

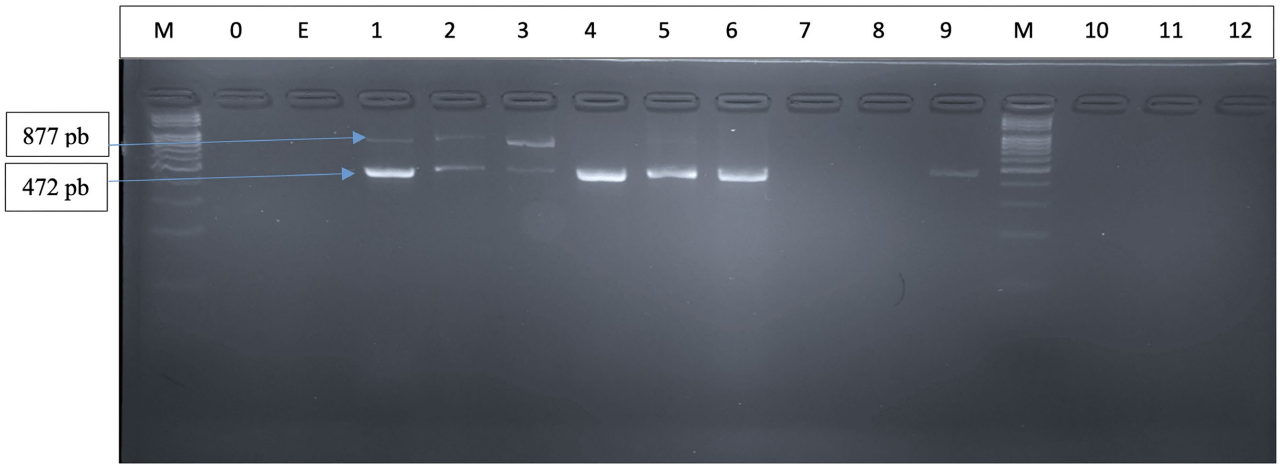

Figure 1. Photo of a gel after migration for the aac (3)-IIc and aac (6)-Ib genes. Source: DJAGBARE *et al.*, CERBA, 2019. Legend: M: Molecular weight marker (100 bp Solis Biodyne Ladder); 0: Negative control strain of *E. coli* ATCC 25,922; E: PCR water; samples 1, 2, 3: positive for aac(6')-Ib and aac(3)-IIc; 4, 5, 6, 9: positive for aac(3)-IIc; samples 7, 8, 10, 11, 12: negative for both genes aac(6')-Ib and aac(3)-IIc.

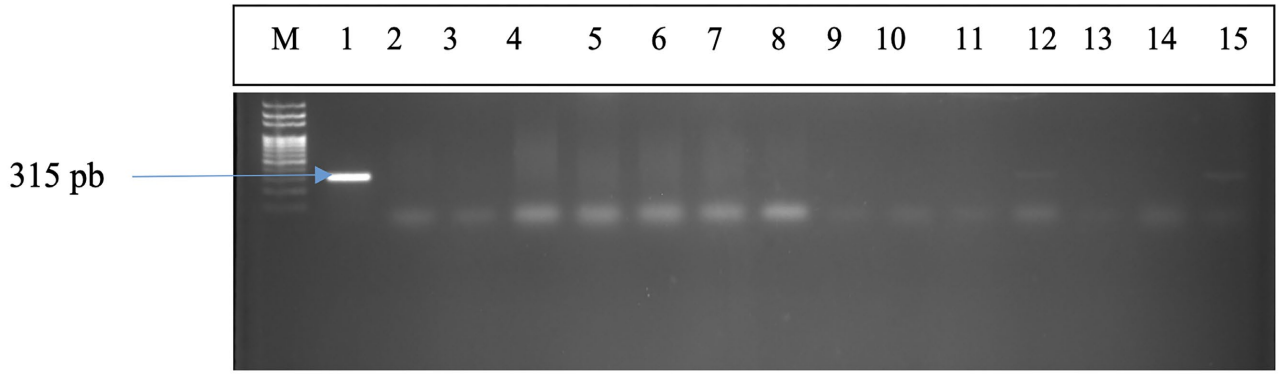

Figure 2. Photo of a gel after migration for the *armA* gene. Source: DJAGBARE *et al.*, CERBA, 2022. Legend: M: Molecular weight marker (100 bp Solis Biodyne Ladder); 1: *E. coli* armA positive control strain; armA negative samples: 2 - 11, 13, 14; Samples positive for armA gene: 12, 15.

The cumulative prevalence gave a resistance of 46.8% to aminoglycosides. In 2011, resistance to gentamicin was also observed in Kumasi, Ghana, where 28% of *E. coli* strains isolated from urine were found to be resistant to this antibiotic [21]. This antibiotic has been on the Ghanaian market for a relatively short period of time compared to other antibiotics such as ampicillin and chloramphenicol. This may be one reason for the relatively low resistance of gentamicin [22].

The highest rate of resistance of Enterobacteriaceae to antibiotics at the Sidi Bel Abbes University Hospital in Algeria was 42.1% for tobramycin, 38.6% for gentamicin, and 35% for Kanamycin [23]. High resistance rates have been observed in Cameroon in 2015. Indeed, aminoglycosides showed low activity against strains of Enterobacteriaceae isolated at the General Hospital of Douala in surgical departments: gentamicin (97.25% of resistant strains), tobramycin (67.3% of resistant strains), and nelltimicin (56.9% of resistant strains) [24]. Our results are comparable to those obtained in Egypt in 2011 on isolated gram-negative bacterial strains (175 strains of Enterobacteria) from infected patients, where a resistance of 4% to amikacin, 40% to gentamicin, and 46% to kanamycin was found [25]. This difference between the resistance rates in Burkina Faso and Egypt could be attributed to selective pressure from aminoglycoside use.

Among the non-β-lactamase producing *E. coli* strains at 57.40% (124/216), the resistance rates were 20.97% (26/124) for Tobramycin; 16.94% (21/124) for Gentamicin; 2.42% (3/124) for Neomycin; 0.81% (1/124) for Netilmicin and 0.81% for Amikacin (1/124), respectively. Our results are similar to those obtained in Poland for 44 strains of non-ESBL *E. coli* isolated from hospitalized patients. Resistance was 13.6% for tobramycin, 59% for gentamicin, and 4.6% for Netilmicin [26].

Among β-lactamase-producing strains, 92 (42.59%) were resistance observed in the 216 strains (33.33% Tobramycin, 22.68% Gentamicin, 11.57%, Kanamycin 1.84% Netilmicin, 0.46%), and amikacin (0%).

Among the 92 ESBL-producing *E. coli* strains (92), resistance rates were 78.26% (72/92), 53.26% (49/92), 27.17% (25/92), 4.35% (4/92), 1.09% (1/92), and 0% (0/92), respectively to tobramycin, gentamicin, kanamycin netilmicin and neomycin. Sensitivity rates were observed for Neomycin, 98.91% for Amikacin at 94.57% and Netilmicin at 91.30% for netilmicin. Aminoglycoside resistance was also investigated by Ebongue *et al*. 30 who tested ESBL-producing *E. coli* clinical isolates (105) and found high rates of resistance to gentamicin (80.6%), netilmicin (89.4%), and tobramycin (94%) [27].

This accumulation of resistance mechanisms indicates the coexistence of multiple resistance mechanisms (β-lactams associated with aminoglycosides). The use of cephalosporins and Amoxicillin + Clavulanic Acid would have favored the increase in aminoglycoside resistance among ESBL strains because this resistance is often conditioned by the presence of plasmids carrying multiple resistance determinants that are transferable to other gram-negative bacteria [28]. ESBL-encoding genes have most often been found on large plasmids with multi-

ple resistance genes. Plasmids encoding multidrug resistance, carrying ESBL genes in addition to genes encoding aminoglycoside resistance, and carrying trimethoprim/sulfamethoxazole resistance genes may explain the coexistence of these resistance mechanisms [28].

The results of this study showed that Amikacin was the most susceptible antibiotic among the aminoglycoside. The high sensitivity of amikacin (96.30%) has been confirmed by previous studies on 91 *E. coli* ESBL strains in Togo, of which 97% were amikacin. In Norway, a low rate of resistance to amikacin (6%) was observed in *E. coli* strains isolated from urine and hemoculture [27]. The low rate of resistance to Amikacin in our study suggests that they may be better therapeutic alternatives for the treatment of drug-resistant enterobacterial infections [28] [29]. The low rate of resistance to amikacin could be explained by the fact that this antibiotic is not commonly used in our setting and that the gene that allows resistance to amikacin is not widespread, unlike other commonly used antibiotics, such as Tobramycin, Kanamycin and Gentamicin.

All strains that showed at least one resistance to aminoglycosides were subjected to PCR for the *aac*(3)-*IIc*, *aac*(6')-*Ib* and *armA* genes (**Figure 1** and **Figure 2**). According to our study, 85.15% and 71.30% of the strains subjected to PCR contained aac3IIc and aac6Ib genes respectively. The strains with both genes together represented 61.38%. This high proportion of *aac*(3)-*IIc* to *aac*(6')-*Ib* has also been found in China and Norway. In China, the prevalence of *aac*(3)-*II* and aac(6')-Ib genes was 79.2% (162/205) and 24.39% (50/205) on *E. coli* strains (uropathogenic, isolated from blood cultures and respiratory infections), respectively [30]. In Norway, the prevalence was 79.3% of *aac*(3)-*II* and 37.9% of *aac*(6')-*Ib* in uropathogenic *E. coli* strains isolated from blood cultures 30. In contrast, in Spain, the prevalence was 420 uropathogenic *E. coli*, 16.2% *aac*(6')-*Ib* and 14.7% *aac*(3)-*IIc* [31].

The frequencies of aminoglycoside-resistant strains carrying the *aac*(3')-*IIc* gene were 95.35% (82/86) for tobramycin, 74.42% (64/86) for gentamicin, 30.23% (26/86) for kanamycin, 4.65% (4/86) for netilmicin, 3.49% (3/86) for neomycin and 1.16% (1/86) for amikacin. The high prevalence of *aac*(3)-*IIc* might explain the high frequency of resistance to Tobramycin, Gentamicin and Kanamycin and Netilmicin. The AAC(3)-II enzyme confers a common resistance mechanism to gram-negative bacteria and was also found to be resistant to Tobramycin, Gentamicin and Netilmicin [13].

The frequencies of aminoglycoside-resistant strains carrying the aac(6')-Ib gene were 98.59% (70/71) for tobramycin, 63.38% (45/71) for gentamicin, 38.03% (27/71) for kanamycin, 5.62% (4/71) for netilmicin, 1.41% (1/71) for amikacin, and 0% for neomycin. The AAC(6)-I group was resistant to Amikacin, Tobramycin, Netilmicin and Kanamycin [13]. AAC(6')-Ib is considered to be the most prevalent Gram-negative bacteria [32] [33]. It is found in nearly 70% of Gram-negative bacteria 36. The *aac*(6')-*Ib* gene has been found in transposons and integrons [34] [35] [36]. Therefore, it can be assumed that its location on

mobile elements facilitates its dissemination among *E. coli* strains.

Aminoglycoside resistance is primarily due to the *aac3IIc* and *aac6Ib* genes. As shown in Table 3, the presence of these two main genes was due to the high resistance of *E. coli* strains to tobramycin (40.98%) and gentamicin (33.33%) for aac3IIc, 38.89% to tobramycin, and 34.57% to gentamicin for aac6Ib. The lowest rate was observed for amikacin, where the presence of *aac3IIc* was found in 1.09% (2/183) of strains with *aac3IIc*. Although Amikacin is currently the most suitable antibiotic for the treatment of various infections, monitoring should be implemented to slow the rapid progression towards resistance to all aminoglycoside antibiotics.

Of the strains tested by PCR, 8.91% (9/101) harbored *armA*. To the best of our knowledge, this is the first time this gene has been identified in Burkina Faso. Methylase genes confer high resistance to aminoglycosides. Despite the low rate of the *armA* methylase gene in this study (19%) compared with that of acetyl aminoglycosides (aac), it appears that the presence of the *armA* gene is linked to resistance to Tobramycin and Gentamicin. All strains (09) with the armA gene were resistant to Tobramycin and Gentamicin, and the highest frequencies of *aacXarmA* were found in the tobramycin- and gentamicin-resistant strains. A study published in 2007 on the emergence of aminoglycoside resistance genes *armA* and *rmtB* in Belgium showed that out of 18 Enterobacteriaceae, only one *E. coli* strain carried the *armA* and *rmtB* genes [37]. The number of *E. coli* strains with the armA gene was 0.4% in 2004 and 11.6% between 2007 and 2009 [38] [39].

The main limitation of our work is the limited number of resistance markers studied, in particular genes involved in resistance to beta-lactam antibiotics, fluoroquinolones and trimethoprim/sulfamethoxazole.

The presence of this methylase gene in our study is a boon to the awareness of the risk of transferring this gene to other clinical strains.

5. Conclusion

This study on the identification of *E. coli* resistance genes in *E. coli* in Ouagadougou showed that these strains were more resistant to Tobramycin and Gentamycin. Among these strains, amikacin was the most active aminoglycoside. The *aac*(3)-*IIc* and *aac*(6)-*Ib* genes are primarily responsible for aminoglycoside resistance in *E. coli*. However, the presence of the RNA methylase resistance gene *armA* found for the first time among *E. coli* strains in Burkina Faso, allowed us to strengthen the monitoring and control of resistant strains.

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Conflicts of Interest

The authors and all participants in this study declare no conflicts of interest.

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