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ORIGINAL RESEARCH

Coexistence and Genetic Location of Aminoglycoside-Modifying Enzyme Genes in Clinical *Escherichia coli* Isolates from a Tertiary Hospital in Ouagadougou

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Introduction: *Escherichia coli* resistance to aminoglycosides is a major obstacle to the treatment of nosocomial infections. This resistance is often promoted by aminoglycoside-modifying enzymes (AMEs) encoded by genes such as aac(6′)-II, aac(3)-II, aph(3′)-VI, and ant(3″)-I.

Objective: To study the coexistence and genetic localization of aac(6′)-II, aac(3)-II, aph(3′)-VI, and ant(3″)-I genes in clinical isolates of *E. coli* obtained at Saint-Camille Hospital in Ouagadougou and exhibiting a phenotype resistant to at least one of the aminoglycosides tested.

Methodology: *E. coli* isolates were identified using standard microbiological methods and tested for susceptibility to aminoglycosides according to CA-SFM recommendations. Resistance genes were detected using conventional PCR, and their genetic support was evaluated by plasmid extraction and targeted amplification.

Results: *E. coli* accounted for 191/300 (63.66%) of the isolates analyzed. The resistance rates observed were 91 (47.6%) for streptomycin, 42 (22.0%) for kanamycin, 36 (18.8%) for gentamicin, and 3 (1.6%) for amikacin. The presence of the aac(6′)-II, aac(3)-II, ant(3″)-I, and aph(3′)-VI genes was observed in 89 (87.3%), 38 (37.3%), 33 (32.4%), and 2 (2.0%) of *E. coli* isolates, respectively. In addition, the aac(6')-II and ant(3")-I genes coexisted in 21 (20.58%) of isolates, while the aac(3)-II and aph(3')-VI genes were simultaneously present in 2 (2.0%) of isolates. A significant correlation was observed between aac(3)-II and resistance to various aminoglycosides, as well as between aph(3')-VI and resistance to neomycin and netilmicin ($p < 0.05$). The majority of aac(6')-II genes 88 (98.88%) were located on chromosomes, while 26 (68.42%) of the aac(3)-II genes identified were found on plasmids.

Conclusion: These results highlight the importance of monitoring the evolution of antibiotic resistance and promoting the judicious use of antibiotics to limit the spread of such resistance.

Keywords: aminoglycoside, bacterial resistance, *Escherichia coli*, plasmid, Burkina Faso

Introduction

Antibiotic-resistant *Escherichia coli* bacterial infections pose a significant threat to public health systems, resulting in high morbidity and mortality rates, and may contribute to a rapid increase in global healthcare costs.[1]

According to data from the World Health Organization's GLASS surveillance system, there has been an increase in the resistance of *Escherichia coli* strains to commonly used antibiotics, particularly aminoglycosides, with significant geographical variation. From 2018 to 2023, antibiotic resistance increased in 40% of the pathogen-antibiotic combinations monitored by GLASS, with an estimated average annual growth rate of between 5% and 15%.[2]

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Aminoglycosides are broad-spectrum antibiotics that act by inhibiting bacterial protein synthesis and have been used to treat life-threatening infections for nearly 80 years.[3] In clinical settings, aminoglycoside resistance is most often mediated by the presence of various aminoglycoside-modifying enzymes (AMEs).[4,5] These AMEs (AAC acetyltransferases, APH phosphotransferases, and ANT nucleotidyl transferases) chemically inactivate aminoglycoside molecules and reduce their affinity for the ribosomal target, resulting in a loss of therapeutic activity. N-acetyltransferases (ACC) inactivate aminoglycosides by acetylation, O-nucleotidyltransferases (ANT) cause adenylation of aminoglycosides, and O-phosphotransferases (APH) can phosphorylate aminoglycosides.[5,6] The clinical impact of AMEs is amplified by the coexistence of several genes encoding these enzymes within the same isolate and by the mobilization of these genes on mobile genetic elements such as plasmids, transposons, and integrons.[7]

In hospital settings in resource-limited countries, where empirical use of antibiotics promotes the selection and spread of multidrug-resistant isolates, the dissemination of these genes is particularly concerning. It is essential to understand not only the diversity of the genes involved, but also their modes of dissemination. This study is part of this dynamic by exploring, for the first time at the Saint Camille Hospital in Ouagadougou, the coexistence of aminoglycoside-modifying enzymes and their genetic carriers in clinically isolated *Escherichia coli*.

Methodology

Collection of Bacterial Strains

A total of 300 non-duplicated Gram-negative bacilli strains were collected at the microbiology laboratory of Saint Camille Hospital in Ouagadougou (HOSCO) between July 2024 and January 2025 by inoculating biological samples onto Uri Select, Hektoen Enteric agar and Brain Heart Infusion (BHI) broth for blood samples. After incubation for 24 hours at 37°C, *Escherichia coli* strains were identified using the API 20E gallery (BioMérieux), in accordance with the manufacturer's recommendations, and constituted the specific sample selected for aminoglycoside susceptibility testing and molecular analysis. The selected colonies were then cultured on Muller-Hinton agar at 37°C for 24 hours to ensure their purity. Details regarding sampling are presented in Table 1.

The sensitivity of bacterial strains to aminoglycosides was evaluated by diffusion on Mueller-Hinton (MH) agar, in accordance with the protocols of the Antibiogram Committee of the French Society of Microbiology (EUCAST/CA-SFM, 2024).

Discs impregnated with Gentamicin (10 µg), Kanamycin (30 µg), Tobramycin (10 µg), Netilmicin (10 µg), Neomycin (10 µg), Amikacin (30 µg) and streptomycin (10 µg) were tested. To validate the antibiogram, a strain of *Escherichia coli* ATCC 25922 provided by the microbiology laboratory at the Princess Sarah Clinic was used in accordance with the protocols of the Antibiogram Committee of the French Society of Microbiology (EUCAST/CA-SFM, 2024). Only strains resistant to at least one of the antibiotics tested were considered for molecular analysis. The isolate selection process is summarized in Figure 1.

Inclusion and Exclusion Criteria for This Study

All patients who attended consultations at HOSCO during the study period and who were prescribed a bacteriological examination as part of their treatment were included in this study.

All isolates not identified as *E. coli* and duplicates from the same patient were excluded from this study.

Table 1 Study Site and Sample Characteristics

Variable	Descriptions
Location	Ouagadougou/Burkina Faso
Hospital	Saint Camille Hospital of Ouagadougou (HOSCO)
Study period	July 2024 to January 2025
Total number of samples	N = 300
Types of specimens	Urine, stool, pus, semen, vaginal samples, blood samples, and catheter tip
Hospital departments	Internal medicine, pediatrics, urology, gynecology

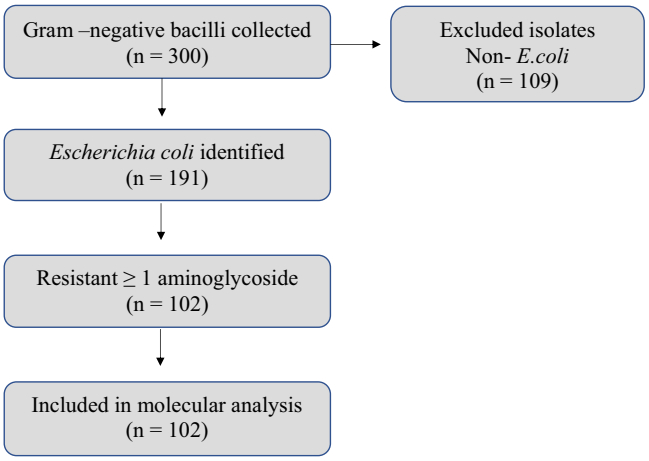

Figure 1 Flow diagram of isolate selection and analysis process. Diagram showing the selection process of bacterial isolates included in the study, from sample collection to phenotypic antimicrobial susceptibility testing and molecular detection of résistance genes.

Molecular Analysis

At the molecular level, the study focused on identifying resistance genes using conventional PCR applied to total genomic DNA extracted from the analyzed strains. In order to elucidate the genetic basis of these genes, PCR was then performed on plasmid DNA obtained from strains that had tested positive during the initial amplification. Genes identified in both genomic and plasmid DNA were considered to be carried by plasmids, while genes amplified only from genomic DNA were considered to be carried by the bacterial chromosome.

This approach allows us to infer the probable location of the detected genes, although definitive confirmation would require genomic sequencing.[8]

Extraction of Total Genomic DNA

Total genomic DNA extraction from bacterial strains was performed by heat shock.[9] Pure colonies, cultured for 24 hours, were selected and suspended in 200 µL of sterile distilled water, then placed in properly labeled Eppendorf tubes. These tubes were then incubated in a water bath at 100°C for 15 minutes to promote the release of bacterial genetic material.

After the procedure, centrifugation was performed for 10 minutes at 13,000 rpm, allowing the supernatant containing the extracted DNA to be recovered, which was then transferred to a new Eppendorf tube.

Plasmid DNA Extraction

Plasmid DNA from the strains was extracted using the alkaline lysis method described by Birnboim.[10]

The selected bacterial strains were cultured in LB (*Luria Bertani*) broth at 37°C for 24 hours. Then, 1.5 mL of each bacterial suspension was collected in labeled cryotubes. After centrifugation at 12,000 g for 3 min, the supernatants were removed. This step was repeated once to obtain a sufficient amount of pellet containing the sedimented bacterial cells.

The pellet was recovered and placed in a sterile microtube and suspended in 150 µL of 25 mM Tris-HCl buffer, pH 8; 10 mM EDTA.

In order to lyse the bacterial cells, 300 µL of 200 mM sodium hydroxide, 1% SDS prepared fresh was added and then incubated at room temperature for 5 min. Next, 225 µL of 3 M sodium acetate, pH 5.5, was added to precipitate the chromosomal DNA initially denatured by the sodium hydroxide and the proteins complexed by the SDS detergent.

The mixture was homogenized by successive inversions (10 times) and the tubes were placed on ice for 10 min. Centrifugation at 12,000 g for 10 min was then performed to separate the genomic DNA and plasmid DNA.

The supernatant containing soluble plasmid DNA was recovered in another sterile Eppendorf tube. Two volumes of fresh absolute ethanol were added and the mixture was incubated at −20°C for 30 min to precipitate the plasmid DNA.

After incubation, the tubes were centrifuged at 12,000 g for 15 minutes and the supernatants were removed. The pellet containing the plasmid DNA was purified by adding 300 µL of 75% ethanol and centrifuging for 15 minutes at 12,000 g at 4°C.

The supernatant was removed and the pellet was dried for a few minutes at 37°C.

The plasmid DNA obtained was eluted in 50 µL of sterile distilled.

DNA quantification and purity assessment were performed using NanoDrop. Consequently, any sample with an A260/A280 absorbance ratio between 1.8 and 2 was considered pure.

Amplification of Extracts by Conventional PCR

DNA extracts were amplified by conventional PCR (polymerase chain reaction) using the GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA). This approach enabled the detection of the aac(6')-II, aac(3)-II, aph(3')-VI, and ant(3")-I genes. The reaction mixture, with a total volume of 20 µL, consisted of 4 µL of Firepol® Master Mix 5X, 0.6 µL of sense primer, 0.6 µL of antisense primer, 12.8 µL of PCR water, and 2 µL of bacterial DNA from each strain.[11] A negative control including the elution buffer was included to ensure that there was no contamination. Table 2 presents a summary of the primer sequences and the PCR program used.

Agarose Gel Electrophoresis

The DNA fragments amplified by PCR were separated by electrophoresis on a 1.5% agarose gel prepared in a 1X Tris-acetate-EDTA solution with 8 µL of ethidium bromide added. A 100 bp molecular weight marker was used to estimate the size of the fragments obtained for the aac(6')-II and ant(3")-I genes. For the aac(3)-II and aph(3')-VI genes, a 1 kb molecular weight marker was used. Migration was performed at a voltage of 100 V for 35 minutes. After migration, the DNA fragments were visualized under UV light using the Vilber E-Box Transilluminator, and the images were recorded.

Characterization of Resistance Genes

The DNA of strains resistant to at least one of the antibiotics tested was analyzed by conventional PCR to detect the presence of the aac(6')-II, aac(3)-II, aph(3')-VI, and ant(3")-I genes. As the primer pairs used were specific to the genes sought, the presence of bands measuring 877 bp, 780 bp, 542 bp and 787 bp was defined as characteristic of the presence of the aac(3)-II and aph(3')-VI (Figure 2), aac(6')-II and ant(3")-I (Figure 3), respectively.

Statistical Analysis

The collected data were entered into Excel 2019 software and then checked for consistency before statistical analysis. Antibiotic resistance rates and the prevalence of resistance genes were calculated using SPSS 21 software. The association between phenotypic resistance and the presence of resistance genes was analyzed using Fisher's exact test. The statistical significance threshold was set at a value of $p < 0.05$.

Table 2 Primers and PCR Program

Genes	Sequences (5'-3')	Size (bp)	PCR Cycle
aac(6')-II	F: CGACCATTTCATGTCC R: GAAGGCTTGTCGTGTTT	542	1 cycle: 95°C for 15 minutes 30 cycles: 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute 1 cycle: 72°C for 5 minutes.[11]
aac(3)-II	F: ATATCGCGATGCATACGCGG R: GACGGCCTCTAACCGGAAGG	877	
aph(3')-VI	F: ATGGAATTGCCCAATATTATT R: TCAATTCAATTCATCAAGTTT	780	
ant(3")-I	F: CATCATGAGGGAAGCGGTG R: GACTACCTTGGTGATCTCG	787	

Abbreviations: aac, aminoglycoside acetyltransferase; aph, aminoglycoside phosphotransferase; ant, aminoglycoside nucleotidyl transferase.

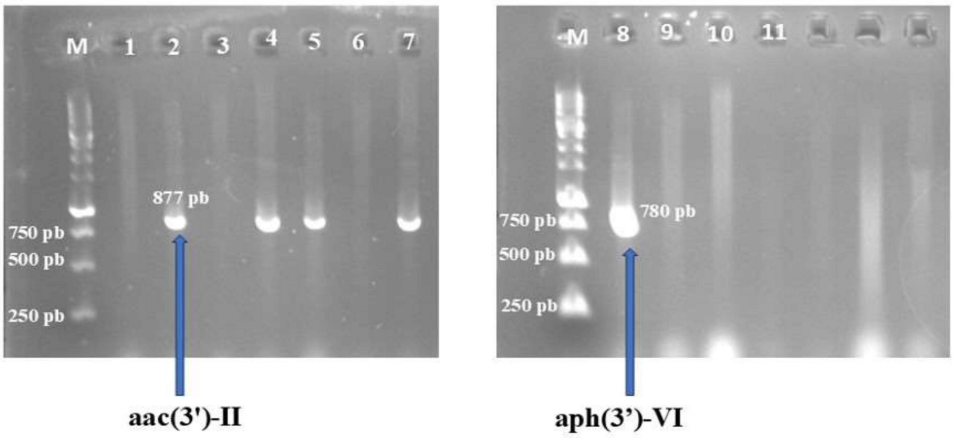

Figure 2 Agarose gel of PCR products aac(3)-II and aph (3')-VI. M: 1 Kb molecular weight marker; 1: negative control 3.6: absence of the aac(3)-II gene; 2,4,5,7: presence of the aac(3)-II gene; 8: presence of the aph (3')-VI gene; 9, 10, and 11: absence of the aph(3')-VI gene.

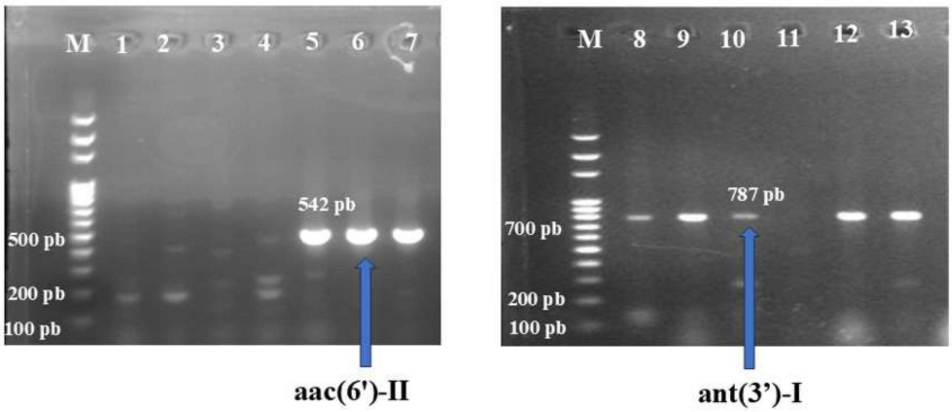

Figure 3 Agarose gel of PCR products aac(6')-II and ant(3")-I. M: molecular weight marker 100 bp; 1: negative control; 2,3: absence of the aac(6')-II gene; 4,5,6,7: presence of the aac(6')-II gene; 8,9,10,12,13: presence of the ant(3")-I gene; 11: absence of the ant(3")-I gene.

Results

Isolation and Identification

Out of a total of 300 Gram-negative bacilli collected, *Escherichia coli* accounted for 63.66% (191/300) of isolates. These *E. coli* strains were isolated in 134 (70.1%) of urine samples, 30 (15.7%) of stool samples, 19 (9.9%) of pus samples, and 5 (2.6%) of semen samples. Vaginal samples, blood samples, and catheter tip samples each accounted for 1 (0.5%).

Antibiotic Sensitivity Tests

Our results reveal a higher resistance rate to streptomycin 91 (47.6%) compared to kanamycin and gentamicin, which recorded resistance rates of 42 (22.0%) and 36 (18.8%), respectively (Table 3). Only 3 (1.6%) of isolates were resistant to amikacin.

Frequency of Resistance Genes

Of the 191 *E. coli* strains analyzed in this study, 102 (53.40%) showed resistance to at least one of the antibiotics tested and were subjected to molecular analysis.

In this study, the aac(6')-II and aac(3)-II genes were the most frequently detected in *E. coli* strains, with frequencies of 89 (87.3%) and 38 (37.3%), respectively (Figure 4). The ant(3")-I and aph(3')-VI genes were present in 33 (32.4%)

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Table 3 Susceptibility of Isolates to Antibiotics

Antibiotics	Susceptibility Test Result				Total	
	Resistant		Susceptible			
	N	%	N	%	N	%
Amikacin	3	1.6	188	98.4	191	100
Gentamicin	36	18.8	155	81.2	191	100
Kanamycin	42	22.0	149	78.0	191	100
Neomycin	17	8.9	174	91.1	191	100
Netilmicin	17	8.9	174	91.1	191	100
Streptomycin	91	47.6	100	52.4	191	100
Tobramycin	18	9.4	173	90.6	191	100

and 2 (2.0%) of isolates, respectively. In addition, 21 (20.58%) of isolates combined the aac(6')-II and ant(3")-I genes, and 2 (2.0%) carried both aac(3)-II and aph(3')-VI.

Association Between Gene and Phenotypic Resistance

Fisher's exact test showed a significant association between the presence of the aac(3)-II gene and resistance to certain antibiotics such as: Gentamicin ($p < 0.001$), Kanamycin ($p = 0.002$), Netilmicin ($p < 0.005$) and Tobramycin ($p < 0.001$) (Table 4).

In addition, the presence of the aph(3')-VI gene was significantly associated with the resistance of *E. coli* strains to Netilmicin ($p = 0.026$) and Neomycin ($p = 0.026$) (Table 5). Conversely, no association was observed between the carriage of the aac(6')-II and ant(3")-I genes and resistance to the antibiotics tested.

Genetic Carriers of Resistance Genes

Molecular analysis revealed that 26 (68.42%) of the aac(3')-II genes detected in this study were carried by plasmids (Table 6). As for the ant(3")-I genes detected, 11 (33.33%) were carried by plasmids, compared to 22 (66.67%) carried by chromosomal DNA. The majority of aac(6')-II genes 88 (98.88%) were carried by chromosomes.

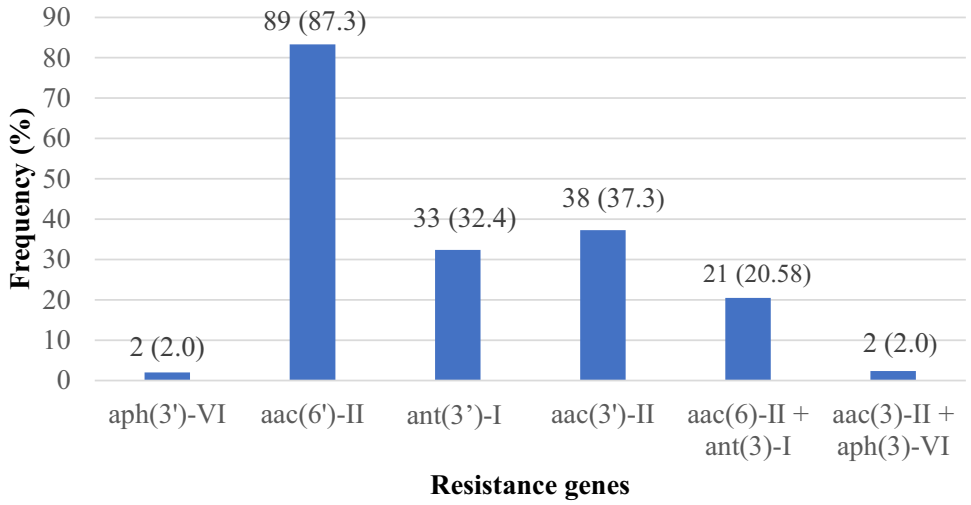

Figure 4 Frequency of resistance genes. aac(6')-II + ant(3")-I: Coexistence of the aac(6')-II and ant(3")-I genes in the same strain; aac(3')-II and aph(3')-VI: Coexistence of the aac(3')-II and aph(3')-VI genes in the same strain.

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Table 4 Association Between the Aac(6')-Il and Aac(3')-Il Genes and Phenotypic Resistance

Genes	Antibiotics		Absence of Gene N (%)	Presence of Gene N (%)	P-value
aac(6')-Il	Amikacin	Resistant	0 (0.0)	3 (100.0)	1.000
		Susceptible	13 (13.1)	86 (86.9)	
	Gentamicin	Resistant	7 (19.4)	29 (80.6)	0.212
		Susceptible	6 (9.1)	60 (90.9)	
	Kanamycin	Resistant	8 (18.6)	35 (81.4)	0.145
		Susceptible	5 (8.5)	54 (91.5)	
	Neomycin	Resistant	2 (11.8)	15 (88.2)	1.000
		Susceptible	11 (12.9)	74 (81.7)	
	Netilmicin	Resistant	3 (17.6)	14 (82.4)	0.451
		Susceptible	10 (11.8)	75 (88.2)	
	Streptomycin	Resistant	10 (10.9)	82 (89.1)	0.115
		Susceptible	3 (30.0)	7 (70.0)	
	Tobramycin	Resistant	2 (11.2)	16 (88.9)	1.000
		Susceptible	11 (13.1)	73 (86.9)	
aac(3)-Il	Amikacin	Resistant	1 (33.3)	2 (66.7)	0.554
		Susceptible	63 (63.6)	36 (36.4)	
	Gentamicin	Resistant	5 (13.9)	31 (86.1)	< 0.001
		Susceptible	59 (89.4)	7 (10.6)	
	Kanamycin	Resistant	19 (44.2)	24 (55.8)	0.002
		Susceptible	45 (76.3)	14 (23.7)	
	Neomycin	Resistant	9 (52.9)	8 (47.1)	0.415
		Susceptible	55 (64.7)	30 (35.3)	
	Netilmicin	Resistant	5 (29.4)	12 (70.6)	0.005
		Susceptible	59 (69.4)	26 (30.6)	
	Streptomycin	Resistant	61 (66.3)	31 (33.7)	0.494
		Susceptible	8 (80.0)	2 (20.0)	
	Tobramycin	Resistant	5 (27.8)	13 (72.2)	0.001
		Susceptible	59 (70.2)	25 (29.8)	

Discussion

This study conducted at Saint-Camille Hospital in Ouagadougou focused on the coexistence and genetic location of aminoglycoside-modifying enzymes in *Escherichia coli*. Of the 300 Gram-negative bacilli isolates analyzed, *Escherichia*

Table 5 Association Between the Aph(3')-VI and Ant(3')-I Genes and Phenotypic Resistance

Genes	Antibiotics		Absence of Gene N (%)	Presence of Gene N (%)	P-value
aph(3')-VI	Amikacin	Resistant	3 (100.0)	0 (0.0)	1.000
		Susceptible	97 (98.0)	2 (2.0)	
	Gentamicin	Resistant	34 (94.4)	2 (5.6)	0.212
		Susceptible	66 (100.0)	0 (0.0)	
	Kanamycin	Resistant	42 (97.7)	1 (2.3)	1.000
		Susceptible	58 (98.3)	1 (1.7)	
	Neomycin	Resistant	15 (88.2)	2 (11.8)	0.026
		Susceptible	85 (100.0)	0 (0.0)	
	Netilmicin	Resistant	15 (88.2)	2 (11.8)	0.026
		Susceptible	85 (100)	0 (0.0)	
	Streptomycin	Resistant	90 (97.8)	2 (2.2)	1.000
		Susceptible	10 (100.0)	0 (0.0)	
	Tobramycin	Resistant	17 (94.4)	1 (5.6)	0.323
		Susceptible	83 (98.8)	1 (1.2)	
ant(3')-I	Amikacin	Resistant	2 (66.7)	1 (33.3)	1.000
		Susceptible	67 (67.7)	32 (32.3)	
	Gentamicin	Resistant	23 (63.9)	13 (36.1)	0.659
		Susceptible	46 (69.7)	20 (30.3)	
	Kanamycin	Resistant	30 (69.8)	13 (30.2)	0.831
		Susceptible	39 (66.1)	20 (33.9)	
	Neomycin	Resistant	12 (70.6)	5 (29.4)	1.000
		Susceptible	57 (67.1)	38 (32.9)	
	Netilmicin	Resistant	12 (70.6)	5 (29.4)	1.000
		Susceptible	57 (67.1)	28 (32.9)	
	Streptomycin	Resistant	61 (66.3)	31 (33.7)	0.493
		Susceptible	8 (80.0)	2 (20.0)	
	Tobramycin	Resistant	14 (77.8)	4 (22.2)	0.410
		Susceptible	55 (65.6)	29 (34.5)	

coli strains accounted for 191 (63.66%). This result confirms the significant presence of this bacterium among Gram-negative bacilli involved in clinical infections.

From a phenotypic point of view, 47.6% of the isolates were resistant to streptomycin, while 22% were resistant to kanamycin, 18.8% to gentamicin, and only 1.8% to amikacin. The high rate of resistance to streptomycin is probably

Table 6 Genetic Carrier of Aminoglycoside Resistance Genes

Genes	Genetic Carrier				Total	
	Plasmid		Chromosome			
	N	%	N	%	N	%
aac(6')-II	1	1.12	88	98.88	89	100
aac(3)-II	26	68.42	12	31.58	38	100
aph(3')-VI	1	50.00	1	50.00	2	100
ant(3")-I	11	33.33	22	66.67	33	100

indicative of its prolonged and widespread use, thus promoting the emergence of resistant strains. Conversely, the low rate of resistance to amikacin suggests that this antibiotic class remains relatively unaffected in our environment. These results are consistent with previous studies that have shown higher levels of resistance to older or more frequently used aminoglycosides, as well as lower rates of resistance to antibiotics that are still relatively rare or underused.[12,13]

At the molecular level, a predominance of the aac(6′)-II gene (87.3%) was observed in our study. This gene codes for an acetyltransferase responsible for modifying aminoglycosides, particularly gentamicin and tobramycin, and has been identified in numerous studies as a key resistance marker. In addition, the aac(3)-II gene was identified in 37.3% of cases, ant(3″)-I in 32.4%, and aph(3′)-VI in 2.0%. This diversity of aminoglycoside-modifying genes highlights the complexity of the resistance mechanisms present in our bacterial strains.

Previous research has shown that the prevalence of these genes varies according to geographical context and local antibiotic use practices.[11,14] To our knowledge, no African publication mentions the presence of the aac(6′)-II gene in clinical isolates of *E. coli*. Therefore, ours is probably the first in the region. In Tunisia, aac(3)-II and aph(3′)-VI were identified in 42% and 32.4% of aminoglycoside-resistant isolates, respectively, while ant(3″)-I was mentioned in 32.4% of the same isolates.[15] In Morocco, aac(3)-II and aph(3′)-VI were found in 27.7% and 8.3% of aminoglycoside-resistant Enterobacteriaceae.[16]

The coexistence of several resistance genes within the same strain was observed in our study. Indeed, the combination of aac(6′)-II and ant(3″)-I was found in 20.58% of isolates, while the combination of aac(3)-II and aph(3′)-VI was observed in 2.0% of cases. This accumulation of resistance determinants implies that the strains do not depend on a single mechanism, but acquire a significant genetic arsenal that enhances their ability to resist various aminoglycosides and potentially other classes of antibiotics. Such associations have also been observed in various clinical and veterinary settings.[16,17]

Our results reveal a significant correlation between the presence of the aac(3)-II gene and resistance to several aminoglycosides, including gentamicin, kanamycin, netilmicin, and tobramycin. Similarly, the aph(3′)-VI gene was associated with resistance to both netilmicin and neomycin. These results confirm the crucial role of these genes in aminoglycoside resistance in *E. coli*, as demonstrated in numerous previous studies. The significant correlation with gentamicin observed in this study indicates that it is a crucial molecular marker of resistance in our hospital environment. In addition, the aph(3′)-VI gene is frequently carried by plasmids, thus promoting horizontal transfer of resistance between different strains.[18,19]

In contrast, the lack of a notable correlation between the aac(6′)-II and ant(3″)-I genes and phenotypic resistance could indicate low gene expression or a reduced role for these mechanisms in local strains. These results suggest that other factors influence the expression of these enzymes. Sequencing analyses would be useful to identify possible point mutations or regulatory elements that could modulate gene activity.

Comparable geographical disparities have been observed in other countries, where the profile of aminoglycoside resistance genes is frequently influenced by antibiotic therapy practices.[20]

Study of the genetic location for these genes reveals that the aac(6′)-II gene is located on a chromosome in 98.88% of cases, indicating stable integration and fixation within the genomes of local strains.[8] Conversely, the aac(3')-II gene is mainly localized on a plasmid in 68.42% of cases, while the aph(3')-VI and ant(3")-I genes are associated with plasmids in 50.0% and 33.33% of cases, respectively.

Chromosomal genes are less likely to be transferred but show greater persistence within a strain, while plasmid genes facilitate their transfer between strains or species, thus promoting their dissemination. This is consistent with existing literature data, which identify plasmids, integrons, or transposons as the main vectors for horizontal resistance transfer.[21]

The significant presence of plasmid genes, particularly aac(3)-II, suggests a considerable risk of dissemination. In a hospital environment characterized by high antibiotic pressure and rapid patient turnover, genetics plays a crucial role. Secondly, although the aac(6′)-II gene is predominantly chromosomal, this does not solve the problem, this gene is already well established within the local bacterial population, posing a significant challenge for its eradication. The genetic location of resistance genes was determined in our study using an indirect approach based on separate amplifications performed using total genomic DNA and plasmid DNA, which limits the generalizability of the results. Although this strategy provides an indication of genetic location, approaches based on whole-genome sequencing will allow the location of these genes to be confirmed with precision.

The simultaneous presence of multiple genes within the same strain can reduce the effectiveness of aminoglycosides, posing a major therapeutic challenge, especially when these agents are the last resort. Therefore, these results highlight the urgent need for rigorous antibiotic stewardship, enhanced hygiene practices in healthcare facilities, and continuous molecular surveillance to identify strains with an increased risk of spread.

The clinical significance of this study lies in the fact that aminoglycosides are frequently used in combination with other molecules for the treatment of invasive infections, one of the main causative agents of which is *Escherichia coli*. Thus, the detection of a high prevalence of resistance genes in our context suggests an increased risk of failure of probabilistic regimens including these molecules. These data therefore provide essential information for adapting local treatment protocols and strengthening rational antibiotic therapy strategies. Despite the high detection of aminoglycoside-modifying genes, the high sensitivity to amikacin in this study suggests that this molecule remains an effective therapeutic option in our context. These data are essential for guiding local probabilistic antibiotic therapy and supporting strategies for the rational use of aminoglycosides.

Conclusions

This study highlights the high prevalence of genes encoding aminoglycoside-modifying enzymes, particularly aac(6′)-II and aac(3)-II, in clinical isolates *of E. coli* from Saint-Camille Hospital in Ouagadougou. The notable correlation between these genes and resistance to various aminoglycosides illustrates their crucial role in the phenotypic resistance observed. The presence of several genes within the same isolate, as well as their plasmid transfer, indicate an increased risk of horizontal transmission in hospital settings. These results highlight the need to strengthen molecular surveillance of resistance genes, limit the empirical use of aminoglycosides, and develop infection control strategies based on bacterial genomics. However, extending this study to other hospitals and other bacterial species would provide a broader view of the regional dynamics of aminoglycoside resistance. In addition, genomic analysis by sequencing would refine our understanding of the mechanisms of resistance and their spread.

Ethical Considerations

This study was approved by the Institutional Ethics Committee of the Saint-Camille Hospital in Ouagadougou (HOSCO) under authorization No. 2024-05-005.

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Disclosure

The authors report no conflicts of interest in this work.

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