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# Characterization of Antibiotic Resistance and Prevalence of Diarrheagenic *E. coli* Strains Isolated from Stool Samples in a Hospital Setting in Mali

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## Abstract

**Introduction:** Antibiotic resistance is a major global health challenge that disproportionately affects low-resource countries, particularly those in West Africa. *E. coli*, a major pathogen in childhood diarrhea, is both a prominent infectious agent and a reservoir of resistance genes, including resistance to last-resort antibiotics, such as carbapenems. **Methodology:** The study focused on 98 clinical *E. coli* isolates collected from stool samples of patients in a hospital setting in Bamako. The analyses included screening for DEC-specific virulence genes, detection of resistance genes across various classes of antibiotics (e.g., beta-lactams, carbapenems, fluoroquinolones), and identification of class 1, 2, and 3 integrons. The $bla_{NDM}$ gene was sequenced to identify mutations associated with carbapenem resistance. **Results:** Among the isolates, 85.7% carried at least one virulence gene. Of these, half involved co-infections, commonly combining EPEC, EAEC, and ETEC strains. Regarding antibiotic resistance,

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94.9% of isolates harbored at least one resistance gene, and 50% were multidrug-resistant. The most frequently detected genes were $bla_{TEM}$, *qnrS1*, and *aphA3*. Class 2 integrons were significantly associated with multidrug resistance ($p = 0.01$). Sequencing of the $bla_{NDM}$ gene revealed point mutations likely to affect protein function, suggesting an evolution toward increased resistance to carbapenems. **Conclusion:** The high prevalence of multidrug-resistant diarrheagenic *E. coli* strains in this study highlights the local antibiotic pressure and the serious health threat it represents. This study shows that the new $\beta$-lactamase $bla_{NDM}$ gene has disseminated in the hospital environment of Bamako. It should be noted that this will become a major challenge for clinicians.

## Keywords

*E. coli*, DEC, Gene, Multidrug Resistance, Integron, Mali

## 1. Introduction

Bacterial resistance to antibiotics is one of the major threats to global public health, with several million deaths estimated each year. Sub-Saharan Africa, particularly its western region, appears to be especially affected by this phenomenon [1]. However, even industrialized countries experience significant health and economic consequences [2] [3].

Among the primary causes of this resistance are the excessive and inappropriate use of antibiotics in both human and animal health, poor management and stewardship, and the intrinsic resistance specific to certain bacterial species [4].

In 2019, *Escherichia coli* (*E. coli*) was one of the six pathogens responsible for the highest number of deaths related to antibiotic resistance, ranking first [1]. As a member of the *Enterobacteriaceae* family, *E. coli* is a commensal bacterium in the mammalian digestive tract. While most strains are harmless, some are pathogenic and can cause severe intestinal or extra-intestinal infections [5]. The World Health Organization (WHO) ranks *E. coli*, alongside other *Enterobacteriaceae*, among the priority pathogens due to the growing threat they pose to human health [6].

*E. coli* is a common cause of diarrhea in children worldwide. In Mali, this bacterium is implicated in more than 30% of diarrhea cases in children under five years of age. These strains show increased resistance to several classes of antibiotics, notably beta-lactams and quinolones, although they remain relatively sensitive to imipenem, a carbapenem [7]. Moreover, multiple multidrug-resistant strains have been isolated from children suffering from acute diarrhea [8].

Beyond its multidrug-resistant profile, *E. coli* also plays a key role as a reservoir of resistance genes, due to its ability to acquire such genes from other bacteria and transmit them through horizontal gene transfer mechanisms [9] [10]. This resistance is more pronounced in clinical strains compared to environmental strains [11].

In this context, this study aims to characterize the molecular profile of virulence and antibiotic resistance genes in clinical *E. coli* strains, with a particular focus on carbapenems, a class that remains understudied in Mali.

## 2. Materials and Methods

The study focused on 98 clinical *E. coli* isolates collected from stool samples of patients between 2020 and 2021 at the laboratory of the CHU of Point G, Bamako, Mali, and stored at −80˚C. The isolates were subsequently analyzed at the Laboratory of Applied Molecular Biology (LBMA) using standard PCR.

Molecular analyses included screening for DEC-specific virulence genes, detection of resistance genes across various antibiotic classes, and identification of class 1, 2, and 3 integrons (Table 1).

**Table 1.** List of primers used.

| DEC type | Target gene | Primer | Primer sequence 5' - 3' | PCR product Size bp | Reference |
|---|---|---|---|---|---|
| ETEC | *eltB* | LT-F | TCTCTATGTGCATACGGAGC | 322 | [12] |
| | | LT-R | CCATACTGATTGCCGCAAT | | |
| | *estA* | ST-F | GTCAAACCAGTA(G/A)GGTCTTCAAAA | 147 | [12] |
| | | ST-R | CCCGGTACA(G/A)GGAGGATTACAACA | | |
| EHEC | *vt1* | VT1-F | GAAGAGTCCGTGGGATTAC | 130 | [12] |
| | | VT1-R | AGCGATGCAGCTATTAATAA | | |
| | *vt2* | VT2-F | ACCGTTTTTCAGATTTT(G/A)CACATA | 298 | [12] |
| | | VT2-R | TACACAGGAGCAGTTTCAGACAGT | | |
| EPEC | *EaeA* | Eae-F | CACACGAATAAACTGACTAAAATG | 376 | [12] |
| | | Eae-R | AAAAACGCTGACCCGCACCTAAAT | | |
| | *bfpA* | bfpA-F | TTCTTGGTGCTTGCGTGTCTTTT | 367 | [12] |
| | | bfpA-R | TTTTGTTTGTTGTATCTTTGTAA | | |
| EIEC | *Ial* | SHIG-F | CTGGTAGGTATGGTGAGG | 320 | [12] |
| | | SHIG-R | CCAGGCCAACAATTATTTCC | | |
| EAEC | *pCVD432* | EA-F | CTGGCGAAAGACTGTATCAT | 630 | [12] |
| | | EA-R | AAATGTATAGAAATCCGCTGTT | | |
| Resistance genes | $bla_{OXA}$ | OXA 1-F | ATGAAAAACACAATACATATC | 890 | [13] |
| | | OXA 1-R | AATTTAGTGTGTTTAGAATGG | | |
| | $bla_{SHV}$ | SHV-F | TTATCTCCCTGTTAGCCACC | 800 | [13] [14] |
| | | SHV-R | GATTTGCTGATTTCGCTCGG | | |

**Continued**

| | | | | | |
|---|---|---|---|---|---|
| | *bla*$_{TEM}$ | TEM-F | ATAAAATTCTTGAAGACGAAA | 850 | [13] |
| | | TEM-R | GACAGTTACCAATGCTTAATC | | |
| | *bla*$_{CTX-M-3/15/22}$ | CTX-M-F | GTTACAATGTGTGAGAAGCAG | 593 | [15] |
| | | CTX-M-R | CCGTTTCCGCTATTACAAAC | | |
| | *catA1* | catA 1-F | CGCCTGATGAATGCTCATCCG | 450 | [14] |
| | | catA 1-R | CCTGCCACTCATCGCAGTAC | | |
| | *tetA* | tetA-F | GTAATTCTGAGCACTGTCGC | 956 | [14] |
| | | tetA-R | CTGCCTGGACAACATTGCTT | | |
| | *aphA-3* | aphA-3-F | GGGACCACCTATGATGTGGAACG | 600 | [14] |
| | | aphA-3-R | CAGGCTTGATCCCCAGTAAGTC | | [16] |
| | *bla*$_{NDM}$ | NDM-1_F | GGTTTGGCGATCTGGTTTTC | 621 | |
| | | NDM-1_R | CGGAATGGCTCATCACGATC | | |
| | *bla*$_{IMP}$ | IMP_F | CACTTGGTTTGTGGAACGTG | 192 | This study GenBank: CP090265.1 |
| | | IMP_R | CAATAGTTAACCCCGCCAAA | | |
| | *Qnrs$_1$* | QnrS1_F | ACGCACGGAACTCTATACCG | 154 | This study GenBank: CP090265.1 |
| | | QnrS1_R | ACGACATTCGTCAACTGCAA | | |
| Integron | *intI1* | Int1_F | ACATGTGATGGCGACGCACGA | 580 | [17] |
| | | Int1_R | ATTTCTGTCCTGGCTGGCGA | | |
| | *intI2* | Int2_F | CACGGATATGCGACAAAAAGGT | 806 | [17] |
| | | Int2_R | GTAGCAAACGACTGACGAAATG | | |
| | *intI3* | Int3_F | AACTCTTGCACCGTTCGGAT | 542 | This study GenBank: CP047278.1 |
| | | Int3_R | CAGGAGGTTCAGACGTTGCT | | |

Frozen isolates were thawed and cultured on Mueller-Hinton (MH) agar for DNA extraction. Then, a full loop of fresh pure *E. coli* culture was stored at −80˚C in 1.5% (v/v) glycerol in enumeration broth for further analysis.

**DNA Extraction**

Reference strains were provided by the National Institute of Public Health (NIPH) of Mali. DNA was extracted from *E. coli* isolates and the reference strain using a full loop of colonies in 100 µL of ultrapure water following the Salting-out method. The purified DNA was eluted in 70 µL of TE buffer and stored at −20˚C for further

amplification.

**Identification of Virulence Genes**

Strains harboring at least one DEC-associated virulence gene were classified as Diarrheagenic *E. coli* (DEC). Several genes characteristic of Diarrheagenic *E. coli* (DEC) were targeted (see Table 1):

- *bfpA* and *eae* for typical enteropathogenic *E. coli* (EPEC),
- *agg* and *aaic* for enteroaggregative *E. coli* (EAEC),
- *lt* and *st* for enterotoxigenic *E. coli* (ETEC).

Three microliters of DNA from the reference strains provided by the NIPH, the negative control (sterile ultrapure water), and the *E. coli* isolates were used for multiplex PCR with specific primers (Table 1), as described by Vilchez *et al.* (2009) [12].

Additionally, to confirm the multiplex PCR results, DNA extracted from freshly cultured colonies on MH agar was subjected to single PCR. Each specific primer was tested independently in a single PCR to confirm suspected DEC isolates identified by multiplex PCR.

**Identification of Resistance and Integron Genes**

Specific resistance genes to different antibiotic families were evaluated: ESBL genes (Extended-Spectrum Beta-Lactamases) for beta-lactams; $bla_{NDM}$ and *bla*IMP for carbapenems; *tetA* for tetracyclines; *qnrS1* for quinolones; *catA1* for chloramphenicols, and *aphA3* for aminoglycosides.

PCR was used to detect the presence of antibiotic resistance genes, as well as class 1, 2, and 3 integron genes, and to assess their distribution among the isolates. Integrons play a major role in the acquisition, expression, and dissemination of antibiotic resistance, particularly resistance integrons.

All amplification was carried out using the PTC 200 thermocycler (MJ Research, USA) with specific primer sequences [13]-[17], as listed in Table 1. The reaction total volume was 25 µL containing 3 µL of DNA, 1× Buffer ($Mg^{2+}$ free), 3 mM $MgCl_2$, 0.4 mM deoxynucleotide triphosphates (dNTPs) (Invitrogen, USA), 0.4 µM of each primer, and 0.025 U of Taq polymerase (Invitrogen, USA).

The PCR program for all reactions was as follows: initial denaturation at 94˚C for 5 minutes, followed by 35 cycles of 94˚C for 30 seconds (denaturation), annealing at 44 to 60˚C for 45 seconds (variable according to the specific primers), and 72˚C for 1 minute (extension). The annealing temperatures were: OXA (44˚C), QnrS1 and IMP (50˚C), NDM (52˚C), SHV (60˚C), TEM (55˚C), catA (59˚C), tetA (55˚C), CTX-M (57.2˚C), aphA-3 (51.9˚C), Int1 (59˚C), Int2 (55˚C), and Int3 (57˚C), respectively. A final elongation step was performed at 72˚C for 10 minutes. All PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

**Sequencing**

Among isolates carrying the $bla_{NDM}$ gene, two with strong PCR amplification signals were considered for Sanger sequencing to confirm the sequence and identify mutations associated with carbapenem resistance, given the first-time detection of this gene and the limited resources.

The amplified product was purified using Exonuclease I and Alkaline Phosphatase enzymes, followed by thermal cycling and ethanol precipitation. Sequencing was performed on the CEQ™ 8000 DNA Analyzer (Beckman Coulter). The resulting sequence was compared to database entries using the NCBI BLAST search tool. Mutations were analyzed using Geneious Prime 2023.0 software.

**Phylogenetic Analysis**

We retrieved the most closely related sequences from GenBank as of June 3rd, 2024. The sequence dataset was aligned using BioEdit, version 7.7.1 (5/10/2021). Based on the resulting alignments, we performed a maximum likelihood (ML) phylogenetic reconstruction using MEGA version 7.0.26, applying the Tamura-Nei model and assessing branch support with 1,000 bootstrap replicates.

**Data Analysis**

Beta-lactam resistance was defined as the presence of at least one beta-lactam resistance gene. Multidrug resistance (MDR) was defined genotypically as the detection of resistance genes belonging to at least three different classes of antibiotics. A genotypic definition was selected because the focus of this study was to characterize the genetic determinants of antimicrobial resistance. Genomic screening provides a sensitive and reproducible means of identifying resistance mechanisms, including those that may not be consistently detectable through phenotypic susceptibility testing, and allows standardized comparison across isolates. Although this definition differs from conventional phenotypic surveillance criteria, it offers a robust framework for assessing MDR potential in the context of genomic analysis.

Data were analyzed using STATA software version 14.0. The chi-square test or Fisher's exact test was used, as appropriate, to determine the statistical significance of the data. A $p$-value less than 0.05 was considered statistically significant.

Figures were generated using Excel and the online platform Flourish (https://flourish.studio/), employing chord diagrams to illustrate associations between variables in cases of co-infections, multi-resistance, and the presence of different integrons.

## 3. Results

This study focused on the molecular characterization of virulence and antibiotic resistance gene profiles of *E. coli* strains isolated in a clinical setting. Of the 98 isolates, 84 (approximately 86%) were identified as DEC, based on the detection of at least one specific virulence gene (**Figure 1**).

Among the 84 DEC strains identified, 50% were mono-infections, predominantly EPEC (21.4%), followed by EAEC (20.2%) and ETEC (8.3%). The remaining 50% involved mixed infections, with the EAEC-EPEC combination being the most common (27.4%), followed by EPEC-ETEC (9.5%), EAEC-ETEC (3.6%), and the triple combination EAEC-EPEC-ETEC (9.5%) (**Figure 2**).

**Detection of Resistance Genes**

A total of 93 out of 98 *E. coli* isolates (94.9%) carried at least one antibiotic

resistance gene, and 50% (49/98) were identified as multidrug-resistant (MDR).

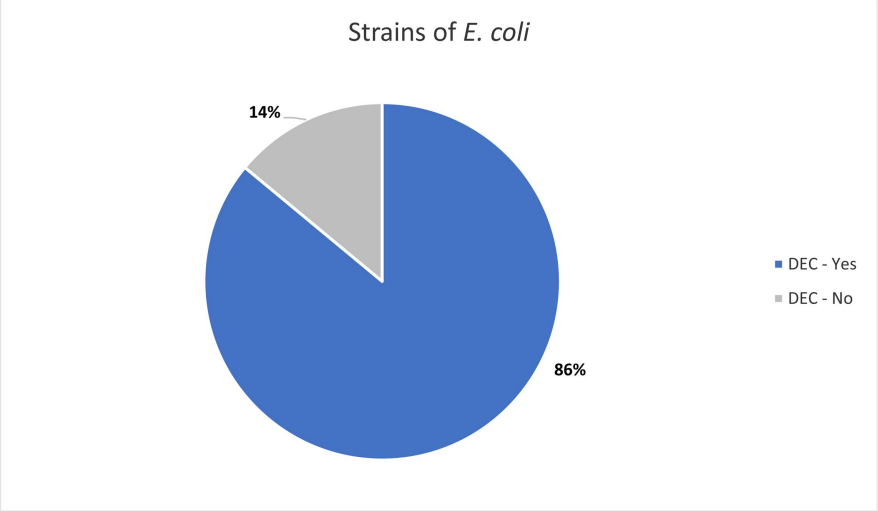



**Figure 1.** Prevalence of *E. coli* strains based on the presence of virulence genes.

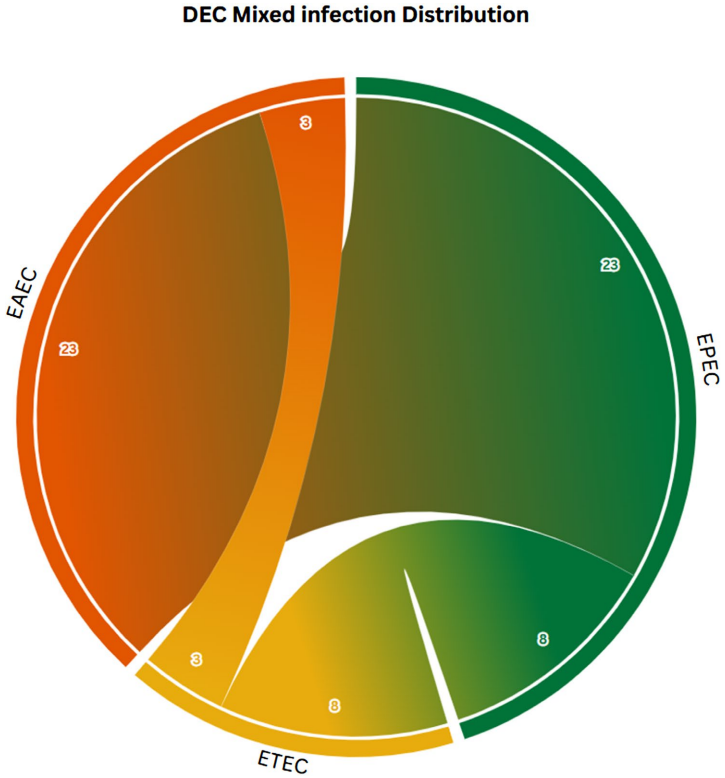



**Figure 2.** Distribution of mixed infections among DEC pathotypes based on detected virulence genes.

Beta-lactam resistance genes were detected in 77.6% (76/98) of isolates, with $bla_{TEM}$ being the most frequently identified gene. Regarding the overall prevalence of resistance genes, *qnrS1* was the most common (66.3%), followed by $bla_{TEM}$ (49%), *aphA3* (45.9%), $bla_{CTX\text{-}M}$ (35.7%), $bla_{IMP}$ (23.5%), *tetA* (22.5%), $bla_{NDM}$ (16.3%), and

*catA1* (10.2%). Carbapenem resistance genes were detected in 38.8% of isolates (Table 2).

Table 2. Distribution of resistance genes according to antibiotic families among *E. coli* isolates.

| Antibiotic families | Gene | Present, n (%) | Absent, n (%) |
|---|---|---|---|
| | Overall, N = 98 | 93 (94.9) | 5 (5.1) |
| Beta-lactams | *Overall* | 76 (77.6) | 22 (22.4) |
| | $bla_{TEM}$ | 48 (49.0) | 50 (51.0) |
| | $bla_{CTX-M}$ | 35 (35.7) | 63 (64.3) |
| | $bla_{SHV}$ | 25 (25.5) | 73 (74.5) |
| | $bla_{OXA}$ | 16 (16.3) | 82 (83.7) |
| Carbapenems | *Overall* | 38 (38.8) | 60 (61.2) |
| | $bla_{IMP}$ | 23 (23.5) | 75 (76.5) |
| | $bla_{NDM}$ | 16 (16.3) | 82 (83.7) |
| Quinolone | *qnrS1* | 65 (66.3) | 33 (33.7) |
| Chloramphenicol | *catA1* | 10 (10.2) | 88 (89.8) |
| Tetracycline | *tetA* | 22 (22.5) | 76 (77.5) |
| Aminoglycoside | *aphA3* | 45 (45.9) | 53 (54.1) |
| Integron | *Overall* | 37 (37.8) | 61 (62.2) |
| | *Int 1* | 35 (35.7) | 63 (64.3) |
| | *Int 2* | 25 (25.5) | 73 (74.5) |
| | *Int 3* | 31 (31.6) | 67 (68.4) |

Multidrug resistance associated with the $bla_{NDM}$ resistance gene was observed in 4 isolates, of which 3 also carried an integron gene. Additionally, 11 isolates harbored the *bla*TEM-*bla*CTX-M-*qnrS1* gene combination, and 8 of them also carried an integron gene. Finally, 7 isolates carried the $bla_{Oxa}$-$bla_{CTX-M}$-*qnrS1* gene combination, all of which were positive for the integron gene.

Co-detection of resistance genes was observed between resistance genes across most classes of antibiotics investigated. However, the most frequent co-detections were observed between beta-lactams and quinolones (53 isolates), followed by beta-lactams and aminoglycosides (37 isolates), beta-lactams and carbapenems (33 isolates), aminoglycosides and quinolones (31 isolates), quinolones and carbapenems (26 isolates), and beta-lactams and tetracyclines (21 isolates) (Figure 3). These co-detections contribute to the high risk of multidrug resistance, significantly reducing therapeutic options.

**Integron Distribution and Resistance Genes**

Integron genes were detected among the *E. coli* isolates as follows: *Int1* (35.7%,

35/98), *Int3* (31.6%, 31/98), and *Int2* (25.5%, 25/98) (Table 3). A statistically significant association was observed between class 2 integrons and isolates harboring more than three resistance genes ($p = 0.01$), suggesting a strong link between class 2 integrons and multidrug resistance. Although class 1 and class 3 integrons were also found among isolates with more than three resistance genes (60% and 61.3%, respectively), their associations were not statistically significant ($p > 0.05$) (Table 3).

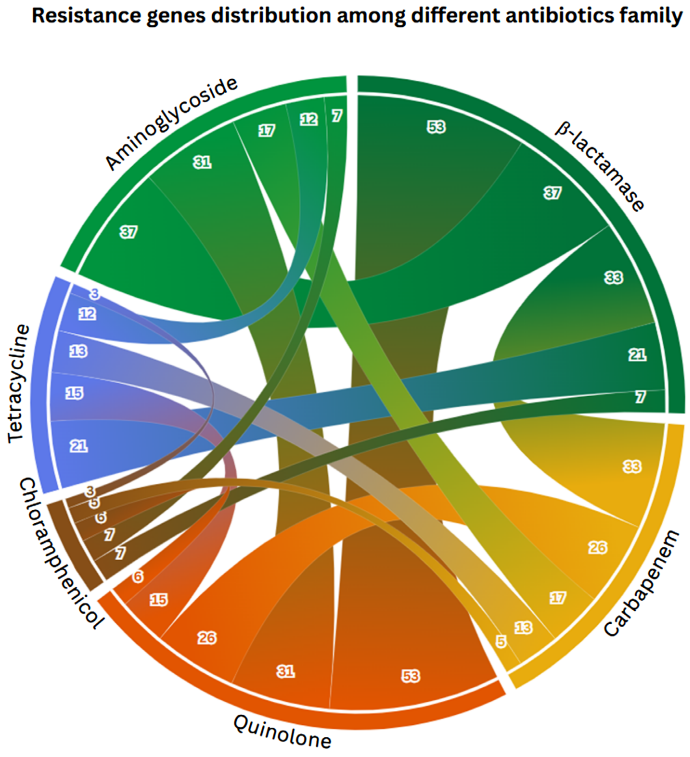



Figure 3. Distribution of resistance genes across different classes of antibiotics in *E. coli* strains.

Table 3. Distribution of integron genes based on the number of co-detection resistance genes.

| Number of resistance genes | Integron 1 (n = 34) | | Integron 2 (n = 25) | | Integron 3 (n = 30) | |
|---|---|---|---|---|---|---|
| | n (%) | *p*-value | n (%) | *p*-value | n (%) | *p*-value |
| **1** | 4 (11.4) | 0.962 | 1 (4) | 0.185 | 3 (9.7) | 0.791 |
| **2** | 9 (25.7) | 0.214 | 6 (24) | 0.236 | 8 (25.8) | 0.262 |
| **3 or more** | 21 (60) | 0.14 | 18 (72) | 0.011 | 19 (61.39) | 0.128 |

### Analysis of $bla_{NDM}$ Sequencing Results

Sequencing of the PCR-amplified $bla_{NDM}$ product was successful for one sample and showed high similarity to $bla_{NDM-5}$. The encoded protein was characterized by several amino acid substitutions, including Val → Leu at position 88 and Met → Leu

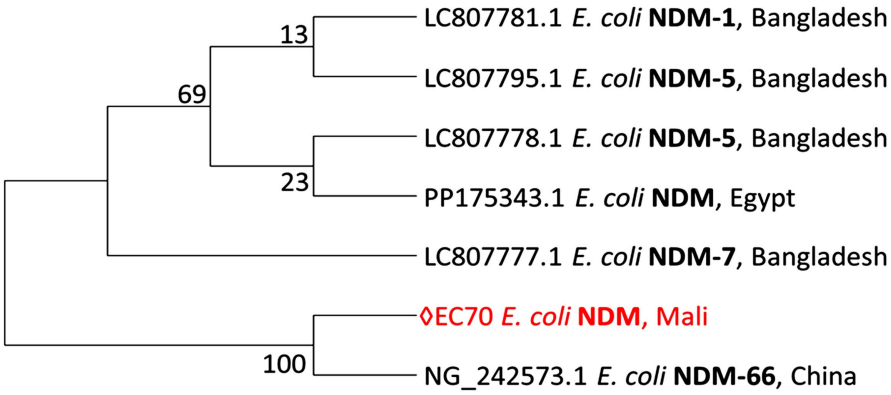
at position 154. It differed from previously described enzymes by additional substitutions at positions 132 (Leu → Met) and 166 (Asp → Hist), which may alter the structure or function of the protein (Table 4). These novel mutations could contribute to reduced susceptibility of *E. coli* strains to expanded-spectrum cephalosporins and carbapenems. However, functional validation studies, such as site-directed mutagenesis and phenotypic susceptibility testing, are required to confirm the impact of these specific amino acid substitutions on enzyme activity and antibiotic resistance.

Table 4. Analysis of mutations in *bla*$_{NDM}$ sequencing results.

| Position | Mutation type | Mutation consequence |
|---|---|---|
| 88 | G → T substitution | Valine-Leucine change |
| 98 | C → A substitution | Silent mutation |
| 132 | C → A substitution | Leucine-Methionine change |
| 154 | A → C substitution | Methionine-Leucine change |
| 166 | A → C substitution | Asparagine-Histidine change |

### Phylogenetic Diversity of NDM-Producing *Escherichia coli* Strains Including a Malian Isolate

The single sequence from this study is closely related to the sequence from China (Figure 4).




Maximum-likelihood phylogeny of *bla*$_{NDM}$ sequence from this study was reconstructed with MEGA version 7.0.26. Phylogeny was inferred by using the Maximum Likelihood method based on the Tamura-Nei model, and branch support was evaluated with bootstrap approximation using 1000 replicates. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. We used six sequences from BLAST in GenBank, along with the single sequence from this study. The sequences from this study is shown in red.

Figure 4. Phylogeny of *bla*$_{NDM}$ sequence from this study.

## 4. Discussion

Enteropathogenic *E. coli* (EPEC) strains play a central role in the occurrence of diarrhea, especially in children [18] [19]. In this study, 86% of the isolates were identified as DEC, representing a higher prevalence than that reported in other African countries: 61.1% in Niger, 55.9% in Ethiopia [20] [21], and 45% in Burkina Faso [22]. Conversely, lower rates have been observed in Colombia (17.9%) [18], Kenya (22.46%) [19], and India (17.4%) [23]. Such variability may be attributed to differences in the target population, sanitation conditions, and detection methods.

In this study, EPEC strains were the most frequently detected, followed by EAEC and ETEC. This trend is consistent with findings from Kenya [19], South Korea [21], and India [23], although the latter did not report any ETEC strains. Other studies, however, have reported the opposite pattern, with a predominance of EAEC followed by EPEC [24]-[26]. Although the overall prevalence of EPEC is generally low, these strains are highly contagious in children and can lead to more severe forms of diarrhea [27]. We did not differentiate between typical and atypical EPEC strains, but previous research suggests that typical EPEC strains are more commonly associated with diarrheal illness in developing countries [28].

Additionally, 50% of DEC strains in this study exhibited mixed infections involving two or three virulence genes. These combinations may enhance the pathogenic potential of the strains and exacerbate the severity of infection [29] [30]. Similar associations have been reported in Sub-Saharan Africa, although at lower frequencies [20] [22] [26]. Regarding antibiotic resistance, 50% of the DEC strains in this study were multidrug-resistant (MDR), defined as the presence of at least one resistance gene in three different antibiotic classes. By comparison, MDR rates were 63.2% in Ethiopia [21], 95.3% in Nigeria [31], and 42.07% in Iran [32]. In Mali, a study also reported high levels of beta-lactam resistance, with 13 different variants identified [7].

The most frequently detected resistance genes in this study were $bla_{\text{TEM}}$ (49%), $bla_{\text{CTX-M}}$ (35.7%), and $bla_{\text{SHV}}$ (25.5%). These results align with those obtained by Saye in Bamako [33] and are consistent with findings from other studies. For instance, in Iran, the prevalence of these genes was 93.2% for $bla_{\text{TEM}}$, 20.5% for $bla_{\text{CTX-M}}$, and 2.3% for $bla_{\text{SHV}}$ [34]. Co-detection of resistance genes across multiple antibiotic classes was also observed, particularly between beta-lactams and quinolones, and between beta-lactams and aminoglycosides. A similar, though less frequent, association was reported in Iran (11.4%) [34]. The coexistence of ESBL genes and fluoroquinolone resistance has also been documented in other studies [35]. We also found isolates carrying both the $bla_{\text{NDM}}$ gene and integron genes.

The high prevalence of multidrug-resistant (MDR) *E. coli* observed in this study may be attributed to the strong selection pressure exerted by excessive and inappropriate antibiotic use in Mali. Historical data support this hypothesis; for instance, a 2002 study conducted in community health centers (CSCOM) reported an antibiotic prescription rate of 61.6%, with a substantial proportion classified as

inappropriate [36]. Such prescribing practices not only promote resistance but also reduce the effectiveness of commonly used treatments over time. Beta-lactams remain the most frequently prescribed class, followed by aminoglycosides [37]. This pattern of antibiotic use is concerning, as it may contribute to the continued selection of resistant strains, particularly in environments with limited diagnostic capacity and antimicrobial stewardship programs.

We also observed a high prevalence of class 1 integrons, followed by class 3 and class 2 integrons. These genetic elements facilitate the acquisition and dissemination of resistance genes. A similar integron distribution profile was reported by Guindo *et al*. in Mali [7]. In contrast, a study conducted in Iran found that class 2 integrons (76.8%) were more strongly associated with multidrug resistance [32]. In our study, class 2 integrons were significantly associated with the presence of more than three resistance genes. For class 1 and class 3 integrons, associations were observed but did not reach statistical significance. This contrasts with the findings of Singh *et al*., who reported a significant association only for class 1 integrons [38].

Clinically, such extensive co-detection has serious implications. The presence of linked resistance determinants can drastically limit empirical therapy: for example, the combination of $\beta$-lactamase and PMQR genes may render both cephalosporins and fluoroquinolones ineffective, while co-carriage of carbapenemase and aminoglycoside resistance genes substantially restricts salvage treatment options. These resistance gene combinations, therefore, pose a major threat to the efficacy of first-line and last-resort antibiotics in both community and hospital settings.

The frequent co-detections of resistance genes across beta-lactams and other antibiotic classes observed in this study strongly suggest that resistance determinants are not acting in isolation and may be mediated by linked elements, such as plasmids harboring multiple resistance genes. In addition, co-transmission of plasmid-mediated quinolone resistance (PMQR) genes with extended-spectrum $\beta$-lactamase (ESBL) genes has been documented in *Enterobacteriaceae* [39]. Such combinations can severely constrain empirical therapeutic choices, as previously noted in ESBL- and PMQR-co-harboring *Enterobacteriaceae* [39].

The co-occurrence of carbapenemase and aminoglycoside resistance genes has also been reported in clinical isolates [40] [41], further compounding the risk of multidrug resistance. Clinically, such extensive co-detection has serious implications. These resistance gene combinations, therefore, pose a major threat to the efficacy of first-line and last-resort antibiotics in both community and hospital settings.

Finally, the detection of the $bla_{\text{NDM}}$ gene, which encodes carbapenemase production, underscores the emergence of highly resistant strains. To our knowledge, this is the first report of the NDM $\beta$-lactamase gene with mutations in Mali, highlighting its spread within hospital settings in Bamako. These findings emphasize the urgent need for enhanced surveillance and molecular studies to mitigate the

clinical impact of this emerging resistance threat. This gene was first identified in 2008 [42], and the sequence obtained in our study reveals two characteristic mutations at positions 88 (Val → Leu) and 154 (Met → Leu) [43]. The presence of *NDM-5* in clinical isolates constitutes a major public health concern, given the limited therapeutic options available for treating infections caused by such strains.

One limitation of this study is the low number of sequenced samples due to the limited resources and its retrospective design. However, a key strength is the large number of resistance genes investigated using a molecular approach. Finally, additional studies using high-throughput sequencing (NGS) are needed to better characterize the genetic diversity of *E. coli* strains and to deepen our understanding of the molecular mechanisms underlying their resistance.

## 5. Conclusion

This study demonstrates the prevalence of diarrheagenic *E. coli* (DEC) strains carrying diverse antibiotic resistance genes, highlighting the public health threat posed by multidrug-resistant DEC. Based on these results, we recommend implementing routine molecular surveillance for carbapenemase genes in Malian hospitals to monitor their spread and inform infection control strategies.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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