Characterization of class 1, 2 and 3 integrons in multidrug-resistant *Escherichia coli* isolated from clinical samples from Niamey, Niger

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Antibiotic resistance is a major public health problem worldwide. *Escherichia coli* is one of the bacteria most frequently isolated in hospital infections and became more resistance to common antibiotics used. This resistance to antibiotics could be attributed to a modification of the genetic supports or the acquisition of mobile genetic elements. A total of 195 multi-drug resistant *E. coli* isolated from clinical samples, were analyzed. Of these multi-drug resistant *E. coli*, 54 isolates were producing extended-spectrum beta-lactamase. The presence of class 1, 2, and 3 integrons was performed using simple PCR. To highlight the different classes of integrons, genomic DNA was extracted with the QIAmpl, DNA mini, and Qiagen kit. The result of the 195 isolates DNA amplification showed that 60.5% isolates were positive for the class 1 integron, while class 2 integron was found in 6 isolates (3.1%) and class 3 integron was found in 24 isolates (12.3%). Among multi-drug resistant *E. coli* producing extended spectrum beta-lactamase, 68.5% carried the class 1 integron, 3.7% for the class 2 integron, and 13% for the class 3 integron. The results of this study showed the presence of three classes of integrons in several clinical isolates of multi-drug resistant *E. coli*. The simultaneous presence of resistance genes and integron classes in several extended-spectrum beta-lactamase-producing isolates demonstrates the need for increased monitoring of antibiotic use.

**Key words:** Integron, multi-drug resistant, *Escherichia coli*, extended-spectrum beta-lactamase.

INTRODUCTION

The increasing of resistance to commonly applied antimicrobial agents is being reflected by growing multiple drug resistance in bacteria and is becoming a growing threat to public health. The use of antimicrobial agents in animal husbandry has been linked to the development and spread of resistant bacteria (Agyare et
Escherichia coli, a conditional pathogen, is one of the most common and important pathogens in medical care settings. It is the most prominent cause of diarrhea, urinary tract infections, septicemia, and various other clinical infections, including neonatal meningitis (Wu et al., 2021). The problem of bacterial antibiotic resistance is one of the World Health Organization’s highest priorities when it comes to threats to human health (Nasif et al., 2022). Beta-lactamase mediated resistance in E. coli is a significant problem that requires immediate attention (Tewari et al., 2022).

Acquiring mobile elements, including plasmids, transposons, and integrons among Gram-negative bacteria, plays an important role in the development of antibiotic resistance (Süterlin et al., 2020). Various classes of integrons possessing a wide variety of gene cassettes are distributed in bacteria throughout the world. The role of integrons as mobile genetic elements playing a central role in antibiotic resistance has been well studied and documented. Integrons are the ancient structures that mediate the evolution of bacteria by acquiring, storing, disposing, and resorting to the reading frameworks in gene cassettes (Sabbaghi et al., 2021).

Several classes of integron have been described, including classes 1 and 2 of the most common integrons of multi-drug resistant. Gram-negative bacteria are associated with antibiotic treatment failure (Kaushik et al., 2018).

The presence of integrons in the clinical E. coli isolates is also highly related to antibiotic resistance, class 1-integron was highly prevalent in these pathogenic isolates (Nasif et al., 2022). Class I integrons of E. coli strains were present in all sources, while the prevalence of intI2 was lower but remarkable in food isolates (Etayo et al., 2018).

The percentage of clinical multi-drug resistant E. coli isolates was higher among those positive for integron II gene followed by integron III gene (Taha et al., 2018).

The gene blaTEM, blaSHV, blaCTX-M as well as integrons (Int1, Int2, and Int3) are involved in the antibiotic resistance of diarrheagenic E. coli (Dembélé et al., 2022).

This study aims to determine the prevalence of class 1, 2, and 3 integrons in multidrug-resistant E. coli isolated from the clinical specimen in two hospitals in Niamey, Niger.

MATERIALS AND METHODS

Study design and samples

It is a cross-sectional study conducted in two hospitals of Niamey, Niger (National and AMIROU BOUBACAR DIALLO hospitals). The study investigated 195 isolates of multi-drug resistant E. coli obtained from various clinical specimens collected from March 2014 to June 2016. The clinical specimens included: urine, stool, blood, vaginal swab, and pus.

Isolation, identification, antimicrobial susceptibility testing of isolates, and phenotypic characterization of extended-spectrum beta-lactamases (ESBL) were described in our previous study (Allo et al., 2017).

Genomic DNA extraction

Genomic DNA extraction was performed with the QIAmp DNA mini kit (Qiagen Germany). Two colonies of E. coli isolates were suspended in 180 µl ATL buffer for the first digestion. The mixture was homogenized, then 20 µl of proteinase K was added, vortexed, and incubated at 56°C. After 1 h of incubation, the tube was centrifuged for 1 min at 8,000 rpm. After, 200 µl of AL buffer was added. The mixture was homogenized and incubated at 70°C for 10 min. Then 200 µl of 100% ethanol was added. The mixture was centrifuged at 8,000 rpm for 3 min. The tube containing 600 µl of the total mixture was placed in the Qiagen column and centrifuged at 8,000 rpm. After 3 min, 500 µl of AW1 buffer was added to the column and centrifuged at 8,000 rpm for 3 min. Once this step was complete, 500 µl of buffer AW2 was added to the column and centrifuged at 14,000 rpm for 3 min. The column was then placed in an Eppendorf tube and 200 µl of buffer AE was added. The Eppendorf tube was incubated at room temperature for 1 min and then centrifuged at 8000 rpm for 3 min. The column was then discarded, and the Eppendorf tube DNA was stored at -20°C for integron analysis.

Characterization of integrons

The presence of class 1, 2, and 3 integrons was tested using simple PCR according to Ploy et al. (2000). Primers sequences and amplicons of the different classes of integrons are listed in Table 1.

Single PCRs were performed with a final reaction volume of 25 µl. The PCR mix contained 2.5 µl of 10 X GC buffer, 0.5 µl of dNTPs (10 mM), 2 µl of MgCl2 (25 mM), 0.25 µl of Taq Polymerase (5 U/l), 14.25 µl of H2O, 1.5 µl of Forward primers, 1.5 µl of Reverse primers and 2.5 µl of DNA lysate. The PCR conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s for denaturation, annealing at 60°C (IntI1) and 62°C (IntI2 and IntI3) for 1 min, and then extension at 72°C for 1 min followed by a final extension of 72°C for 7 min. Amplicons were stored at 4°C for electrophoretic separation. After PCR, 10 µl of each amplicon was mixed with a drop of blue loading buffer and then separated by electrophoresis on agarose gel (1%) with tris borate EDTA buffer (1X) at 130 V and 300 mA during 1 h.

Ladder of 100 and 200 bp (HyperLadder I, Bioline) were used. Once migrated, ethidium bromide gels were visualized under UV light. The molecular weight of the amplified fragment was checked against the expected fragment using several ladders. For the positive control, DNAs from the reference strains R3 and R7 were used for class 1 and 2 integrons, respectively.

Data analysis

Data were processed and analyzed using Microsoft Excel 2013 and

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The PCR amplification results showed that, of the 195 isolates, 118 were positive for the class 1 integron (IntI1) which represented 60.5% of all tested strains while class 2 Integron (IntI2) was found in 6 isolates (3.1%) and the class 3 integron (IntI3) was found in 24 isolates (12.3%) (Table 2).

The results in Table 2 indicated a higher prevalence of IntI1 in stool isolates (89.8%) than in other isolates from urine (50.7%), pus (57%), and blood (50%) \((p = 0.0006)\).

In contrast, the prevalence of IntI2 observed in pus isolates (14.3%) was higher than that observed in stool isolates (4.1%) and urine isolates (2.2%) \((p = 0.0020)\).

On the other hand, results of this study reported the presence of IntI3 only in stool isolates with a prevalence of 49%. Figure 1 shows amplicons sizes of the different classes of integrons.

### Prevalence of class 1, 2, and 3 integrons in ESBL-producing \(E.\) coli isolates

Among the multidrug resistant \(E.\) coli isolates, 54 of them were producing extended spectrum beta-lactamases.

From stools samples, the results indicate that there was no significant difference \((p = 0.7637)\) between the prevalence of IntI1 in ESBL-producing \(E.\) coli (85.7%) and that observed in multidrug-resistant \(E.\) coli strains that did not express ESBL (91.4%). No ESBL-producing \(E.\) coli contained IntI2 gene was observed. However, a prevalence of 5.7% of these integrons was observed in \(E.\) coli which does not express ESBL. Moreover, for IntI3, a prevalence of 50 and 48.6% was observed in ESBL-producing and non-ESBL-producing \(E.\) coli isolates, respectively \((p = 1.00)\).

In urine samples, the prevalence of IntI1 was 59.5% in ESBL-producing \(E.\) coli and 47.4% in multidrug-resistant \(E.\) coli which do not express ESBL \((p = 0.2460)\). The

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**Table 1.** Primers used for the detection of integrons.

<table>
<thead>
<tr>
<th>Integrons</th>
<th>Primer sequence (5'-3')</th>
<th>Amplicon size (PB)</th>
<th>Annealing temp. (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IntI1</td>
<td>F: ATTTCTGTCTCTGGCTGGGA</td>
<td>600</td>
<td>60</td>
<td>Ploy et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>R: ACATGATGATGCGACGGACGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IntI2</td>
<td>F: CACCGGATATGCGACAAAAAGG</td>
<td>806</td>
<td>62</td>
<td>Ploy et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>R: GTAGCAAACGACTGACGAAATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IntI3</td>
<td>F: GCCCCGGCGGAGGACTTTCAG</td>
<td>600</td>
<td>62</td>
<td>Ploy et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>R: ACGGCTCTGCGAAACCTGACT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Prevalence of class 1, 2 and 3 integrons among MDR \(E.\) coli.

<table>
<thead>
<tr>
<th>Integrons class n (%)</th>
<th>Stool N=49</th>
<th>Urine N=134</th>
<th>Pus N=7</th>
<th>Blood N=4</th>
<th>Vaginal swabs N=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IntI1</td>
<td>44 (89.8)</td>
<td>68 (50.7)</td>
<td>4 (57)</td>
<td>2 (50)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IntI2</td>
<td>2 (4.1)</td>
<td>3 (2.2)</td>
<td>1 (14.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IntI3</td>
<td>24 (49)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**RESULTS**

**Bacterial isolates and antimicrobial susceptibility testing**

A total of 195 multi-drug resistance (MDR) \(E.\) coli were collected and analysed during the study period. Among these isolates, 54 (27.7%) were extended-spectrum beta-lactamases producers. Therefore, 49 (25.1%) strains of multi-resistant \(E.\) coli were isolated from stool samples, 134 (68.7%) strains from urine samples, 7 (3.6%) from pus samples, 4 (2.1%) from blood samples, and one strain from vaginal swabs.

As shown in our previous study, high resistance to beta-lactams was observed, mainly with ampicillin (100%), amoxicillin + clavulanic acid (93.1%), cephalothin (98.2%), cefotaxime (92.6%), ceftazidime (97.2%), and ceftriaxone (83.9%) as compared to quinolone with ofloxacin (77.4%), ciprofloxacin (84.9%), and nalidixic acid (91.2%). Resistance to the monobactams was 77.4% to aztreonam, and the sulphonamides were 95.4% to trimethoprim-sulfamethoxazole (Alio et al., 2017).

**Prevalence of class 1, 2 and 3 integrons in multidrug-resistant \(E.\) coli isolates**

The PCR amplification results showed that, of the 195 isolates, 118 were positive for the class 1 integron (IntI1) which represented 60.5% of all tested strains while class

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Med Cal version 11.0.1.0. \(p < 0.05\) was considered to be statistically significant.
prevalence of \textit{IntI2} was 5.4 and 1\% in ESBL-producing \textit{E. coli} and non-ESBL-producing \textit{E. coli}, respectively ($p = 0.2207$). No \textit{IntI3} was detected in urine isolates. Only \textit{IntI1} in ESBL-producing isolates from pus and blood was detected with a prevalence of 100\% (Table 3).

**Combination of different resistance integron classes**

Results in Table 4 indicated that only isolates from stool and urine carry two or three classes of integrons simultaneously. In stool isolates, the prevalence of \textit{IntI1 + IntI3} (49\%) was significantly higher ($p < 0.0001$) than the other types of combinations \textit{IntI1 + IntI2} (4.1\%) and \textit{IntI2 + IntI3} (4.1\%). However, the combination of all three integron classes (\textit{IntI1 + IntI2 + IntI3}) was only observed in stool isolates with a prevalence of 2\%. For urine isolates, only a prevalence of 1.2\% of \textit{IntI1 + IntI2} was observed.

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**Table 3.** Prevalence of class 1, 2, and 3 integrons in ESBL-producing and non-producing \textit{E. coli} isolates.

<table>
<thead>
<tr>
<th>Integrons class</th>
<th>Stools</th>
<th>Urine</th>
<th>Pus</th>
<th>Blood</th>
<th>Vaginal swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESBL + N=14</td>
<td>ESBL - N=37</td>
<td>ESBL + N=97</td>
<td>ESBL - N=5</td>
<td>ESBL - N=1</td>
</tr>
<tr>
<td>\textit{IntI1} n (%)</td>
<td>12 (85.7)</td>
<td>32 (91.4)</td>
<td>22 (59.5)</td>
<td>46 (47.4)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>\textit{IntI2} n (%)</td>
<td>0 (0)</td>
<td>2 (5.7)</td>
<td>2 (5.4)</td>
<td>1 (1.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>\textit{IntI3} n (%)</td>
<td>7 (50)</td>
<td>17 (48.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Table 4.** Combination prevalence of different resistance integron classes.

<table>
<thead>
<tr>
<th>Integrons class</th>
<th>Stools N=49</th>
<th>Urine N=134</th>
<th>Pus N=7</th>
<th>Blood N=4</th>
<th>Vaginal swabs N=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{IntI1 + IntI2}</td>
<td>2 (4.1)</td>
<td>2 (1.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>\textit{IntI1 + IntI3}</td>
<td>24 (49)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>\textit{IntI2 + IntI3}</td>
<td>2 (4.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>\textit{IntI1 + IntI2 + IntI3}</td>
<td>1 (2.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Figure 1. Integrons class \textit{IntI1} (A), \textit{IntI2} (C) and \textit{IntI3} (B) of stool samples gel on agarose.
Prevalence of integron classes associated with resistance genes

Results of stool samples showed a high prevalence (95.5%) of E. coli isolates that harboured both the IntI1 and bla TEM genes. This prevalence was higher (p < 0.0001) than that of isolates that harboured both IntI1 and bla CTX-M (70.5%), bla OXA-1 (75%), and bla SHV (18.2%). The prevalence of isolates harbouring IntI2, IntI3, and the bla TEM gene was also higher (p < 0.0001) than those harbouring IntI2 and IntI3 with the bla CTX-M, bla OXA-1, and bla SHV genes.

For urine isolates carrying IntI1 and the bla TEM, bla CTX-M, and bla SHV genes showed a prevalence of 82.4, 42.6, and 4.4%, respectively. These results showed that there was a significant difference in isolates harbouring IntI1 and bla TEM, bla CTX-M, and bla SHV genes simultaneously (p < 0.0001). For isolates carrying IntI2, 100 and 33.3% prevalence was observed with bla TEM and bla CTX-M genes, respectively. For isolates from pus and blood, only isolates carrying IntI1 harboured bla TEM, bla CTX-M, and bla OXA-1 genes (Table 5).

Table 5. Prevalence of isolates harbouring integron classes and resistance genes.

<table>
<thead>
<tr>
<th>Integrons class</th>
<th>Isolates origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stools</td>
</tr>
<tr>
<td></td>
<td>bla TEM</td>
</tr>
<tr>
<td>IntI1 n (%)</td>
<td>42 (95.5)</td>
</tr>
<tr>
<td>IntI2 n (%)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>IntI3 n (%)</td>
<td>24 (100)</td>
</tr>
</tbody>
</table>

DISCUSSION

Integrons are genetic elements that play a major role in antibiotic resistance transmission. They can carry several resistance genes at the same time. Integrons play an essential role in disseminating drug-resistance genes among bacteria isolates (Barzegar et al., 2022). The co-occurrence of these genetic elements significantly contributes to the dissemination of antibiotic resistance in Enterobacteriaceae and has been associated with specific genes conferring resistance to β-lactams, quinolones, and aminoglycosides (Tewari et al., 2022).

The results obtained in strains isolated from stool samples showed a higher prevalence of IntI1 (89.8%) than IntI2 (4.1%) and IntI3 (49%). Similar results were reported by a study in Iran where the prevalence of IntI1(78.26%) was higher than IntI2 (76.81%) (Kargar et al., 2014). Furthermore, the results of a study in Spain reported by Vinue et al. (2008) showed a higher prevalence of IntI1 than IntI2 detected in isolates from stool (Vinue et al., 2008). Otherwise, the prevalence of IntI3 was higher than that observed in a study in Burkina Faso (Dembelé et al., 2022). Globally, these results showed that class I integrons are extremely important for the development and transmission of resistance genes in clinical E. coli strains. Overall, given the high prevalence of IntI1, it can be suggested that multidrug resistance is associated with the presence of these IntI1.

Regarding urine isolates, the results showed a higher prevalence of IntI1 (50.7%) than IntI2 (2.2%). However, IntI3 was not found. The results of the present study are similar to those reported by a study that was done in Iran by Khoramrooz et al. (2016), where a prevalence of IntI1 of 52 and 2.5% for IntI2 was reported. The same study reported the absence of IntI3 in urine isolates (Khoramrooz et al., 2016).

However, the results of this study are lower than those of Zeighami et al. (2014) who reported a prevalence of 78.8 and 4.5% for IntI1 and IntI2, respectively (Zeighami et al., 2014). A recent study in Iran reported the incidence of class 1 and 2 integrons was obtained in 39.9 and 14.1% of the isolates, respectively. Class 3-integron was not detected in any of the Uropathogenic E. coli isolates (Nasif et al., 2022; Barzegar et al., 2022). However, results of this study were contradicted by those reported by Lin et al. (2015) in which any isolates from urine carried IntI2 and IntI3 (Lin et al., 2015). Overall, the results showed an absence of IntI3 in isolates from urine, pus, blood, and vaginal swabs. This suggests that IntI3 appears to play a minor role in resistance in these E. coli strains (Moura et al., 2010).

The results of this study also showed the coexistence of two or even three integrons class in certain isolates. Integrons of class 1 and 3 were found simultaneously in 24 (49%) stool isolates. Etayo et al. (2018) reported the coexistence of IntI1 and IntI2 in 8% in ESBL-producing E. coli (Etayo et al., 2018). Rizk and El-Mahdy (2017) reported the co-existence of more than one type
of integron in 36.9% of isolates, and a prevalence of 38% was reported by Kargar et al. (2014) in a study performed in 69 multidrug-resistant (MDR) E. coli. Kor et al. (2013) found only one isolate carrying both integrons among clinical isolates. Odetoyin et al. (2017) reported a prevalence of 2.4% in fecal E. coli isolated from mother–child pairs in Nigeria. Results of the present study revealed a prevalence of 1.2% for IntI1 and IntI2 simultaneously in urine isolates. Previous studies have reported the simultaneous occurrence of IntI1 and IntI2 in 3.3% (Alkhuheidary et al., 2019). Integrons, capable of integrating, expressing, and disseminating gene cassettes carrying resistance determinants, play a critical role in facilitating the multidrug resistance (MDR) phenotype in these bacteria (Sabbagh et al., 2021).

Conclusion

This study reported the existence of class 1, 2 and 3 integrons in clinical isolates of multi-resistant E. coli obtained from different biological samples. Thus, class 1 integrons were observed with a high percentage. The co-existence of these integrons with resistance genes in ESBL-producing strains of E. coli had also been demonstrated. Hence, it is necessary to set up a surveillance system in order to better control the dissemination of resistance genes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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