Multidrug resistance, prevalence and phylogenetic analysis of genes encoding class II and III integrons in clinically isolated *Escherichia coli*

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Abstract: The present study was conducted to evaluate the susceptibility, multidrug resistance and genetic characteristics of *Escherichia coli* (E. coli) strains, isolated from clinical samples from Jazan Hospital, Jazan, Saudi Arabia. A total of 95 samples were recruited from wound, urine, stool and blood. The isolates were assessed for their antibiotic susceptibilities and the presence of class II and III integrons was studied using PCR technique. Integron II-positive PCR products were further confirmed using partial sequencing. Ampicillin (84.2%) was found to be the most resistant antibiotic followed by ciprofloxacin (57.9%), gentamicin (56.8%), nalidixic acid (50.5%), cefotaxim (49.5%), ofloxacin (45.3%), amikacin (26.3%) and imipenem (16.8%), respectively. Seventy-six isolated strains were further confirmed using partial sequencing. Ampicillin (84.2%) was found to be the most resistant antibiotic followed by ciprofloxacin (57.9%), gentamicin (56.8%), nalidixic acid (50.5%), cefotaxim (49.5%), ofloxacin (45.3%), amikacin (26.3%) and imipenem (16.8%), respectively. Seventy-six isolates of E. coli were assessed for their antibiotic susceptibilities and the presence of class II and 5 (6.5%) were positive for class III integron. Bioinformatics’ analysis have shown that the sequences of our samples aligned along with many published sequences of integron II. Our findings further strengthen the significance of hospital-based and community E. coli strains on rising the burden of antimicrobial resistance.

Key words: Integron II; Integron III; *Escherichia coli*; Saudi Arabia; Clinical samples; Multidrug resistance.

Introduction

*Escherichia coli* is a common occupant of the human and animal gastrointestinal tract, but can also be found in the surrounding environment such as water, soil and vegetation. It is the leading pathogen causing urinary tract, blood stream, wounds, *otitis media* infections and other health problems in human (1, 2). *E. coli* is the most common cause of food and water-borne human diarrhea, leading to many deaths in children (3). Incidence and vulnerability profiles of *E. coli* show considerable geographic differences as well as significant variations in diverse populations and environments (4).

Resistance to antimicrobial drugs has recently been known as a international burden in human and veterinary remedies (5). Moreover, extensive utilization of antibiotics in medicine and crops is established as a main discriminating factor in the high incidence of antimicrobial resistance among gram-negative bacteria (6). In general, up to 95% of cases with severe symptoms are treated without bacteriological investigations (7, 8). An increase in microbial resistance to antibiotics complicates treatment of infections. Resistance in *E. coli* has been documented internationally and its increasing rate is being noticed as a growing alarm (6, 9).

Biochemical mechanisms of resistance involve mutational alterations, enzymatic inactivation, genetic acquisition and bypassing of the potential targets (10). Multidrug resistance is often caused by the accumulation of genes on R plasmids with each codes for resistance to a single drug (11). The discovery that many resistance genes in R plasmids contain a unique 59-base 3′-sequence tag led to the discovery of a remarkable apparatus called an integron (12). The integron contains a gene coding for an integrase enzyme, which catalyzes the insertion of resistance genes at a predetermined site. Integrons, for example, are especially powerful in producing multidrug resistance because they assemble several resistance genes in a correct orientation and supply a strong promoter for their expression. Furthermore, the resistance gene once incorporated into an integron becomes tagged, so that it could easily become a part of another integron (11).

*E. coli* is the most common pathogen isolated from both nosocomial and community-acquired infections. A considerable relationship between the presence of integrons and antibiotic resistance has been reported (13). The association between multidrug resistance and existence of integrons in *E. coli* has been studied in many areas of the world (14). An inadequate research has been performed regarding the distribution and existence of integrons amongst multi-drug resistant *E. coli* in Saudi Arabia. Therefore, the current study was intended to examine the multidrug resistance and distribution of integrons amongst clinically isolated *E. coli*. PCR and
Materials and Methods

Isolates

Ninety five clinical isolates of E. coli were collected from Jazan General Hospital, Jazan, KSA. Isolates were recruited from patients' clinical specimens including urine, wound, stool and blood after following conventional clinical and safety procedures. Significant bacterial growth was included in this study and it was identified on the basis of cultural characteristics, gram stain and conventional biochemical tests. When confirmed as E. coli, the isolates were preserved at −70 °C in tryptic soy broth containing 20% sterile glycerol for further investigations.

Antimicrobial susceptibility test

The antibiotic susceptibility test was conducted using disk diffusion method on the suitable agar plates as recommended by the Clinical Laboratory Standards (Jazan General Hospital, Ministry of Health, KSA). The disks were impregnated with ampicillin, ofloxacin, nalidixic acid, gentamycin, imipenem, cefoxacin, amikacin and ciprofloxacin. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate susceptible or susceptible. Multi-drug resistance was defined as resistance for more than three of the tested antimicrobials.

Polymerase Chain Reaction (PCR) Analyses and Sequencing

DNA was isolated using Promega DNA Kit (Madison, USA) following manufacturer’s instructions. PCR was carried out for detection and identification of integrons genes (II and III). Five µL of extracted DNA was used as sample for the amplification. DNA samples were amplified by oligonucleotide primers (Integrated DNA Technologies, Belgium) as shown in Table 1. PCR conditions are shown in Table 1. Initial denaturation was performed at 94°C for 3 min, 35 cycles of denaturation was programmed at 94°C for 60 second, annealing was done at 60°C for 60 second, extension was performed at 72°C for 120 second while final extension was held for 5min. PCR were carried out in total 25 µL reaction volume, each contain 12.5µL master mix and 25µM of each primer. PCR products were analyzed using gel electrophoresis, stained with ethidium bromide and visualized under gel documentation system.

Sequencing and bioinformatics analysis

Purification and standard sequencing for PCR products were performed by Macrogen Company (Seoul, Korea). Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using ABI PRISM® Big Dye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using D1 (forward) primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with Big Dye®X Terminator™ purification protocol. The samples were suspended in distilled water and subjected to electrophoresis in a sequencer. The sequences were searched for sequence similarity.

Statistical analysis

Data were entered and managed using SPSS software, (IBM, USA). All descriptive and inferential statistical analyses were performed using SPSS. Alpha value was set as 0.05.

Results

Disc diffusion method and MDR findings

E. coli was isolated from 95 clinical specimens of...
isolates were identified as being positive for class II and III integrons, respectively, as shown in Table 3, Figure 1 and Figure 2. The percentage of MDR *E. coli* isolates was higher among those positive for integron-II gene (91.1%) followed by integron III gene (83.3%). The current results revealed statistical significant association between MDR and integron-II while there was no significant association for integron-III. The presence of integron –II and III genes amongst non-MDR *E. coli* is shown in Table 3. Whereby, 4 strains were found to be positive for integron II.

### Sequencing and Phylogenetic Analysis
To confirm the PCR results, the partial sequencing was done for forty five PCR product samples represent positive integron II. The Blast search showed that the sequences of our samples aligned along with many published sequences of integron II as shown in Table 4, Figure 3 and the similarity search which illustrates the Genbank accession numbers and the country of isolates.

### Table 3. Distribution of MDR E. coli according to the presence of integrons.

<table>
<thead>
<tr>
<th>Antibiotic resistance pattern</th>
<th>Integron –II</th>
<th></th>
<th>Integron –III</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+VE</td>
<td>%</td>
<td>-VE</td>
<td>%</td>
</tr>
<tr>
<td>Multi-drug resistant</td>
<td>41</td>
<td>91.1</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>Non multi-drug resistant</td>
<td>4</td>
<td>8.9</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 4. Accession numbers of sequences aligned along with tested samples.

<table>
<thead>
<tr>
<th>Gen bank accession No</th>
<th>Gen bank accession No</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC417300.1</td>
<td>KC417300.1</td>
</tr>
<tr>
<td>FJ591049.1</td>
<td>FJ591049.1</td>
</tr>
<tr>
<td>FJ591049.1</td>
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<td>EF560799.1</td>
<td>EF560799.1</td>
</tr>
<tr>
<td>JN987180.1</td>
<td>JN987180.1</td>
</tr>
</tbody>
</table>

![Figure 1](image1.png)  
**Figure 1.** PCR amplification products and agarose gel electrophoresis of integrons II gene (789bp): Lane 1: 100 base-pair (DNA Marker); Lane 2: negative control; Lane 3: positive control; Lanes 4 to7: were integrons II. DNA marker ranges from 100 to 1000 base pair.

![Figure 2](image2.png)  
**Figure 2.** PCR amplification products and agarose gel electrophoresis of integrons III gene (717bp). Lane 1: 100 base-pair (DNA Marker); Lane 2: negative control; Lane 3: positive control; Lanes 4 to7: were integrons III. DNA marker ranges from 100 to 1000 base pair.

![Figure 3](image3.png)  
**Figure 3.** Identities between integrin II from Jazan and integron II of UK (dbj|AB608788.1) *Escherichia coli* isolate P187.11.99.C2 class II integrin. Partial sequence length=2349; Score= 850 bits (460), Expect = 0.0 Identities = 460/460 (100%), Gaps = 0/460 (0%) Strand=Plus/Plus. Subject; reference; Query: Sample.

### Multidrug-resistance and the presence of integrons genes
Among the studied isolates of *E. coli*, the two classes of integrons (II and III) were detected using PCR. Of the 76 MDR *E. coli* isolates, 41 (53.9%) and 5 (6.5%) patients. Moreover, the relation between presence of integrons and susceptibility to eight different antibiotics within *E. coli* strains was investigated. Tables 2 and 3 show antimicrobial resistance profiles of integron-positive and integron-negative *E. coli* strains. As shown in Table 2, findings were expressed as sensitive, intermediate, resistant categories. Susceptibility testing showed that the most resistant category was found against ampicillin (84.2%) followed by ciprofloxacin (57.9%), gentamicin (56.8%), nalidixic acid (50.5%), cefotaxim (49.5%), ofloxacin (45.3%), amikacin (26.3%) and imipenem (16.8%), respectively. Out of the tested sample, 76 (80%) were more sensitive against imipenem. In case of intermediate, the most frequent antimicrobial was amikacin (5.3%) and the lowest was nalidixic acid and ciprofloxacin as depicted in Table 2. Intermediate antibiotics susceptibility statistics of *E. coli* using disk diffusion was noticed to be very low compared to the other categories. Whereby, amikacin showed 5% which observed to the highest percentage. Seventy-six isolated strains were designated as MDR, while non-MDR was found in 19 (20.0%). The percentage of the resistant isolates to the tested antimicrobials is presented in Table 2.
Discussion

In recent years, dissemination of antibiotic resistance genes through integrons in E. coli strains causes difficulties in cure of infections caused by these bacteria (18). The current study was designed to investigate the multidrug resistance and prevalence of genes encoding class II and III integrons in E. coli clinical isolates and their phylogenetic analysis. No previous studies have been conducted on class II and III integrons of the clinical isolates of E. coli from Saudi Arabia, where the available studies were conducted on meat and meat products (19, 20). Therefore, there is a necessity for better survey for resistance and its methods of spreading and persistence and mobility of resistance genes in the community and clinical settings (21).

The results of this paper are in line with previous research (22, 23) which showed that antibiotic resistance in E. coli isolates is rising due to unrestrained utilization of antibiotics. Whereby, considerable percentage of investigated E. coli indicated multidrug- resistance (MDR) phenotype. In this study, antibiotics susceptibility of clinically isolated E. coli was performed using disk diffusion. Our findings showed that the most resistant category was found against ampicillin followed by ciprofloxacin, gentamicin, nalidixic acid, cefotaxim, ofloxacin, amikacin and imipenem, respectively. In this study, a noteworthy correlation between existence of integrons and resistance to the used antibiotics was noticed, whereby, integron-positive strains showed more resistance to these drugs. Moreover, our findings showed that the overall resistance of E. coli to antimicrobials was considered high. Resistance in E. coli isolated from retail raw chicken meat in Taif, Saudi Arabia was most frequently observed against sulphafurazole, ampicillin, nalidixic acid, streptomycin, chloramphenicol and gentamicin (19). The results of this study are in line with the findings of other studies conducted in different parts of the world (22, 23).

In the present study, similar to previous studies (24, 25), PCR technique was utilized to identify integron genes. Overall, these studies showed that amplification of integron II and III genes using PCR was more potent and responsive in recognition of different classes of integrons. Moreover, in this study, PCR was able to detect integrons in all MDR E. coli isolates. The percentage of MDR E. coli isolates was higher among those positive for integrin II genes (91.1%) followed by integron III gene (83.3%). The prevalence of class II integron in our isolates is in agreement with previous report where frequency of class II integron is reported much more than class III. This fact suggests that individuals in the community could be a reservoir of integron containing E. coli isolates. The current results revealed statistically significant association between MDR and integron II while there was no significant association for integron III. Multidrug resistance in the Enterobacteriaceae has been associated with the existence of integrons. The elevated prevalence of integron occurrence principally of the class II integron in the Klebsiella spp. and E. coli harmonize with earlier studies in other geographical areas including Europe, Northern America and Asia (13, 26-30). In all these studies, integrons are considerably linked to the resistance to multiple classes of antibacteri-


