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Research Article

Class 1 Integrons in Clinical Multi Drug Resistance *E. coli*, Sana'a Hospitals, Yemen

¹Mukhtar A. Al-Hammadi, ²Hassan A. Al-Shamahy, ¹Abdulaziz Q. Ali, ³Mahfoudh A.M. Abdulghani, ⁴Hassan Pyar and ¹Ibrahim AL-Suboal

¹Department of Medical Microbiology, Faculty of Medicine and Health Sciences, University of Sciences and Technology, Taiz, Yemen

²Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, P.O. Box 775, Sana'a, Yemen

³Department of Pharmacology, Unaizah College of Pharmacy, Qassim University, 51911 Unaizah, Kingdom of Saudi Arabia

⁴College of Environmental Science and Marine Biology, Hadramout University, Al Mukalla, Yemen

Abstract

Background and Objectives: The occurrence of multi-drug resistance (MDR) *Escherichia coli* is one responsible for raised mortality and morbidity and was reported as major health problem. Class 1 integrons has crucial role in distributing antibiotic resistance genes among bacteria. Present work was aimed to determine the prevalence of class 1 integrons and its association with antibiotic resistance in MDR *E. coli* isolated from patient's body fluid and tissues from 6 health centers in Sana'a, Yemen. **Materials and Methods:** A cross-sectional study a total of 198 *E. coli* from patients diagnosed with infection that had been referred to 6 hospitals and medical diagnostic from July, 2017 to August, 2017 in Sana'a, Yemen. Susceptibility of *E. coli* isolates to 15 antibiotics using the disc diffusion method. Conventional polymerase chain reaction was used for detection of class 1 of integrons in 100 randomly selected MDR *E. coli*. **Results:** Overall 174 (87.9%) of 198 *E. coli* isolates were MDR. Class 1 integrons were detected in 67% of the randomly selected 100 of 198 MDR *E. coli*. A significant range ($p < 0.05$ - $p < 0.0001$) was identified between presence of class 1 integrons and resistance to ceftriaxone, aztreonam, cefepime, amoxicillin-clavulanic acid, cefotaxime, cefepime-clavulanic acid, ceftazidime-clavulanic acid, ciprofloxacin, ceftazidime, norfloxacin and trimethoprim-sulfamethoxazole, while no significant difference were identified between integron class 1 and resistance to gentamicin, amikacin, nitrofurantoin and imipenem. **Conclusion:** High MDR *E. coli* isolates were detected in this study, among them the prevalence of class 1 integrons is the most common. The significant association between class 1 integrons and resistance to common prescribed antibiotics in hospitals in Sana'a, Yemen.

Key words: *Escherichia coli*, Integron, multi-drug resistance, Yemen

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Corresponding Author: Hassan A. Al-Shamahy, Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, P.O. Box 775, Sana'a, Yemen Tel: +967-1-239551, +967-770299847

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antimicrobial resistance in the *E. coli* strains and its association with the presence of integrons have been studied in some Arabian regions, however, little is known of the prevalence of integrons and related gene cassettes in *E. coli* strains isolated from patients in Yemen. This study was conducted for 2 important reasons. 1st: It shows the clinical laboratory's ability to conduct molecular studies in Yemen, 2nd: This study also provides baseline data on the mechanisms of resistance of many antimicrobials as mediated by the integrons. The emergence of resistance to several antibiotics in particular, multi-drug resistance (MDR) has been reported in clinical isolated bacteria as a major health problem and has received more attention in Yemen recently¹⁻⁵.

The emergence of resistance to several antibiotics particularly, multidrug resistance (MDR) in clinical isolated *Escherichia coli* was reported as major health problem and got more attention globally in the last decade^{6,7}. Because occurrence of *E. coli* with multi-drug resistance is associated with raised mortality and morbidity^{6,8}. The *E. coli* strains is recognized with MDR when they show resistance to ≥ 3 antibiotics and to ≤ 3 corresponding antibiotic classes, they categorized as MDR isolates⁹⁻¹¹.

Clinical isolated susceptible *E. coli* strains, which can acquire resistance via the mutations or class 1 integrons^{12,13}. The integrons which was defined as amino acid sequence which work as expression systems and gene capture¹⁴⁻¹⁶. The integrons gene is one of horizontal genes transfer of mobile genetic elements is responsible for spreading, recombination and emergence of MDR in pathogenic microbes^{11,17}. The integrons genes have 4 classes (class 1-4) which are connected with antibiotic resistance¹².

The integrons class 1 is consist of 2 preserved sections (5'-CS) and (3'-CS), that are segregated by changeable region that contain gene cassettes and a promoter (p)¹⁸. Integrons comprise three essential components located within the 5-conserved segment (CS): An integrase gene, *IntI1* which encodes a site-specific recombinase, an adjacent *attI1* site, which is recognized by the integrase and acts as a receptor for gene cassettes and a promoter region, P_c^{12,15,19}. Gene cassettes exist either in a circular cassette when it is in a free form or linear form when turn into integrons^{13,18,20}.

According to the author's knowledge, study was not found on antibiotic resistance pattern of *E. coli* isolates and their association with presence of class 1 integrons in MDR *E. coli* isolates obtained from clinical specimens in Yemen. This information are importance on contribution to general

antibiotics resistant of *E. coli* locally and internationally, the aim of the current study was to determine the prevalence of class 1 integrons and its association with antibiotic resistance in *E. coli* isolated from patient's clinical specimens collected from 6 health centers Sana'a, Yemen.

MATERIALS AND METHODS

Sample collection and bacterial isolation: A total of 198 non-duplicate *E. coli* strains were isolated from clinical specimens that collected from 6 Hospitals and Medical diagnostic labs in Sana'a, Yemen, between July, 2017 and August, 2017 in the study. The hospitals include University of Science and Technology, Modern German and Saudi German Group. The medical diagnostic labs include Aulagi Specialized and Al-Mamoon. The isolates of *E. coli* were from both sexes including 83 male and 115 female and were recovered from infected patient's body fluids/tissues including urine (mid stream urine how was collected), sputum, wound, blood, pus, vaginal swab (H.V.S), semen, cerebrospinal fluid (CSF), catheter tip/tube, ear swab, stool and breast discharges, for all age groups. The collection of CSF was performed by clinicians under aseptic conditions. This study was approved by the ethical committee of the Faculty of Medicine and Health Sciences, University of Sciences and Technology, Yemen (MECA N0: 2014/33).

The isolates were identified as *E. coli*, after culturing on MacConkey agar (Oxoid, UK) media and incubation at 37°C for 24 h. Single colony for sub-cultured on EMB agar incubation at 37°C for 24 h. Characteristic each colonies of *E. coli* were confirmed by standard biochemical tests (Triple sugar, Indole, Citrate, Motility and Urease tests). The confirmed *E. coli* strains were kept with 15% v/v glycerol at -20°C for future examinations²¹.

Antimicrobial susceptibility testing: *Escherichia coli* isolates were tested for susceptibility to antimicrobial agents and identification of MDR strains were performed by using Kirby-Bauer standard described by the Clinical and Laboratory Standards Institute (CLSI) guidelines²². The 15 antibiotics were selected based on common prescription at hospitals in Sana'a, Yemen as follows: Aztreonam (30 µg), cefepime (30 µg), amoxicillin-clavulanic acid (30 µg), cefotaxime (30 µg), cefepime-clavulanic acid (30 µg), imipenem (10 µg), ceftazidime-clavulanic acid (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), ceftazidime (30 µg), norfloxacin (10 µg) and trimethoprim-sulfamethoxazole (25 µg), gentamicin (10 µg), amikacin (30 µg) and nitrofurantoin (300 µg) (Oxoid, UK). The reference strain used was *E. coli* ATCC 25922.

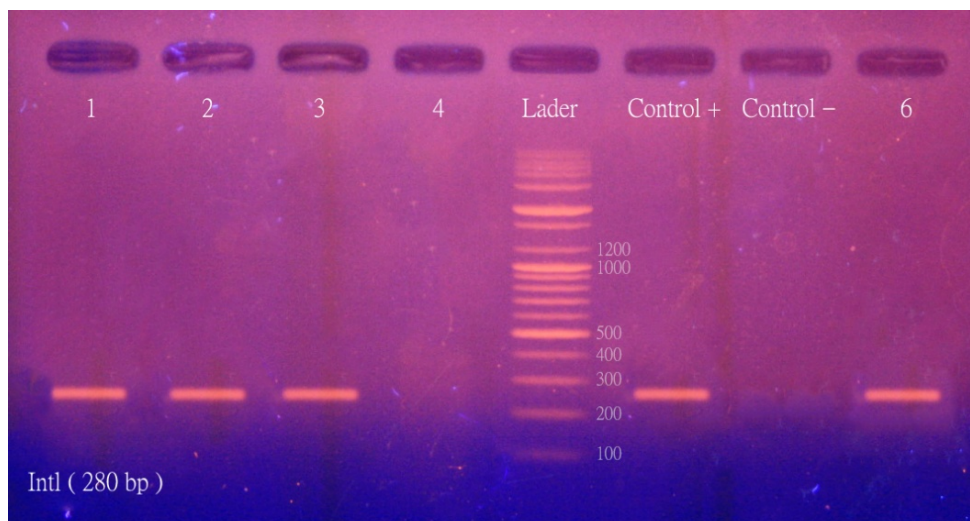


Fig. 1: PCR detecting class 1 integrons variable regions in *E. coli*MDR-clinical isolates

Lane 5: DNA ladder, with molecular weight size in the range of 100-2000 bp, 6 positive control, Lane 7: Negative control, Lane 1, 2, 3, 4, 8: *E. coli* isolates a fragment of 280 bp was detected (1 from left to 8 on the right)

Table 1: Sequences of oligonucleotide primers used for detection and amplification of class 1 integrons (*Int1*), among 100 *Escherichia coli* isolates from clinical specimens, Sana'a hospitals, Yemen, 2017

Gene target	Primer sequence (5-3)	PCR conditions		Product size	References
		Temperature	Time		
<i>Int1</i> .F	Forward: TCT CGG GTA ACA TCA AGG			280 bp	Collis and Hall ¹⁸
<i>Int1</i> .R	Reverse: GTT CTT CTA CGG CAA GGT				
Initial denaturation		94°C	5 min		
Denaturation		94°C	20 sec		
Annealing		50°C	30 sec		
Followed by: 30 cycles					
Extension		72°C	1 min		
Final extension		72°C	10 min		

Results were interpreted according CLSI and the manufacturer protocols (Mast, UK) and each *E. coli* isolate which showed resistance to ≥ 3 antibiotic classes was identified as MDR¹¹. A 100 multi-drug resistant (MDR) *E. coli* were randomly selected for class 1 integrons evaluation.

DNA extraction: DNA of each MDR *E. coli* isolates was extracted by the extraction kit (Cat. No. k-3032. Bioneer's AccuPrep®, South Korea) according to the instruction of the manufacture.

Polymerase chain reaction-detection of class 1 integron on the isolates of *E. coli*: The presence of class 1 integron in MDR *E. coli* was investigated by amplification of integrase gene *int1* by PCR using *int1* F and *int1* R primers (forward and reverse) (Table 1)²³. The amplification was performed by PreMix PCR assay (Bioneer, South Korea) under

the guidelines of the manufacturer using a Techne Progene thermocycler (TC-512) verity 96 well as follows: PCR reaction mixture contained 1 μ L of DNA template with 1 μ L of each primer, then 17 μ L of nuclease free water was added to the mixture. The PCR conditions were as follows, 94°C for 5 min, followed by 30 cycles at 94°C for 20 sec, 50°C for 30 sec, 72°C for 1 min and final extension at 72°C for 10 min showed in Table 1.

The PCR product was analyzed by 1% agarose gel (Bioneer, South Korea), the gels then photographed using gel documentation system (Syngene Ingenius). The DNA ladder (Bioneer, South Korea), with molecular weight size in the range of 100-2000 bp was used as weight markers (Positive control) for determining the PCR products size with expected amplified products 280 bp, whereas, sample free of DNA template was used as a negative control (Fig. 1).

Statistical analysis: Statistical analysis were accomplished by SPSS software version 15 (SPSS Inc., Chicago, USA). The Chi-square test was used to estimate the association between antibiotic resistance and existence of integrons. Proportions were compared using the Chi-square test. The significance level was defined as $p < 0.05$.

Ethical considerations: This study was approved by the ethical committee of the Faculty of Medicine and Health Sciences, University of Sciences and Technology, Yemen (MECA N0: 2014/33).

RESULTS

The 11 types of clinical samples were collected from 198 patients with age ranged between 10 days and 120 years and the *E. coli* were isolated (Table 2). A 174 (87.9%) of 198 *E. coli* isolates showed multi-resistance patterns (resistant to 3 or more antibiotics). The remaining 24 MDR *E. coli* isolates showed multi-resistance patterns for <3 antibiotics. None of the *E. coli* isolates were fully susceptible or resistant to all of the 15 tested antibiotics (Table 3). Class integron 1 were detected in 67 (67%) of the 100 MDR *E. coli*

isolates which selected randomly from 198 MDR *E. coli* isolates from clinical specimens. The carriage of class 1 integron was found to be significantly higher in ciprofloxacin ($p = 0.0002$), amoxicillin-clavulanic acid ($p = 0.003$), aztreonam ($p = 0.006$), cefepime ($p = 0.01$), cefepime-clavulanic acid ($p < 0.0001$), cefotaxime ($p = 0.0003$), ceftazidime ($p = 0.002$), ceftazidime-clavulanic acid ($p = 0.01$), ceftriaxone ($p = 0.03$), norfloxacin ($p = 0.0002$) and trimethoprim-sulfamethoxazole ($p < 0.0001$). While there was no significant association between resistance to amikacin ($p = 0.98$), gentamicin ($p = 0.06$) and nitrofurantoin ($p = 0.2$) (Table 4).

Table 2: Percentage of collected specimens

Specimens	Number	Percentage
Urine	133	67.2
Pus	12	6.1
Wound	11	5.6
Sputum	11	5.6
Blood	7	3.5
Body fluid	2	1.0
Catheter tip	2	1.0
Stool	2	1.0
Semen	5	2.5
Breast discharge	2	1.0
High vaginal swap	11	5.6
Total	198	100.0

Table 3: Multi-resistance patterns of *E. coli* isolated from clinical sources at Sana'a city main hospitals and medical laboratories

Number of antibiotics	Antimicrobial patterns	Resistance	
		Number	Percentage
14	AK, AMC, AT, CPM, CEC, CTX, CAZ, CAC, CTR, CIP, GEN, NIT, NX, COT	3	1.5
12	AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, GEN, NX, CIP, COT AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, AK, NX, CIP, COT AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, NIT, NX, CIP, COT	39	19.5
11	AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, CAT, NX, CIP, COT AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAT, NX, GEN, NIX	49	24.7
10	AMC, TX, CTR, CAZ, PM, CEC, CAC, NX, CIP, COT AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, NX, CIP AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, NX, GEN	13	6.6
9	AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, COT AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC, NX	25	12.6
8	AMC, CTX, CTR, CAZ, AT, CPM, CEC, CAC AMC, CTX, CTR, CAZ, CPM, CEC, CAC, COT AMC, CTX, CTR, CAZ, AT, CPM, CAC, COT AMC, CTX, CTR, AT, CEC, CAC, GEN, NX	23	11.6
7	AMC, CTX, CAZ, CEC, CAC, GEN, COT AMC, CEC, CAC, GEN, NX, CIP, COT AMC, CTX, CAZ, AT, CPM, CEC, CAC AMC, CTX, CTR, CAZ, CEC, CAC, COT	16	8.1
5	AMC, CEC, CAC, AK, COT	3	1.5
3	AMC, CAC, COT	3	1.5
	Less than 3 antibiotics	24	12.1
	No resistance to all antibiotics	0	0.0
	Total	198	100.0

NIT: Nitrofurantoin, AK: Amikacin, GEN: Gentamicin, CIP: Ciprofloxacin, NX: Norfloxacin, COT: Trimethoprim-sulfamethoxazol, AT: Aztreonam, CPM: Cefepime, CTR: Ceftriaxone, CTX: Cefotaxime, CAZ: Ceftazidime, CEC: Cefepime-clavulanic Acid, AMC: Amoxicillin-clavulanic Acid

Table 4: Correlation between presence of integron class 1 and antibiotic resistance among 100 *E. coli* isolates selected randomly from 198 clinical samples of patients

Antibiotics	Integron Class 1 Positive 67(100%) ^a	Other classes of Integron 33(100%) ^a	Total resistant n = 100 ^a	Association With Class 1 Integrons (p-value)
Imipenem (10 µg)	0 (0.0)	0 (0.0)	0	NA
Nitrofurantoin (300µg)	3 (4.5)	0 (0.0)	3	0.2
Amikacin (30 µg)	4 (6.0)	2 (6.1)	6	0.98
Gentamicin (10 µg)	19 (28.4)	4 (12.1)	23	0.06
Ciprofloxacin (5 µg)	40 (59.7)	7 (21.2)	47	0.0002
Norfloxacin(10 µg)	42 (62.7)	8 (24.2)	50	0.0002
Aztreonam (30 µg)	54 (80.6)	18 (54.5)	72	0.006
Cefepime (30 µg)	59 (87.8)	22 (66.7)	81	0.01
Trimethoprim-sulfamethoxazol (25 µg)	59 (88.1)	7 (21.2)	66	<0.0001
Ceftriaxone (30 µg)	60 (89.6)	24 (72.7)	84	0.03
Cefotaxime (30 µg)	63 (94.0)	22 (66.7)	85	0.0003
Ceftazidime (30 µg)	63 (94.0)	24 (72.7)	87	0.002
Cefepime-clavanic acid (30 µg)	65 (97.0)	26 (78.8)	91	<0.0001
Amoxicillin-clavulanic acid (30 µg)	67 (100.0)	29 (87.9)	96	0.003
Ceftazidime-clavulanic acid (30 µg)	67 (100.0)	30 (90.9)	97	0.01

NA: Not available, ^aValues are expressed as No. (%)

DISCUSSION

Consistent with previous reports, in this study class 1 integrons were the most quite prevalent in MDR *E. coli* isolates obtained from hospitals in Sana'a, Yemen. The frequency of class 1 integrons was 67%. The value of class 1 integrons of our study is either equivalent and lower or higher than the results obtained in studies at different countries. Shehabi *et al.*²⁴ and Murshed *et al.*²⁵ in Jordan and Bangladesh, respectively reported that the percentage of class 1 integrons in *E. coli* was 67.0% which is similar to the value of class 1 integrons in our study. Moreover, other studies in Pakistan, Egypt and Burkina Faso have shown that class 1 integrons values as 69.0, 64.0 and 61.5%, respectively Bashir *et al.*²⁶, El-Hendawy *et al.*²⁷ and Kpoda *et al.*²⁸ which were comparable to the value of class 1 integrons in this study. While the value of class 1 integrons in our study was lower than the values obtained by studies conducted in Iran (87.0%), China (85.5%), Egypt (83.3%), Beni-Suef Egypt (82%), Burkina Faso (80%), Karachi Pakistan (79%), Iran (79%) and Southwest Iran (78.26%)²⁹⁻³⁶. In contrast, the class 1 integrons value in this study higher than obtained by several studies in Southwestern Iran, Southwest Nigeria and Jazan area Kingdom of Saudi Arabia showed shown that class 1 integrons values as 59.5, 57 and 54.4%, respectively, Ebrahim-Saraie *et al.*³⁷, Odumosu *et al.*³⁸ and Abdelhaleem *et al.*³⁹.

In addition, the size of class 1 integrons in the this study was 280 bp which is similar to the size of class 1 integrons detected by Kpoda *et al.*²⁸, Abdel Aziz *et al.*²⁹, Khoramrooz *et al.*⁴⁰, Tahou *et al.*⁴¹ and Kashif *et al.*⁴².

In this study, 87.9% of the multi-drug resistance isolates which has a comparable rate to the MDR isolates found studies conducted in Nigeria, Sudan, Cairo Egypt, Iran, Jazan

area Kingdom of Saudi Arabia, India and Southwest of Iran which were 98.5, 90.2, 87.0, 84.2, 79.0, 77.0, 45.8 and 42.1% respectively (Rezaee *et al.*⁶, Kargar *et al.*³⁶, Abdelhaleem *et al.*³⁹, Khoramrooz *et al.*⁴⁰, Odetoyin *et al.*⁴³, Ibrahim *et al.*⁴⁴, Salem *et al.*⁴⁵ and Singh *et al.*⁴⁶).

The results of association between class 1 integrons and MDR of isolates in this study showed that class 1 integron presence has meaningfully donated to the rises in multidrug resistance among clinical strains obtained from hospitals which is similar with other studies which reported the relationship between integrons carriage and multidrug resistance Malek *et al.*³² and Kargar *et al.*³⁶. The class 1 integrons of *E. coli* is strictly linked to human associated environments that are very probably influenced by antimicrobial selective pressures.

The integrons emphasize the progression of MDR, as using of one antimicrobial could energize the expression and spread of a gene cassette stressing connection of class 1 integrons to multidrug resistance^{15,47}. The results in our study showed that high resistance to amoxicillin-clavulanic acid with percentage of 96%. The upper-level resistance in *E. coli* isolates were reported in other countries such as Iran Kargar *et al.*³⁶, Rezaee *et al.*⁴⁸ and China, Yang *et al.*⁴⁹. The rates of high resistance to trimethoprim-sulfamethoxazole and aztreonam in MDR *E. coli* isolates were observed with 66 and 77% respectively. On the other hand, MDR *E. coli* strains showed resistance range from 81-97% to cephalosporins group Abdelhaleem *et al.*³⁹ reported that the percentage was (77%) for MDR and the significant different with connection between the MDR and integrons class 1 was 98.9%. In Pakistan, Bashir *et al.*²⁶ In MDR *E. coli* isolates there were 69% class 1 integrons, which showed high, resistant towards ampicillin, ciprofloxacin, tetracycline,

chloramphenicol, nalidixic acid, streptomycin and trimethoprim, sulfamethoxazole. In previous studies were reported that the integron class 1 occurrence was low in MDR as 25.6% Farshad *et al.*⁵⁰, 47% Jones *et al.*⁵¹, 26.03% Rezaee *et al.*⁶. In contrast, Ranjbaran *et al.*⁵² reported higher carriage rates of integrons class 1 in MDR *E. coli*. Khoramrooz *et al.*⁴⁰ reported that *E. coli* strains resistant toward cephalothin (99%) and amoxicillin (76%) trimethoprim/sulfamethoxazole (62%), tetracycline (50%), nalidixic acid (48.5), ceftazidime (40.5%), ciprofloxacin (29%), gentamicin (15.5%), chloramphenicol (13%) amikacin (3%) and imipenem (1%). Study reported in Sudan by Ibrahim *et al.*⁴⁴ that rate of class 1 integrons were 40.6% and the MDR *E. coli* isolates carriage of class 1 integrons confer higher levels of resistance than any other isolates such as trimethoprim-sulfamethoxazole (98.1%), tetracycline (88.9%), ciprofloxacin (70.4%), amoxicillin-clavulanic acid (66.7%), ceftazidime (46.3 %) and chloramphenicol (29.6%).

The incidence of integrons is strictly linked to antimicrobial resistance like, aminoglycosides, β -lactam, co-trimoxazole, chloramphenicol and quinolones^{31,46,49,52-55}. The result in our study, it was observed that the relationship was significant between class 1 integrons gene cassettes and resistance to aztreonam, cefepime, amoxicillin-clavulanic acid, cefotaxime, cefepime-clavulanic acid, ceftazidime-clavulanic acid, ciprofloxacin, ceftriaxone, ceftazidime, norfloxacin and trimethoprim-sulfamethoxazole. The relation between resistance phenotypes patterns of the MDR *E. coli* isolates and the presence of the *int1* gene was presented in Table 4. It was clear that out of 198 collected samples, 6 isolates were fully susceptible to all test antibiotics, 20 isolates were resistant to 1-2 antibiotics only, 174 isolates were resistant to antibiotics in the range of 3-14 antibiotics and classified as MDR *E. coli*. On the other hand, none of the isolates were fully resistant to all 15 tested antibiotics. This result is in the same line with Khoramrooz *et al.*⁴⁰ Additionally, phenotypes were presented in one isolate only resistant to 14 antibiotics, which was class 1 integrons carriage. On the other hand, one isolate only integrons carriage was resistant to 3 antibiotics, whereas, 20 isolates integrons carriage were resistant to 11 antibiotics were observed.

The implications and applications of the study: Understanding the molecular mechanism of resistance genes may help introduce new antimicrobial strategies and some preventive measures to prevent further spread of resistance determinants among pathogens. The results of the present study indicate that there are at least two resistance mechanisms in our isolates, which can be transferred to other

clinical strains and therefore it is very important to identify and control resistant strains. There may be a relationship between increased resistance, lack of appropriate research, abuse of chemotherapeutic agents, general misuse of antibiotics and little or no preventive action. However, further studies are needed to be undertaken in this area. The limitations of the study were due to the small size of the sample, the geographical limitations of the samples were studied in Sana'a city only and the association of class 1 integrons was studied only in the clinical isolates *E. coli* and others *Enterobacteriaceae* were not tested.

CONCLUSION

An integrons class 1 is considered as common among MDR clinical isolates *E. coli* obtained from hospitals, which can be utilized as a marker for the detection of MDR isolates. Further studies is recommended to be conducted in other governors of Yemen with high population. Measurement integrons class 1 can be implemented to minimize and prevent the spreading of the integrons.

SIGNIFICANCE STATEMENT

This study discovers the widespread prevalence of the integrons on the *E. coli* clinical isolates in Yemen, which can be a marker for the detection of *E. coli*-MDR isolates. Researchers in Yemen were unable to explore this and this study was the first to determine the prevalence of class 1 integrons and their association with antibiotic resistance in *E. coli* isolated from patient clinical samples. This study will help to introduce new antimicrobial strategies and some preventive measures to prevent further spread of determinants of resistance among pathogens in Yemen.

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