# Distribution of Class I integrons and their effect on the prevalence of multi-drug resistant *Escherichia coli* clinical isolates from Sudan

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## ABSTRACT

**الأهداف:** قياس نسبة انتشار جزيئات الانتغرون النوع الأول وتحليل محتوياتها الجينية ودراسة أثرها على أنماط المقاومة للمضادات الحيوية وسط بكتيريا الإشريشية القولونية.

**الطريقة:** أجريت دراسة مقطعية على 133 سلالة من بكتريا الإشريشية القولونية من مختلف العينات السريرية للمرضي في 6 مستشفيات تعليمية بولاية الخرطوم، السودان خلال الفترة من أبريل إلي أغسطس 2011م. تم عزل وتمييز بكتيريا الاشريشية القولونية باستخدام طرق مخبريه قياسية ثم اختبار مقاومة البكتريا ل 15 نوعاً من المضادات الحيوية باستخدام طريقة القرص المنتشر. طبقت تقنية تفاعل البلمرة المتسلسل للكشف عن جزيئات الإنتغرون النوع 1 وتم تمييز محتوياتها من خلال تحليل التسلسل الجيني.

**النتائج:** باستخدام تقنية تفاعل البلمرة المتسلسل للكشف عن الإنتغرون، كان معدل انتشاره بنسبة 40.6% العدد=45 في البكتيريا المعزولة. كل السلالات الحاملة للأنتغرون كانت من النوع المقاوم لمجموعة متعددة من المضادات الحيوية. لاحظت هذه الدراسة وجود علاقة هامة ( $p<0.05$ ) بين تواجد الإنتغرون النوع 1 وزيادة نسبة المقاومة المتعددة للمضادات الحيوية في البكتيريا. أعطت السلالات الحاملة للإنتغرون معدلات مقاومة عالية مقارنة بالتي لا تحمله للمضادات الحيوية التاليه: كلافيونالات الأموكسيسلين ( 66.7% بالمقابل 36.7% )، السيفتازيديم ( 46.3%بالمقابل 17.7% ) السيفترياكسون( 66.7% بالمقابل 36.7% )، الكلورامفينيكول ( 29.6% بالمقابل 7.6% )، السيبروفلوكساسين ( 70.4% بالمقابل 43% ) ، التيتراسيكلين ( 88.9% بالمقابل 57% )، والترايميثوبريم–سلفاميثوكسازول( 98.1% بالمقابل 69.6% ). عند دراسة التسلسل الجيني للإنتغرون وجد أن الجينات الشائعة في البكتيريا المعزولة هي التي تحدث المقاومة للمضادات الحيوية من نوع الترامبثوبريم والأمينوجلايكوسيد نوع الإستربتوميسين.

**خاتمة:** أوضحت هذه الدراسة ارتفاع نسبة انتشار الإنتغرون النوع 1 ومساهمتها في ظهور البكتيريا المقاومة لمجموعة متعددة من المضادات في السودان. بالرغم من إنتشارالإنتغرون بنسب عالية أوصت الدراسة بإجراء المزيد من الأبحاث للكشف عن المسببات التي تساعد في انتشار نسبة الأنتغرون وسط البكتيريا الممرضة.

**Objectives:** To analyze integrons gene cassettes Class I among *Escherichia coli* (*E. coli)* isolates from Sudan and to determine their effect on the prevalence of resistance to antimicrobials.

**Methods:** This cross-sectional study was conducted at 6 hospitals in Khartoum State, Sudan between April and August 2011. *Escherichia coli* (n=133) isolated from clinical specimens of patients were included. Isolates were identified and tested for antimicrobial susceptibility following standard procedures. Multi-drug resistance (MDR) patterns was defined as non-susceptibility to ≥3 antimicrobials. Class I integrons was detected by polymerase chain reaction, and gene cassettes were characterized via sequencing analysis.

**Results:** Of the 133 *E. coli* isolates, 40.6% (n=54) harbored Class I integrons. All the 54 integron carriage, *E. coli* was found to be MDR strains. Integron carriage isolates confer higher levels of resistance than any other isolates ($p<0.05$) such as amoxicillin-clavulanic acid (66.7% versus 36.7%), ceftazidime (46.3% versus 17.7%), chloramphenicol (29.6% versus 7.6%), ciprofloxacin (70.4% versus 43%), tetracycline (88.9% versus 57%) and trimethoprim-sulfamethoxazole (98.1% versus 69.6%). Sequencing of gene cassettes harbored mostly dihydrofolate reductase (dfrA), which encodes resistance to trimethoprim and aminoglycoside adenyltransferase (aadA) that encodes resistance to streptomycin. The most frequent combination types were dfrA17 and aadA5 genes.

**Conclusions:** Class I integrons were quite common and its carriage contributed significantly to the emergence of MDR among *E. coli*. Nevertheless, factors leading to the wide spread of integrons are still to be determined.


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Antimicrobial resistance, particularly, multidrug resistance (MDR) is an emerging serious health concern worldwide.[1,2] Multidrug resistance patterns among members of the family of *Enterobacteriaceae* either can happen by mutations in chromosomal DNA or through acquisition of horizontal resistance genes transfer carried by plasmids or transposons.[3,4] Among these genes were the integrons, which are mobile elements that contain gene cassettes. Such cassettes can be mobilized to other integrons or to secondary sites in the bacterial genome.[5] The majority of the known gene cassettes encode for resistance to antimicrobial agents. Four classes of integrons have been identified of which Class I integrons make the majority of the integrons found in clinical isolates and associated with the MDR patterns.[2,6] During the last 2 decades, antimicrobial resistance in *Escherichia coli* (*E.coli)* strains has been linked to the acquisition of integrons. As integrons have the ability to capture, integrate and express gene cassettes encoding proteins associated with antimicrobial resistance.[6] The presence of integrons in clinical multi-resistant *E.coli* isolates recovered from clinical materials has been frequently reported.[2] A prevalence of Class I integron in clinical *E.coli* strains of up to 49% was documented.[7] Antimicrobial resistance in the *E.coli* strains and its association with the presence of integrons have been studied in some African regions including Central African Republic,[8] Tunisia,[9,10] and Nigeria.[11]

Studies have established a strong association between the presence of integrons and antimicrobial resistance either MDR or a single-drug resistance.[12] Few studies have analyzed the antimicrobial resistance in *E.coli* in Sudan.[13-16] However, little is known of the prevalence of integrons and related gene cassettes in *E.coli* strains isolated from patients in Sudan. Examination of the role of mobile elements and the distribution of resistance genes is important towards understanding the epidemiology of antimicrobial resistance. The present study describes the distribution of integron gene cassettes Class I among *E.coli* clinical isolates from Sudan, and analyzes their effect on the prevalence of resistance to antimicrobials.

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**Methods.** This was a cross sectional study conducted between April and August 2011. A total of 133 pathogenic *E.coli* isolates were studied. The isolates were obtained from various clinical specimens and infected body sites of patients of all age groups at different educational hospitals in Khartoum State, Sudan, including Khartoum North Educational Hospital, Khartoum Teaching Hospital, National Health Laboratory, Omdurman Educational Hospital, Soba University Hospital and Turkish Teaching Hospital. The related hospitals are referral hospitals, including different specialties; therefore, serving various patient groups and covering most population of different areas. The study was approved by the Research Council Board of Faculty of Medical Laboratory Sciences, University of Khartoum.

*Bacterial isolates.* The microbiology laboratory of each hospital undergoes the routine processing of the clinical specimens, namely: urine, stool, blood, high-vaginal swabs, ear discharges, wounds pus, seminal fluids and other miscellaneous body fluids. Isolation and identification of significant bacterial pathogens followed standard conventional procedures.[17,18] Only single non-duplicate significant growth of *E.coli* isolate per patient was included in this study. Cultures plates, which yielded more than 2 organisms per specimen, were excluded from the study.

*Antimicrobial susceptibility testing.* Antimicrobial susceptibility testing of *E.coli* isolates was performed by the Kirby-Bauer disk diffusion assay on Mueller-Hinton agar medium (Oxoid, Basingstoke, England) against 15 antimicrobial agent disks following the guidelines of the Clinical Laboratory Standard Institute (CLSI).[19] The antimicrobial agents which were tested including: amikacin (30 μg), amoxicillin (10 μg), amoxicillin-clavulanic acid (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg) cefuroxime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), nalidixic acid (30 μg), nitrofurantoin (50 μg), ofloxacin (5 μg), tetracycline (30 μg), tobramicin (10 μg), and trimethoprim-sulfamethoxazole (25 μg) (Oxoid, Basingstoke, England). *Escherichia coli* ATCC 25922 was used as control strains, and was tested each time when susceptibility testing was performed. Test results were only validated in cases where inhibition zone diameters of the control strains were within performance ranges in accordance to CLSI guidelines.[19] Multidrug resistance patterns of *E.coli* isolates were defined as non-susceptibility to at least one agent in 3 or more antimicrobial categories according to the standard criteria.[20]

*Detection of Class I integrons by polymerase chain reaction (PCR).* Deoxyribonucleic acid was extracted from *E.coli* isolates by the boiling method as described by Yu et al.[21] A single pure colony of each isolate was emulsified in 200 µl sterile distilled water. The suspension was boiled in water bath at 100ºC for 10 min, then the cell debris was precipitated by centrifugation at 13,000 rpm for 5 minutes. The supernatant was removed to new sterile eppendorf tube and used directly as template during screening process for the presence of Class I integrons. The forward and reverse specific oligonucleotide primers used in PCR reaction were 5'CS: 5-GGCATCCAAGCAGCAAG-3 and 3'CS: 5-AAGCAGACTTGACCTGA-3 (First Base Laboratories, Selangor, Malaysia) as described previously,[22] which they amplified the variable region between 5' conserved segment and 3' conserved segment of Class I integrons.

*Screening PCR for Class I integrons.* The PCR amplification was carried out using HotStarTaq Plus Master Mix (Qiagen GmbH, Hilden, Germany) with a total reaction volume of 20 µl. Each reaction mixture contained 4 µl of DNA template, 1 µl of each primer, 10 µl of master mix reagent and 4 µl of nuclease-free water. We use Eppendorf Mastercycler Gradient instrument (Eppendorf-Netheler-Hinz, Hamburg, Germany), then optimal cycling condition included initial heat activation for 5 min of incubation at 95°C, followed by incubations at 94°C for 45 seconds (denaturation), 50°C for 45 seconds (annealing) and 72°C for 1 min (extension), for 30 cycles and then, a final extension at 72°C for 10 min. The amplification products were detected by gel electrophoresis, 5 µl amplified product of each reaction was loaded on 1.2% agarose gel in 1X tris-acetate (TAE) buffer containing ethidium bromide (1 µg ml 1). The DNA ladder marker (Qiagen GmbH, Hilden, Germany) with size 10.0 kb was used as a standard molecular weight (MW) for determining the size of PCR products. The running condition was 85 volts for 90 minutes. After electrophoresis, the PCR products of agarose gel containing ethidium bromide were visualized under ultraviolet illumination. Positive and negative controls were run with each patch. The DNA extracted from Salmonella enterica serovar typhimurium, which is known Class I integrons-carriage was used as a positive control. A sample with no DNA template was used as a negative control.

*Deoxyribonucleic acid sequencing for characterization of resistance gene cassettes.* For sequence analysis, the gene cassettes inserted within Class I integrons were amplified subsequently with 5'CS and 3'CS primers.[22] Exactly 32 µl of PCR products of each integrons-positive sample (n=25) were sealed in sterile eppendorf tubes and sent to the DNA sequencing services at First Base Laboratories, Selangor, Malaysia. Polymerase chain reaction products were sequenced on Applied Biosystems 3730 xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence results were obtained online at company website. Similarities of the nucleotide sequences were compared to the GenBank database online of the National Center for Biotechnology Information BLAST network.[23]

*Statistical analysis.* Collected data of antimicrobial susceptibility of *E.coli* isolates and their molecular characterization were analyzed using Statistical Package for Social Sciences program (SPSS Inx., Chicago, IL., USA) Version 10. Comparisons between resistance patterns of integron carriage isolates and non-integron carriage isolates were determined. Proportions were compared using the Chi-square test. All *p*-values less than 0.05 were considered as statistically significant.

**Results.** A total of 133 *E.coli* isolates were collected from patient's clinical specimens. The isolates were obtained from patients of all age groups: 86 (64.7%) were females while 47 (35.3%) were males. Of these, 77.4% (103/133) were adult patients. Most isolates were recovered from clinical specimens of urine (n=87) followed by wounds (n=25), ear swabs (n=5), high-vaginal swabs (n=5), blood (n=4), miscellaneous body fluids (n=3), seminal fluids (n=3) and stool (n=1) at different hospitals in Khartoum State of Sudan.

*Amplification of Class I integrons.* Out of the 133 *E.coli* strains (120 MDR and 13 susceptible) screened for the presence of Class I integron with the 5'CS and 3'CS specific primers, 40.6% (54/133) harbored Class I integrons. All the 54 integrons carriage, *E.coli* were found to be MDR strains.

*The associations between resistant rates and the presence of integrons.* Overall, the 54 integrons-positive MDR *E.coli* strains carried different amplicons sizes, ranging between 0.25 and 2.0 kb. The most frequent amplicon was that with size of 1.6 kb (n=19), followed by 1.7 kb (n=10), 2.0 kb, 1.8 kb (n=7 each), 0.7 kb (n=6), 0.6 kb (n=4), and 0.25 kb (n=1).

**Table 1 -** Antimicrobial susceptibility of integrons carriage and non-integrons carriage *Escherichia coli* isolates at different hospitals in Khartoum State, Sudan (N=133).

| Antimicrobial agent | Integrons carriage (n=54) Resistant% | Non-integrons carriage (n=79) Resistant% | *P*-value |
|---|---|---|---|
| Amikacin | 0.0 | 5.1 | 0.245 |
| Amoxicillin | 100.0 | 86.1 | 0.011 |
| Amoxicillin-clavulanic acid | 66.7 | 36.7 | 0.001 |
| Ceftazidime | 46.3 | 17.7 | 0.001 |
| Ceftriaxone | 75.9 | 46.8 | 0.002 |
| Cefuroxime | 94.4 | 77.2 | 0.015 |
| Chloramphenicol | 29.6 | 7.6 | 0.002 |
| Ciprofloxacin | 70.4 | 43.0 | 0.003 |
| Gentamicin | 40.7 | 27.8 | 0.172 |
| Nalidixic acid | 77.8 | 58.2 | 0.031 |
| Nitrofurantoin | 20.6 | 16.5 | 0.729 |
| Ofloxacin | 68.5 | 39.2 | 0.002 |
| Tetracycline | 88.9 | 57.0 | 0.000 |
| Tobramicin | 24.1 | 16.5 | 0.387 |
| Trimethoprim-sulfamethoxazole | 98.1 | 69.6 | 0.000 |

**Table 1** shows the antimicrobial susceptibility of integron carriage and non-integron carriage *E.coli*. There were significant differences ($p$<0.05) of resistance rates between integron carriage and non-integron carriage *E.coli*. Integron positive isolates were more resistant than integron negative isolates for the most tested antimicrobial agents, namely: amoxicillin ($p$=0.011), amoxicillin-clavulanic acid ($p$=0.002), ceftazidime ($p$=0.001), ceftriaxone ($p$=0.002), cefuroxime ($p$=0.015), chloramphenicol ($p$=0.002), ciprofloxacin (p=0.003), nalidixic acid (p=0.031), ofloxacin ($p$=0.002), tetracycline ($p$<0.000) and trimethoprim-sulfamethoxazole ($p$<0.000).

Overall, the 54 *E.coli* isolates carrying Class I integrons were found to be MDR strains (yielded resistant to 3 or more of the total 15 tested antimicrobial agents from different categories) (**Table 2**). Whereas, no integron was found among the susceptible *E.coli* isolates. Resistant profiles of 11 and 12 different antimicrobials were the most frequent among *E.coli* carrying integron (**Table 2**).

In order to characterize the gene cassettes array within Class I integrons, the variable gene cassettes regions were amplified and sequenced with the 3'CS and 5'CS specific primers pair. Of the 54 integron positive *E.coli* isolates, gene cassette regions were sequenced in 25 amplified products, which were randomly selected. The results of nucleotide sequences produced match 95 to 100% identity to the GenBank database (**Table 3**). The nucleotide sequences were submitted to GenBank database and have been designated the following accession numbers: JX122990 to JX123014 and JX113684.

**Table 2 -** Frequency of drug resistance phenotypes among integrons-carriage *Escherichia coli* (n=54) isolated at different hospitals in Khartoum State, Sudan.

| No. of drug resistance (No. of isolates)/ Phenotype | Frequency |
|---|---|
| ***3 drugs (2 strains)*** | |
| AML-AMC-SXT | 1 |
| AML-SXT-TE | 1 |
| ***4 drugs (3 strains)*** | |
| AML-CXM-SXT-TE | 3 |
| ***5 drugs (3 strains)*** | |
| AML-AMC-CXM-SXT-TE | 2 |
| AML-AMC-CXM-CRO-SXT | 1 |
| ***6 drugs (3 strains)*** | |
| AML-AMC-CXM-CRO-SXT-TE | 1 |
| AML-AMC-CXM-NA-SXT-TE | 1 |
| AML-CXM-NA-SXT-TE-C | 1 |
| ***7 drugs (7 strains)*** | |
| AML-AMC-CIP-OFX-NA-SXT-TE | 2 |
| AML-CXM-CRO-CIP-OFX-NA-SXT | 2 |
| AML-CXM-CRO-CAZ-F-SXT-C | 1 |
| AML-CXM-CIP-OFX-NA-TE-GN | 1 |
| AML,CXM,CRO,CAZ,SXT,TE,C | 1 |
| ***8 drugs (5 strains)*** | |
| AML-CXM-CRO-CIP-OFX-NA-SXT-TE | 2 |
| AML-CXM-CRO-SXT-TE-GN-TOB-C | 1 |
| AML-CXM-CRO-CIP-NA-SXT-TE-TOB | 1 |
| AML-AMC-CXM-CRO-NA-SXT-TE-C | 1 |
| ***9 drugs (3 strains)*** | |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-TE | 3 |
| ***10 drugs (7 strains)*** | |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE | 4 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-TE-GN | 1 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-GN-C | 1 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-TE-C | 1 |
| ***11 drugs (8 strains)*** | |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN | 3 |
| AML-CXM-CRO-CIP-OFX-NA-F-SXT-TE-GN-TOB | 1 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE | 1 |
| AML-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB | 1 |
| AML-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-GN | 1 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-C | 1 |
| ***12 drugs (8 strains)*** | |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB | 3 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-C | 1 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-F-SXT-TE-GN-TOB | 1 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-TOB | 1 |
| AML-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-GN-C | 1 |
| AML-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB-C | 1 |
| ***13 drugs (4 strains)*** | |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-GN-C | 2 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB-C | 2 |
| ***14 drugs (1 strain)*** | |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-GN-TOB-C | 1 |
| **Total** | 54 |

AK - amikacin, AML- amoxicillin, AMC - amoxicillin-clavulanic acid, CAZ - ceftazidime, CRO - ceftriaxone, CXM - cefuroxime, C - chloramphenicol, CIP - ciprofloxacin, GN - gentamicin, NA - nalidixic acid, F - nitrofurantoin, OFX - ofloxacin, TOB - tobramicin, TE - tetracycline, SXT - trimethoprim-sulfamethoxazole

Among the 25 amplified products, 49 different genes encoding resistance to different agents were characterized (**Table 3**). Of these genes, 25 (51%) were dihydrofolate reductase A (dfrA) genes which encode resistance to trimethoprim-sulfamethoxazole, including 6 types of dfrA17 (n=16), dfrA12 (n=2), dfrA1 (n=4), dfrA2 (n=1), dfrA5 (n=1) and dfrA7 (n=1). Followed by

**Table 3 -** Antimicrobial resistance patterns, gene cassettes contents and sequence identity in Class I integrons carriage *Escherichia coli* (n=25) isolated at different hospitals in Khartoum State, Sudan.

| Resistance pattern[a] | No. of resistant drugs | Gene cassette(s)[b] | ID %[c] | Accession No. of GenBank reference |
|---|---|---|---|---|
| AML-AMC-SXT | 3 | dfrA1, aadA1 | 98 | JN108887-JF806496-FJ215857 |
| AML-SXT-TE | 3 | dfrA17, aadA5 | 99 | AM937244-JN108894-JN108888 |
| AML-AMC-CXM-SXT-TE | 5 | dfrA1, aadA1 | 99 | JN108887 - JN108886 - JF806496 |
| AML-AMC-CXM-CRO-SXT-TE | 6 | dfrA5 | 99 | EU523055 |
| AML-CXM-NA-SXT-TE-C | 6 | dfrA17, aadA5 | 99 | HQ880260-GQ896501 - GQ896500 |
| AML-CXM-CRO-CAZ-F-SXT-C | 7 | dfrA17, aadA5 | 98 | JN108894 JN108885 - JF806495 |
| AML,CXM,CRO,CAZ,SXT,TE,C | 7 | dfrA17, aadA5 | 99 | DQ322597-DQ838665 -HQ880260 |
| AML-CXM-CRO-SXT-TE-GN-TOB-C | 8 | dfrA17, aadA5 | 100 | HQ880260-GU055937-GQ896501 |
| AML-CXM-CRO-CIP-NA-SXT-TE-TOB | 8 | dfrA7 | 99 | JN645875-EU339236- EU250577 |
| AML-CXM-CRO-CIP-OFX-NA-SXT-TE | 8 | dfrA17, aadA5 | 97 | JF806495- JN108894 - HQ880278 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-TE | 9 | dfrA17, aadA5 | 96 | HQ880260-GQ896500-GQ896499 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-TE-C | 10 | dfr2d | 99 | HQ902143-AY968808 -AY973253 |
| AML-AMC-CXM-CRO-CIP-OFX-NA-SXT-TE-GN | 10 | dfrA12, aadA2, orfF | 99 | HQ880263 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE | 10 | dfrA17,aadA5,qacEΔ1 | 99 | HM367091 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE | 10 | dfrA12, aadA2 | 99 | HM569734-GU304661- GU001949 |
| AML-CXM-CRO-CIP-OFX-NA-F-SXT-TE-GN-TOB | 11 | dfrA17, aadA5 | 95 | HQ880278-HQ880260- DQ322597 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN | 11 | dfrA17, aadA5 | 96 | HQ880260 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE | 11 | dfrA17, aadA5 | 98 | HQ880260- GU055937 |
| AML-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-GN | 11 | dfrA17, aadA5 | 97 | JN108894 -JN10888 -JN108885 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-C | 11 | dfrA17, aadA5 | 97 | JF806495-HQ880265 -JN108894 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-C | 12 | dfrA17, aadA5 | 99 | AM937244- JN108894 - JF806495 |
| AML-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB-C | 12 | dfrA17, aadA5 | 99 | DQ322597 -HQ880260 -DQ838665 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB | 12 | dfrA17, aadA5 | 99 | JN108894E-JN108888E-JN108885E |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-F-SXT-TE-GN-C | 13 | dfrA17, aadA5 | 97 | FN396368-JN108894 - JN108885 |
| AML-AMC-CXM-CRO-CAZ-CIP-OFX-NA-SXT-TE-GN-TOB-C | 13 | dfrA17, aadA5 | 99 | HQ880260 - JF806495 - GU055937 |

[a] AK - amikacin, AML - amoxicillin, AMC - amoxicillin-clavulanic acid, CAZ - ceftazidime, CRO - ceftriaxone, CXM - cefuroxime, C - chloramphenicol, CIP - ciprofloxacin, GN - gentamicin, NA - nalidixic acid, F - nitrofurantoin, OFX - ofloxacin, TOB - tobramicin, TE - tetracycline, SXT - trimethoprim-sulfamethoxazole, [b] aadA - aminoglycoside adenyltransferase A, dfrA - dihydrofolate reductase A, orfF - open reading frame, qacEΔ1- quaternary ammonium compound, [c]ID% - percentage of identity match to the GenBank database

22 (44.9%) genes of aminoglycoside adenyltransferase A (aadA) that confer resistance to streptomycin and spectinomycin, which included 3 types of aadA5 (n=15), aadA1 (n=4), aadA2 (n=3). Moreover, only one gene for open reading frame (orfF), encoding unknown product, and another one encoding resistance to quaternary ammonium compounds (qacΔ1) were detected. These identified genes were found either alone or in combination arrays, mostly between dfrA and aadA. The most prevalent combination type were dfrA17, aadA5, which were detected in 68% (17/25) strains, followed by 8% (2/25) of dfrA1, aadA1, whereas other gene cassettes were detected in lower frequencies, but always in combination with aminoglycoside and/or trimethoprim resistance cassettes (**Table 3**).

**Discussion.** The results of the present study indicated that Class I integron was quite prevalent (40.6%) in the isolates from hospitals in Sudan. Class I integron carriage has significantly contributed to the increases in MDR among studied *E.coli* isolates from Sudan. Integron carriage isolates confer higher resistant than other isolates ($p$<0.05). Notable examples were amoxicillin-clavulanic acid, ceftazidime, chloramphenicol, ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole. Previous reports in Sudan have referred to a high numbers of resistant *E.coli* from clinical sources.[13-16] The prevalence of Class I integron in Gram-negative bacteria has been studied in various regions. In Jordan,[24] recorded 67% prevalence; 76% in the Netherlands,[2] 44.8% in Iran,[25] 23.3% in Australia[26] and 22.2% in Tunisia.[27] Such ranges, 22.2 to 76%, are comparable to our present findings (40.6%). Obviously, these results reflect geographic, demographic and clinical variations. Nevertheless, the known fact is that these results demonstrated high frequencies of integron in Gram-negative bacteria, regardless of variations in health setting, and their remarkable effect on the increasing resistance to antimicrobials.

In this study, all of the 54 *E.coli* isolates carrying Class I integron were found to be resistant to 3 or more of the total 15 tested antimicrobial agents. Integrons were not found in antimicrobial-susceptible *E.coli* isolates indicating a significant association between the presence of integrons and multiple antimicrobial resistant among clinical *E.coli* isolates. Similar findings have been documented worldwide.[6,12,25] Previous study by Martinez et al[3] demonstrated a significant association between integron carriage and reduced susceptibility to some aminoglycosides, quinolones, and β-lactam compounds. In addition, MDR was more common in integron positive strain. Phongpaichit et al[28] have described that the use of one antibiotic may activate the expression and transfer of a whole resistance gene cassette. As a result, a bacterial strain may become multidrug resistant due to exposure to only one antibiotic. In this study, integron carriage isolates were significantly more resistant to amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, nalidixic acid, ofloxacin, tetracycline and trimethoprim-sulfamethoxazole when compared to non-integron carriage *E.coli*. Similar findings have been previously described elsewhere.[12,21,29] White et al[30] suggested that the association of the older antibiotics ampicillin, chloramphenicol, and tetracycline with the presence of an integron is likely to be due to genetic linkage between integron and conjugative plasmids and transposons.

Currently, there are over 130 gene cassettes mediating resistance to different class of antimicrobial agents.[31] Several members of dfrA and aadA gene cassettes families, which encode resistance to trimethoprim and spectomycin, have been discovered.[29,31] In this study, our clinical isolates commonly harbored the dfrA and aadA gene cassettes families, alone or in combination. Similar to the finding by Grape et al[32] and in contrast to reports by White et al,[30] who detect the aadA cassette is predominant which represented 53%, while 27% for dfrA gene family. The most prevalent gene cassette arrays in combination found in Sudanese hospitals was dfrA17-aadA5. This is in agreement with Solberg et al[29] report in which gene cassettes arrays of dfrA17-aadA5 was found the most prevalent among uropathogenic *E.coli*. Similar to the results obtained from this study, Class I integron carrying dfrA17-aadA5 cassette were seen most frequently in Korean,[21,33] USA[34] and Australian *E.coli* isolates,[26] whereas the cassette arrays either an aadA1 cassette alone or dfrA1-aadA1 cassettes were seen most frequently in the *E.coli* isolates from Europe.[2,6] In Central African Republic, Frank et al[8] have determined the most prevalent dfr genes were dfrA7 (49%), dfrA1 (17%), and dfrA2d (13%), associated with Class I integrons in clinical isolates of *Enterobacteriaceae*. Similar finding have been documented in Tunisia.[10] In this study, The possible explanation of high frequent of trimethoprim aminoglycoside resistance genes in Sudan could be due to the widely used of trimethoprim to treat many infection such as urinary tract infections, diarrheal diseases and as well as to prevent malarial infections. In addition, the use of streptomycin in combination with other drugs is used to treat tuberculosis. Moreover, excessive and unnecessary prescribing of antibiotics, self medication and poor quality of available antibiotics has been noted in Sudan.[35] Although, there were a great association between the presence of integron and increase of resistance to different class of antimicrobial compounds.[12,21,29] Our clinical isolates did not carry any types of gene encoded resistance to quinolones, chloramphenicol or tetracycline agents. Perhaps resistance to these compounds in our isolates is a result of chromosomal mutations rather than being carried on any mobile genetic elements as in quinolones,[4] or could be due to other factors such as resistant plasmid, bacteriophages, or transposons.[33,36]

***Study limitations and recommendations for future research.*** The study was a cross sectional study conducted during a limited period of time (April and August 2011) at 6 hospitals in Khartoum State, Sudan. Although the sample size (n=133) of this study is reasonably and representative, the study did not consider samples from hospitals in other states of the Sudan. Besides, other classes of integrons have not been investigated, which might be prevalent.

Nevertheless, this study generated a number of useful conclusions: Class I integrons were found common among *E.coli* in the major Sudanese hospitals in Khartoum state. Such spread has significantly reduced susceptibility to a wide range of commonly used antimicrobial agents. Factors leading to the wide spread of integrons in these health settings are not known. Further surveillance program is required to determine factors contributing to the acquisition and transmission of linked resistance genes.

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