Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

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

Manal Mohamed Elhassan Taha[1*], Hisham Elraih Homeida[2], Omer Mohammed Elhassan Dafalla[3], Siddig Ibrahim Abdelwahab[4]

[1] Medical Research Centre, Jazan University, Kingdom of Saudi Arabia
[2] Faculty of Dentistry, Jazan University, Kingdom of Saudi Arabia
[3] National Center for Vector-Borne Diseases, Ministry of Health, Jazan, Kingdom of Saudi Arabia
[4] Substance Abuse Research Centre, Jazan University, Kingdom of Saudi Arabia

Correspondence to: manalroa@yahoo.com

Received October 22, 2017; **Accepted** April 26, 2018; **Published** April 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.5.21

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 designated as multidrug resistant (MDR), while non-MDR was found in 19 strains (20.0%). Out of 76 MDR *E. coli* isolates, 41 (53.9%) were identified as positive for 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

Table 1. Primers and PCR conditions.

Primers names	Sequence (5'-3')	PCR product size (bp)	PCR condition
Integron II	F-CACGGATATGCGACAAAAAGGT R-GTAGCAAACGAGTGACGAAATG	789	Initial denaturation at 94°C for 3 min,35 cycles of denaturation at 94°C for 60 second,annealing at 60°C for 60 second,extension at 72°C for 120 second and final extension for 5min.
Integron III	F-CATTTGTGTTGTGGACGGC R-GACAGATACGTGTTTGGCAA	717	

DNA sequencing were used for phylogenetic analysis of integrons genes (II and III).

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 (15). 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 (16) 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, impenim, cefoxacim, amikacin and ciprofloxacin. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate susceptible or susceptible. Multidrug resistance was defined as resistance for more than three of the of tested antimicrobials (17).

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 Technology, 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

Table 2. Antibiotics susceptibility test of *E. coli* using disk diffusion.

Antibiotics	*E. coli*					
	Sensitive[1]		Intermediate[2]		Resistant[3]	
	Number	Percentage	Number	Percentage	Number	Percentage
Imipenem	74	77.9	5	5.3	16	16.8
Nalidixic acid	47	49.5	00	00	48	50.5
Amikacin	65	68.4	5	5.3	25	26.3
Ofloxacin	51	53.7	1	1	43	45.3
Gentamicin	38	40.0	3	3.2	54	56.8
Ciprofloxacin	40	42.1	00	00	55	57.9
Ampicillin	11	11.6	4	4.2	80	84.2
Cefotaxim	45	47.4	3	3.1	47	49.5

1, 2 and 3 according to the Clinical Laboratory Standard Institute.

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

Antibiotic resistance pattern	Integron –II				Integron -III			
	+VE		-VE		+VE		-VE	
	N	%	N	%	N	%	N	%
Multi-drug resistant	41	91.1	35	70	5	83.3	71	79.8
Non multi-drug resistant	4	8.9	15	30	1	16.7	18	20.2
Total	45	100	50	100	6	100	89	100

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.

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%) 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.

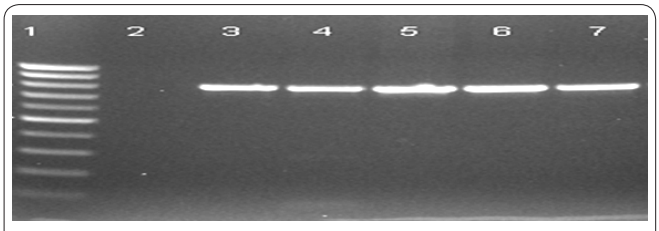

Figure 1. PCR amplification products and agarose gel electrophoresis of integrons II gene (789bp): Lane 1: 100 base-pair (DNA Marker); Lane 2: negative control; Lane 3: positive control; Lanes 4 to7: were integrons II. DNA marker ranges from 100 to 1000 base pair.

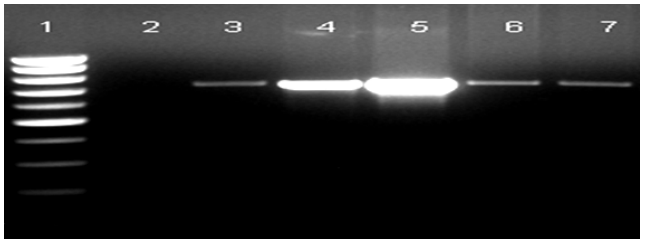

Figure 2. PCR amplification products and agarose gel electrophoresis of integrons III gene (717bp). Lane 1: 100 base-pair (DNA Marker); Lane 2: negative control; Lane 3: positive control; Lanes 4 to7: were integrons III. DNA marker ranges from 100 to 1000 base pair.

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

Integron–II	
Gen bank accession No	Gen bank accession No
KC417300.1	KC417300.1
KF534916.1	KF534916.1
FJ591049.1	FJ591049.1
NG_035774.1	NG_035774.1
KJ184348	KJ184348
EF560799.1	EF560799.1
KF921594.1	KF921594.1
JN987180.1	JN987180.1

```
Query  345  TATCAAAATCAAAATCTTTAACCCGCAAACGCAAGCATTCATTAATGCGCAAACCTGCAC  404
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TATCAAAATCAAAATCTTTAACCCGCAAACGCAAGCATTCATTAATGCGCAAACCTGCAC  60

Query  405  CATACAGCAGCGTAAAAATAACTTGGTTGCGAGTATCCATAACCTGCAAAATGCGTTGCA  464
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CATACAGCAGCGTAAAAATAACTTGGTTGCGAGTATCCATAACCTGCAAAATGCGTTGCA  120

Query  465  CTTCATTTGCAGAGATAACAGAGGGTAGCCGTCTAGGCTTGCTTGCAGGGATATAATCAA  524
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTTCATTTGCAGAGATAACAGAGGGTAGCCGTCTAGGCTTGCTTGCAGGGATATAATCAA  180

Query  525  TATCGCCCAACGGCTGTTGTAAAAAACCTGTTGTACAAAAAAGCTAGGGCATTTAAAGCGA  584
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TATCGCCCAACGGCTGTTGTAAAAAACCTGTTGTACAAAAAAGCTAGGGCATTTAAAGCGA  240

Query  585  TTTTCTGCGTGTTTATGGCTACATGTCTGCTGTTTGCTAAGCTGGATAAAAACAGCCTGA  644
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTTTCTGCGTGTTTATGGCTACATGTCTGCTGTTTGCTAAGCTGGATAAAAACAGCCTGA  300

Query  645  CCTCTTCACTGCCCATGGTCTGAGGATGACGTTTTTTGTGAAACAGAATAAAACGCTTAA  704
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CCTCTTCACTGCCCATGGTCTGAGGATGACGTTTTTTGTGAAACAGAATAAAACGCTTAA  360

Query  705  TCCAGTGCAGGTAAGTTTTTTCAGTTTTCAGCGCATAACCTTTTTG  750
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TCCAGTGCAGGTAAGTTTTTTCAGTTTTCAGCGCATAACCTTTTTG  406
```

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.

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 antibacterial drugs (31, 32).

There are various researches documenting integron positive *E. coli* isolates principally from non-clinical settings in Saudi Arabia. The current study remains the first of its kind to expose the status of *E. coli* strains from Saudi patient's population in Jazan region and supports the scientific literature of genetically meditated antimicrobial resistance in this region. This kind of findings predicts that antimicrobial resistance and resistance genes are popular extensively in the community. PCR results were confirmed with partial sequencing for samples represented positive integron II. The Blast search showed that the sequences of our samples aligned along with many published sequences of integron II. Intermittent examination of antimicrobial resistance both in the community and hospital settings is highly recommended.

Conflicts of interest

No conflict of interest is declared.

Acknowledgments

This research was funded by Deanship of Scientific Research, Jazan University, Saudi Arabia (Project No. 164).

Author's contribution

MME Taha conducted the experiment and wrote the manuscript, HE Homeida conducted the experiment and wrote the manuscript, MME Dafalla conducted the experiment, SI Abdelwahab revise the manuscript.

References

1. DePas WH, Syed AK, Sifuentes M et al. Biofilm formation protects Escherichia coli against killing by Caenorhabditis elegans and Myxococcus xanthus. Applied and environmental microbiology. 2014; 80(22): 7079-7087.
2. Monteiro A, Verde SC. Bacterial Bioburden in Hospital Environment. Exposure to Microbiological Agents in Indoor and Occupational Environments: Springer; 2017: 321-328.
3. Scallan E, Hoekstra R, Mahon B, Jones T, Griffin P. An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. Epidemiology & Infection. 2015; 143(13): 2795-2804.
4. Franz E, Delaquis P, Morabito S et al. Exploiting the explosion of information associated with whole genome sequencing to tackle Shiga toxin-producing Escherichia coli (STEC) in global food production systems. International journal of food microbiology. 2014; 187: 57-72.
5. Laxminarayan R, Duse A, Wattal C et al. Antibiotic resistance—the need for global solutions. The Lancet infectious diseases. 2013; 13(12): 1057-1098.
6. Venieri D, Gounaki I, Bikouvaraki M et al. Solar photocatalysis as disinfection technique: Inactivation of Klebsiella pneumoniae in sewage and investigation of changes in antibiotic resistance profile. Journal of environmental management. 2017; 195: 140-147.
7. Elseviers MM, Van Camp Y, Nayaert S et al. Prevalence and management of antibiotic associated diarrhea in general hospitals. BMC infectious diseases. 2015; 15(1): 129.
8. *Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. African Health Sciences. 2011; 11(Suppl 1): S40-S45.
9. Abraham S, Wong HS, Turnidge J, Johnson JR, Trott DJ. Car-

bapenemase-producing bacteria in companion animals: a public health concern on the horizon. Journal of Antimicrobial Chemotherapy. 2014; 69(5): 1155-1157.
10. Harnvoravongchai P, Pipatthana M, Chankhamhaengdecha S, Janvilisri T. Insights into drug resistance mechanisms in Clostridium difficile. Essays In Biochemistry. 2017; 61(1): 81-88.
11. Kasimanickam V, Owen K, Kasimanickam R. Detection of genes encoding multidrug resistance and biofilm virulence factor in uterine pathogenic bacteria in postpartum dairy cows. Theriogenology. 2016; 85(2): 173-179.
12. Chen B, Yuan K, Chen X et al. Metagenomic analysis revealing antibiotic resistance genes (ARGs) and their genetic compartments in the Tibetan environment. Environmental science & technology. 2016; 50(13): 6670-6679.
13. Fluit A, Schmitz FJ. Resistance integrons and super-integrons. Clinical Microbiology and Infection. 2004; 10(4): 272-288.
14. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxacin resistance in community-and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies. BMC infectious diseases. 2015; 15(1): 545.
15. Dureja C, Mahajan S, Raychaudhuri S. Phylogenetic distribution and prevalence of genes encoding class I integrons and CTX-M-15 extended-spectrum β-lactamases in Escherichia coli isolates from healthy humans in Chandigarh, India. PloS one. 2014; 9(11): e112551.
16. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant Staphylococcus aureus (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. Journal of clinical microbiology. 2002; 40(8): 2766-2771.
17. Magiorakos AP, Srinivasan A, Carey R et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012; 18(3): 268-281.
18. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi journal of biological sciences. 2015; 22(1): 90-101.
19. Altalhi AD, Gherbawy YA, Hassan SA. Antibiotic resistance in Escherichia coli isolated from retail raw chicken meat in Taif, Saudi Arabia. Foodborne pathogens and disease. 2010; 7(3): 281-285.
20. Hessain AM, Al-Arfaj AA, Zakri AM et al. Molecular characterization of Escherichia coli O157:H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences. 2015/11/01/ 2015; 22(6): 725-729.
21. Odetoyin BW, Labar AS, Lamikanra A, Aboderin AO, Okeke IN. Classes 1 and 2 integrons in faecal Escherichia coli strains isolated from mother-child pairs in Nigeria. PLoS ONE. 08/22 10/19/received
08/03/accepted 2017; 12(8): e0183383.
22. Bharathi M, Ramakrishnan R, Maneksha V, Shivakumar C, Nithya V, Mittal S. Comparative bacteriology of acute and chronic dacryocystitis. Eye. 2008; 22(7): 953.
23. Briscoe D, Rubowitz A, Assia EI. Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. Orbit. 2005; 24(1): 29-32.
24. Gonzalez G, Sossa K, Bello H, Dominguez M, Mella S, Zemelman R. Presence of integrons in isolates of different biotypes of Acinetobacter baumannii from Chilean hospitals. FEMS microbiology letters. Apr 1 1998; 161(1): 125-128.
25. Koeleman JG, Stoof J, Van Der Bijl MW, Vandenbroucke-Grauls CM, Savelkoul PH. Identification of epidemic strains of Acinetobacter baumannii by integrase gene PCR. Journal of clinical microbiology. Jan 2001; 39(1): 8-13.
26. Martinez-Freijo P, Fluit A, Schmitz F, Grek V, Verhoef J, Jones M. Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. The Journal of antimicrobial chemotherapy. 1998; 42(6): 689-696.
27. Chang C-Y, Chang L-L, Chang Y-H, Lee T-m, Chang S-f. Characterisation of drug resistance gene cassettes associated with class 1 integrons in clinical isolates of Escherichia coli from Taiwan, ROC. Journal of medical microbiology. 2000; 49(12): 1097-1102.
28. Xu Z, Li L, Shirtliff ME et al. First report of class 2 integron in clinical Enterococcus faecalis and class 1 integron in Enterococcus faecium in South China. Diagnostic microbiology and infectious disease. 2010; 68(3): 315-317.
29. Yao F, Qian Y, Chen S, Wang P, Huang Y. Incidence of Extended-Spectrum β-Lactamases and Characterization of Integrons in Extended-Spectrum β-Lactamase-producing Klebsiella pneumoniae Isolated in Shantou, China. Acta biochimica et biophysica Sinica. 2007; 39(7): 527-532.
30. Bhattacharjee A, Sen M, Prakash P, Gaur A, Anupurba S, Nath G. Observation on integron carriage among clinical isolates of Klebsiella pneumoniae producing extended-spectrum β-lactamases. Indian journal of medical microbiology. 2010; 28(3): 207.
31. Baker-Austin C, Wright MS, Stepanauskas R, McArthur J. Co-selection of antibiotic and metal resistance. Trends in microbiology. 2006; 14(4): 176-182.
32. Oliveira-Pinto C, Diamantino C, Oliveira PL et al. Occurrence and characterization of class 1 integrons in Escherichia coli from healthy individuals and those with urinary infection. Journal of medical microbiology. 2017; 66(5): 577-583.