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Detection of Integron CS Class 1 Type Metallo-β-Lactamase Gene in Clinical Isolate of Escherichia Coli at Thi-Qar , Iraq

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ABSTRACT

Class 1 integrons play a role in the emergence of resistant bacteria by facilitating the recruitment of gene cassettes encoding antibiotic resistance genes. inhibition zone diameters were determined in tests with imipenem (IPM) and meropenem (MEM) discs alone and in combination with 1000 µg of EDTA Combined disk diffusion method was used for detection of integron CS type metallo-β-lactamase-producing isolates, they were emplace each of imipenem and meropenem antibiotics with EDTA on Mueller-Hinton agar, because EDTA working to inhibit of metallo-β-lactamase enzymes and increase the diameter of inhibition zones. The results revealed that out of 0 ⁄ 22 (0 %) isolates produce these enzymes with imipenem and out of 0 ⁄ 22 (0 %) isolates produce these enzymes with meropenem. All the 22 isolates were submitted to molecular detection of Integron CS by using PCR assay. The results showed that only 1 (4.5%) isolates were carried Integron CS.

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Introduction

The term integron was originally coined to describe the group of apparently mobile elements which contain one or more antibiotic-resistance genes located at a specific site, and also contain the determinants of the site-specific recombination system responsible for insertion of the resistance genes (Stokes and Hall, 1989). Class 1 integrons have been examined the most extensively. They consist of a variable region bordered by 5' and 3' conserved regions. The 5' region is made up of the int gene, attI and the promoter Pant which drives transcription of genes within the variable region. The 3' region consists of an ethidium bromide resistance locus (qacED1), a sulfonamide resistance gene (sulI) and an open reading frame containing a gene of unknown function. The integras Class2 integrons is located within the 3' conserved region. Class 3 integrons have yet to be thoroughly studied (Collis and Hall, 1992). Class 1 integrons play an important role in the Emergence of multi-resistant bacteria via the stockpiling of resistance determinants (Recchia and Hall, 1995). Integrons, while not mobile themselves are often carried by mobilizing elements such as conjugative plasmids, transposons or phages, which ensure their horizontal transfer (Toussaint and Merlin, 2002). A substantial proportion of resistance determinants in Gram negative bacteria reside on class 1 integrons that are capable of capturing and expressing genes contained in cassette-like structures (Johnson et al., 2006). Integrons are often found in pathogenic gram-negative bacilli including P. aeruginosa, P. putida, Acinetobacter spp., Escherichia coli, Serratia marcescens, Citrobacter freundii, and Salmonella spp. (Ploy et al.,2003).

Materials and Methods

Bacterial strains. Out of 105 sample have been collected from patients only Twenty-two samples have been gave growth for Escherichia coli , 10 (58.8%) urine, 2 (11.7%) wounds, 5 (29.4%) stool and 5(22.7%) vagina . All swabs were labeled and transported to laboratory within one hour then streaked on nutrient agar , Blood agar, MacConkey agar and (EMB) . All plates were incubated aerobically in incubator at 37ºC for 24 .hrs

Identification

The grown colonies on the culture media with characterized green metallic pigments on Eosin Methylene blue were selected for further diagnostic tests (Morphological and Biochemical) according to (MacFaddin , 2000). Diagnosis of the isolates was

confirmed by API 20 E system.

Antibiotic susceptibility

The susceptibility of Escherichia Coli isolates to 9 antibiotics was determined by disc diffusion method (Bauer et al., 1966). The antibiotics (content per disc) used in the study are Ak: Amikacin (30 µg); Ceftazidime (30 µg); CF: Cephalothin (30 µg); FEP: Cefepime (30 µg); IMP: Imipenem (10 µg); LEV: Levofloxacin (5 µg); MEM:Meropenem (10 µg); TCC: Ticarcillin-clavulanic acid (75/10 µg); TOB: Tobramycin (10 µg). The antibiotic discs were purchased from Bioanalyse, Turkey. The results were recorded according to (CLSI, Screening of MBL producers and .(2007 PCR assay. MBL-CD test (metallo-β-lactamases -combined disk test) , in MBL-CD, imipenem and combined imipenem/EDTA (IPM ⁄ EDTA, 10 ⁄ 1000 µg, Becton Dickinson ⁄ Sigma) discs were placed on the agar plates. After overnight incubation at 35 Cº, inhibition zones of the imipenem with and without EDTA were compared. The test is considered positive if a ≥ 6 mm increase in the zone diameter for imipenem/EDTA is observed (Yong et al., 2002 ; Berge`s et al., 2007). All the isolates were screened by PCR for class 1 integrons using the primers 5'CS (GGCATCCAAGCAGCAAG) and 3'CS (AAGCAGAC TT GA CCTGA). In the this study analysis of the integron gene was carried out for the detection of class 1 integrons, as reported by (Pitout et al., 2005). DNA was extracted from E. coli isolates obtained from the samples by using commercial DNA extraction kits (Promega ⁄ USA) according to the manufacturer's instructions.. Amplification was performed for 35 cycles as: initial denaturation at 94°C for 5min, denaturation at 94°C for 1min, primer annealing at 54°C for 1min, extension

at 72°C for 1.5min and final extension at 72°C for 5min. The PCR products were visualized on a UV Transilluminator. Analysis of the findings was performed using Chi .(Square test and (SPSS, version 16

Results

Out of 105 hospitalized patients from urine, wound, stool and vagina . 22 (20.9%) found to have Escherichia Coli in their isolates. Table 1 shows the antibiotic susceptibility pattern of the Escherichia Coli isolates indicating that the isolates have acquired high level resistance against Cephalothin, Cefepime and Ticarcillin-clavulanic acid was (100%). an intermediate level resistance was found against the Ceftazidime (45.5%) , Levofloxacin (22.7%) and Tobramycin (18.2%). While don't appearance any resistance was recognized for imipenem and meropenem was (0%).

Table 1: Antibiotics resistance percentage of Escherichia Coli according to CLSI 2007. (n=22)

Type of Antibiotics	No.(%) of Resistant Isolates		No. (%) of Intermediate Isolates		No. (%) of Sensitive Isolates	
	No.	%	No.	%	No.	%
Amikacin (AK)	1	4.5	2	9.1	19	86.4
Ceftazidime (CAZ)	10	45.5	5	22.7	7	31.8
Cephalothin (CF)	22	100	0	0	0	0
Cefepime (FEP)	22	100	0	0	0	0
Imipenem (IMP)	0	0	1	4.5	21	95.5
Levofloxacin (LEV)	5	22.7	2	9.1	15	68.2
Meropenem (MEM)	0	0	0	0	22	100
Ticarcillin-clavulanic acid (TCC)	22	100	0	0	0	0
Tobramycin (TOB)	4	18.2	3	13.6	15	68.2

Detection of MBLs Production. (CD test)

CD test (combined disc) was used for detection the effect of β-Lactamases inhibitor (EDTA) on the enhancement of the zone of inhibition in the area between the antimicrobial disc and the inhibitor disc. This test was considered as indicator positivity of MBLs production. The results showed absence Metallo-β-Lactamases for imipenem and meropenem in Molecular .Escherichia Coli isolates Detection of integron CS in Escherichia ColiIn the present study, PCR assay was used for detection of the presence of (bla CS (5CS and 3CS) integron) in Escherichia Coli isolated from clinical and hospital environmental specimens of Thi-Qar province . The entire procedure for the PCR assay was carried out with a negative control containing all the reagents without a DNA template. Class 1 are encoding for Metallo-β-Lactamases enzymes produced for β-Lactams inhibition. Although phenotypic method of antibiotic susceptibilty (Table 1) showed that 100% isolates resistance to Cephalothin, Cefepime and Ticarcillin-clavulanic acid, only 1(4.5%) isolates were positive for presence of integron CS genes (figure 1).

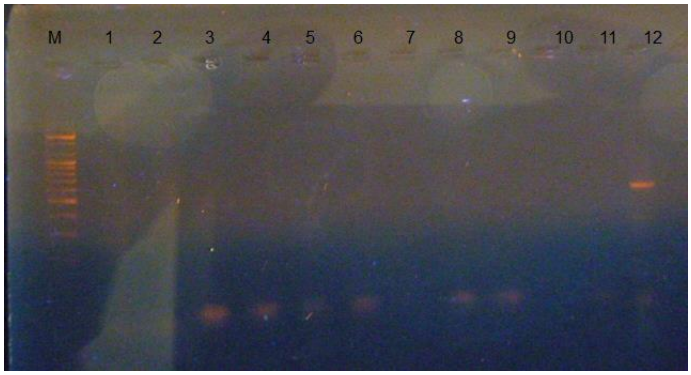

Figure (1): PCR amplification of integron CS. of Escherichia Coli. Lanes: M: Molecular . size marker 100-2000 bp

Discussion

Integrons are natural expression vectors that permit the insertion of antibiotic resistance genes by a site-specific recombinational mechanism. With the potential of integrons to capture and collect gene cassettes there is possibility that antibiotic resistant, genes can be widespread in nature (Mehedi , 2010) . In this study we have characterized antibiotic resistance gene cassette arrays in class 1 integrons that could not be amplified using standard PCR primer, using primer 5CS and 3CS. In the present study, 4.5 % of the 22 E. coli isolates screened harbored class 1 integron. China had the highest levels of antibiotic resistance (mean prevalence of resistance, 41% in hospital-acquired infections and 26% in community-acquired infections) in a comparative analysis with Kuwait and the United States (Zhang et al., 2006).

Furthermore, China had a higher rate of resistance development (22% average annual growth from 1994 to 2000) than Kuwait (17% from 1999 to 2003) or the United States (6% from 1999 to 2002) (Zhang et al., 2006). E. coli, which are deadly pathogens if antibiotic resistant can be very dangerous for the environment. Antimicrobial resistance genes allow a microorganism to expand its ecological niche, allowing its proliferation in the presence of certain noxious compounds. The problem can be further increased with the widespread existence of integrons since antibiotic resistant genes can be Since we .propagated by integrons detected integrons in lots of isolates of humans in the Iraq, our future work will be directed to the identification of the transposon structures that contain these integrons in E. coli and other .bacterial isolates

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