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MACROLIDE –LINCOSAMIDE -STREPTOGRAMIN B RESISTANCE IN *ENTEROCOCCUS* SPP. ISOLATES IN BAGHDAD

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ABSTRACT

In the present study, Forty isolates of *Enterococcus* species (*E. faecalis*, *n* = 20 and *E. faecium*, *n* = 20*)* were isolated from urine samples of patients suffering from Urinary Tract Infections from different hospitals in Baghdad. All isolates were screened for their susceptibility to Antibiotics, majority of isolates were resistant toward ceftriaxone. In addition, 75% of *E.faecium* isolates were resistant to clindamycin and erythromycin and 80 % of *E.faecalis* isolates were resistant to clindamycin and 75% to erythromycin. Phenotypic screening about MLSB was showed 45% of *E. faecium* and 40% of *E. faecalis* isolates is constitutive resistance to erythromycin (cMLSB) phenotype ,10 % for each *E. faecium* and *E. faecalis* isolates inducible resistance to erythromycin with D-shape , 15% of *E. faecium* and 10% of *E. faecalis* isolates showed MS phenotype which is resistance to erythromycin and sensitive to clindamycin without D-shape .The polymerase chain reaction (PCR) was used to study the prevalence of the macrolide resistance genes *ermA, ermB, ermC* and *msrA* in *E. faecium* and *E. faecalis* isolates that were erythromycin resistant. Positive PCR amplifications of *ermB* were obtained for only one erythromycin-resistant *E. faecium* isolate . 15% of *E . faecalis* isolates were positive for PCR amplification of *ermC* but was negative for PCR amplification of the *ermB* and *ermA* genes .Macrolide resistance by efflux due to the *msrA* gene was detected in 10% of erythromycin-resistant *E.faecium* isolates and 20% of erythromycin-resistant *E. faecalis* isolates.]

KEYWORDS: *faecalis, E.faecium, ermA, ermB, ermC* and *msrA.*

INTRODUCTION

Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen.[1] In the last two decades, particularly virulent strains of *Enterococcus* spp. that are resistant to vancomycin (vancomycin-resistant enterococcus, or VRE) have emerged in nosocomial infections of hospitalized patients especially in the US.[2]

Macrolide-lincosamide-streptogramin (MLS) antibiotics constitute an alternative therapy for the treatment of insidious enterococcal infections. Erythromycin and clindamycin inhibit protein synthesis in a wide range of bacteria by binding to a single site the large ribosomal subunit located near the entrance to the growth of the polypeptide chain in bacterial ribosome.[3]

Three different mechanisms account for the acquired resistance to MLS antibiotics in gram-positive bacteria: modification of the drug target, inactivation of the drug, and active efflux of the antibiotic. In the first case, a single alteration of the 23S rRNA confers broad cross-resistance to macrolide-lincosamide-streptogramin B (MLS_B) antibiotics, whereas the inactivation mechanism confers resistance only to structurally related MLS antibiotics. Regarding the pump mechanisms, the *mefA*, *mefE*, *msrA*, and *mreA* genes have been involved in the active efflux of macrolides in gram-positive bacteria.[4,5]

The msrA-mediated resistance mechanism is responsible for resistance to macrolides and streptogramin B only (MS phenotype), while the *erm* genes-mediated resistance genotype is associated with resistance to macrolides, lincosamides, and streptogramin B (MLSB phenotype). The *erm*A and *erm*C are most frequently found in staphylococci. This mechanism confers cross-resistance to MLSB antibiotics, the so-called MLSB phenotype. Expression of MLSB resistance can be either constitutive (cMLSB) or inducible (iMLSB).[6]

It has been demonstrated that clindamycin treatment in patients with $iMLS_B$ may lead to $cMLS_B$ and therapeutic failure.[7] The best way to detect inducible clindamycin resistance (ICR) is a test known as disk approximation test or D-test. The aim of this study was to determine the incidence of Macrolide- Lincosamide- Streptogramin B resistance phenotypes by using D-test method and genotype by using PCR in *Enterococcus* species isolates from Baghdad/Iraq.

MATERIALS AND METHODS

Bacterial isolates

Urine samples were collected from some hospitals in Baghdad /Iraq. The isolates were initially characterized as Enterococci, based on biochemical tests and Gram staining, according to the criteria established by.[8] Species identification was performed by API Rapid ID 32-Strep using mini API (Biomérieux, France).

Antimicrobial susceptibility test and Phenotypic detection of MLSB

Susceptibility of *E.faecium* and *E. faecalis* isolates was tested by the disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[9] with commercially available antimicrobial discs (Bioanalyse/Turkey).Isolates were tested against the following antimicrobial agents: Ceftriaxone (30 µg), Cefoxitin (30 µg), Cephalexin (30 µg), Erythromycin (15 µg), Azithromycin (15 µg) , Clindamycin (2 µg) , Streptomycin(30 µg) ,Vancomycin (30 µg) and Chloramphenicol (10 µg). Clindamycin and erythromycin disks were placed 15-26mm apart from each other on the Muller Hinton Agar plates. After 18h incubation at 37°C, plates were checked. Flattening of inhibition zone (D-shaped) around clindamycin was considered as inducible clindamycin resistance, The test allows for identification of four different phenotypes.[10]

The inducible MLS_B phenotype (D^+)

Resistant to erythromycin and susceptible to clindamycin with a D-zone of inhibition around the clindamycin disk.

The constitutive MLS_B phenotype: Resistant to both erythromycin and clindamycin.

The MS_B phenotype: Resistant to erythromycin and susceptible to clindamycin.

The susceptible phenotype: Susceptible to both clindamycin and erythromycin.

Molecular Detection of MLSB

The presence of genes involved in MLS resistance with a methylation mechanism was determined by PCR amplification of known *erm* genes by using primers specific for *erm*A, *erm*B and *erm*C, and the presence of the *msr*A gene involved in antibiotic efflux systems was also examined (Table 1) .PCR condition for each primer was started the process with initial denaturation step at 96 C/ 3 min was followed by 30 cycles of amplification with denaturation at 94 C for 30 s, annealing at 55 C for 45 s, and extension at 72 C for 1 min, with a final extension at 72 C for 7 min. PCR product was resolved on a 1.5 % agarose gel stained with ethidium bromide and visualized under UV transillumination.

Table1. Primers used for detection of genes encoding antibiotics resistance in *Enterococcus* species.

Gene	Sequence of forward Primer(5´- 3´)	Sequence of reverse primer (5´- 3´)	Reference
*erm*A	TAT CTT ATC GTT GAG AAG GGA TT	CTA CAC TTG GCT TAG GAT GAA A	[11 , 12]
*erm*B	CTA TCT GAT TGT TGA AGA AGG ATT	GTT TAC TCT TGG TTT AGG ATG AAA	[11 , 12]
*erm*C	CTT GTT GAT CAC GAT AAT TTC C	ATC TTT TAG CAA ACC CGT ATT C	[11 , 12]
msrA	TCCAATCATAGC CAAAATC	AATTCCCTCTATTTGGTGGT	[11 , 12]

RESULTS AND DISCUSSION

Enterococci are common causes of nosocomial infections and are ranked second (after staphylococci) as aetiological agents of hospital-associated infections in US hospitals,[13] In the present study, Forty isolates of different *Enterococcus* species (*E. faecalis*, *n* = 20 and *E. faecium*, *n* = 20*)* were isolated from Urinary Tract Infections from different hospitals in Baghdad. Antimicrobial susceptibilities were determined by the agar diffusion procedure and the MLS_B phenotypes by the double disk induction test, 75% of *E.faecium* isolates were resistant to clindamycin and erythromycin and 80 % of *E.faecalis* isolates were resistant to clindamycin and 75% to erythromycin (Table 2).

Table 2: Susceptibility of Enterococcus spp isolates to the Antibiotics.

Antibiotic	Resistance (%) of *E.faecium* isolates	Resistance (%) of *E.faecalis* isolates
Azithromycin	55	65
Cefoxitin	55	40
Clindamycin	75	80
Erythromycin	75	75
Vancomycin	35	40
Cephalexin	60	60
Streptomycin	60	75
Chloramphenicol	45	55
Ceftriaxone	85	95

Phenotypic screening about MLSB was showed 45% of *E. faecium* and 40% of *E. faecalis* isolates is constitutive resistance to erythromycin (cMLSB) phenotype, 10 % for each *E. faecium* and *E. faecalis* isolates inducible resistance to erythromycin with D-shape, 15% of *E. faecium* and 10% of *E. faecalis* isolates showed MS phenotype which is resistance to erythromycin and sensitive to clindamycin without D-shape The *Enterococcus* isolates were

analyzed for the presence of *erm* methylase genes and *msrA* gene by PCR by using specific conditions. Positive PCR amplifications of *ermB* were obtained for only one erythromycin-resistant *E. faecium* isolate; 15% of *E . faecalis* isolates were positive for PCR amplification of *ermC* but was negative for PCR amplification of the *ermB* and *ermA* genes .Macrolide resistance by efflux due to the *msrA* gene was detected in 10% of erythromycin-resistant *E.faecium* isolates and 20% of erythromycin-resistant *E. faecalis* isolates (Table 3).

Table 3: Prevalence of *erm* A, B and C among erythromycin-resistant Enterococci isolates

Enterococci species	cMLSB	iMLSB	MS	*erm*A	*erm*B	*erm*C	*msr*A
E . faecalis	40%	10%	10%	0	0	15%	20%
E. faecium	45%	10%	15%	0	5%	0	10%

The prevalence of $iMLS_B$ phenotype among Enterococci isolates in our study was 10% , and 45% cMLSB phenotype was showed among *E. faecium* and 40% among *E. faecalis* isolates. A study by Schmitz *et al.*[14] showed that 6.6% of the *E. faecium* isolates tested were susceptible to erythromycin, with all erythromycin-resistant isolates displaying the constitutive MLS_B resistance phenotype.

Expression of MLSB resistance can be constitutive or inducible. In inducible resistance, the bacteria produce inactive mRNA that is unable to encode methylase. The mRNA becomes active only in the presence of a macrolide inducer. By contrast, in constitutive expression, active methylase mRNA is produced in the absence of an inducer. Induction is related to the presence of an attenuator upstream from the structural *erm* gene for the methylase.[15]

Jensen et al.[16] recently analysed 113 erythromycin-resistant enterococcal isolates of human and animal origin and found the *ermB* gene to be present in 88%. In other study carried by Zhong *et al.*[17] the *ermC* gene was detected in 13 isolates (22%). Furthermore, 9 (15%) strains were *ermA* positive and 18 strains harboured *ermB* gene (30%).

The spread of *erm* genes belonging to the *erm*(B) class and, rarely, to the *erm*(TR) subset of the *erm*(A) class accounts for the vast majority of resistance caused by ribosomal methylation in streptococci and enterococci.[18] The *msr*(A) resistance determinant was originally detected in *Staphylococcus epidermidis*, and, since then, it has been found in a variety of staphylococcal species, including *S. aureus*. Among the various *erm* genes so far detected in staphylococci, the Tn554-associated gene *erm*A, the Tn917/Tn551 associated gene *erm*B, and the gene *erm*C often located on small plasmids.[19] Previous studies showed that these three

genes(*erm*A ,*erm*B and *erm*C) alone or in various combinations are also present in MRSA CC398 isolates from pigs, cattle, and food of animal origin.[20 ,21,22] Each of these genes is sufficient to confer clinical levels of macrolide–lincosamide resistance to the corresponding isolate. Thus, the presence of more than one *erm* gene may point towards the acquisition of these erm genes by the respective strains at different times and/or under different conditions.[19] In conclusion, we found a high prevalence of constitutive clindamycin resistance phenotype in Enterococci isolates in our region.

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