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RESEARCH ARTICLE

Phenotypic Investigation of Vancomycin, Teicoplanin and Linezolid Resistance Among *Enterococcus* spp. Isolated from Children Diarrhea

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Abstract

Vancomycin-resistant Enterococci(VRE) were common among *Enterococcus. faecalis* and *Enterococcus faecium.* Teicoplanin resistance or sensitivity can determine the VRE phenotypes whether VanA (Van^R/Tec^R) or VanB (Van^R/Tec^S). Linezolid resistance among VRE regards an newly emerged health problem. Infection with LRVRE or TRLRVRE pushan hazardous alert for hard to heal illness. Twenty eight Enterococcus spp. isolates were recovered from children diarrhea after their inoculation on m-EI chromogenic agar. Antibiotics susceptibility and phenotypic detection of antibiotics resistance were performed according to CLSI 2016. The results revealed 92.86% resistance to rifampin, 85.71% to erythromycin. VRE were 46.42%, TRE were 25% and LRE were 35.71% while co-existed resistance for Vancomycin/Teicoplanin/Linezolid(TRLRVRE) were detected 25% in. concern antibiotics resistant patterns, the MDR compile (85.7%) while XDR compile (10.7%) and there is no PDR among *Enterococcus*. spp. isolates were PDR. The presentstudy conclude that VanA and VanB phenotypes were common among MDR and XDR and although there is no using of linezolid but the emergence of TRLRVRE isolates were stated.

Keywords: *Enterococcus* spp., VRE, MDR, XDR, LRVRE, TRLRVRE.

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INTRODUCTION

Enterococci werea Gram-positive, non-spore-forming,catalase-negativefacultative-anaerobe, Which normally dwellthe alimentary tract of humans. even if *Enterococcus* spp. is a coexistence organism of the intestinal tract. However although, It may be the causative agent of diarrhea in the elderly and children and immune compromised patients[1,2]. Enterococci, particularly relevant *Enterococcus faecium* and *Enterococcus faecalis*, have arise as objects of importance because of the distinctness of resistant strains of many drugs[3-5]. Enterococcus which includes some of nosocomial multidrug-resistant organisms. Vancomycin-resistant enterococci (VRE) is now one of the leading causes of nosocomial infections and represent approximately one-third of *Enterococcus* isolates[6-8]. There are three main patterns of resistance: Multi-drug resistance (MDR) was indicated as acquired non-susceptibility to asminimumas one agent in three or more antimicrobial classes, extensive-drug resistance (XDR) was clear as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan-drug resistance (PDR) was defined as resistance to all classesof anti-microbial[9-12]. Vancomycin and teicoplanin resistance via one or more of nine genes (*vanA-vanE, vanG, vanL, vanM* and *vanN*) which express for enzymes needed for the synthesis of new peptidoglycan precursors and enzymes that disrupt the normal d-Ala-d-Ala-ending precursors[13-15]. Enterococci resistant to erythromycin by main two mechanism: enzyme production like ribosomal methylases (coded for by *erm* genes) methylate the bacterial ribosome, impairing the binding of macrolide and macrolide efflux, coded for by *mef* genes [16]. Eflux pumps encoded by *tetK*and *tetL*were responsible for tetracyclines[17]. Three mechanisms were described well includes mutation in gyrA gene, production of NorA efflux pump and encoding for Qnr proteins which guard DNA-gyrase by diminishing DNA binding of the quinolone and succeeding formation of the quinolone–gyrase complex. Tell yet Rifampicin-resistance get up from a range of mutations in the *rpoB* gene that encodes for the polymerase RNA β-subunit. Two mechanism of resistance were well described among Enterococcus sp. to linezolid : genes in which mutations occurencoding the 23S rRNA, (which is an important part of the drug-binding site on the ribosome) and Enzymatic modification of the 23S rRNA by methylase[17-24]. In this study aims to check the antibiotic resistance patterns along with resistance phenotypes of diarrheal Enterococcus faecalis and Enterococcus faecium.

MATERIALS AND METHODS

Sample Collection and Processing

Fifty eight stool samples (diarrhea) were collected from children with diarrhea with age ranged from 1-7 years. Swab were used to take the sample and put it in brain heart infusion broth for transportation and incubated at 37°C for 24 hrs. and then inoculated to mEI chromogenic agar[25].

Cultivation on m-EI chromogenic agar

Agar chromogenic mEI is a chromogenetic agar for recovery and distinctionof faecalis and faecium enterococci. It containnutrients and cycloheximidefor fungi inhibition. Incubation for 18-24 hours and then Enterococcus faecium itGrowth will appears greenish-blue, while give blue colonies for Enterococcus faecalis.

Antibiogram

Antibiotic susceptibility test were done according to CLSI 2016[26] using standard disk diffusion method upon Muller-Hinton agar after normalization of broth to 0.5 McFarland ($1x10^8$ CFU/ml at OD=0.08).

Biosafty Aspects

The biosafty aspects include decontamination of swabs, broth, contaminated disposable and culture medium[27].

RESULTS AND DISCUSSION

The result of Enterococcus sp. Isolation revealed that, enterococcal diarrhea compile 28(48.3%) Fig. 1. Enterococcus spp. is intestinal opportunistic bacterium with virulence possibilities like protease gelatinase (GelE). It is not naturally virulent but their resistance arrays of antibiotics classes leading to infections in susceptible individual like immuno-compromised, children and elderly and cancer patients. Infected patients with diarrhea can be the source of MDR-enterococci especially VRE[28,29].

Enterococcus spp. regards reservoir of intrinsic and inherently resistance to various antibiotics classes. The results of the current

Table 1. Antibiotics resistance percentage among *Enterococcus* spp.

Antibiotic	Symbol	Potency (μg)	Resistance %
Rifampin	RA	5	**92.86**
Erythromycin	E	15	85.71
Nitrofurantion	F	300	64.29
Ciprofloxacin	CIP	5	60.71
Tetracycline	TE	30	57.14
Penicillin	P	10	53.57
Vancomycin	VA	30	46.43
Linezolid	LNE	30	35.71
Teicoplanin	TEC	30	25
Chloramphenicol	CHL	30	21.34
Doxycycline	DO	30	17.86

Table 2. Co-existence resistance among *Enterococcus* spp.

Antibiotics co-existence	No.(%)
Vancomycin^{R} total (VRE)	13/28 (46.42)
Teicoplanin^{R} total (TRE)	7/28 (25.00)
Linezolid^{R} total (LRE)	10/28 (35.71)
Vancomycin^{R}/Teicoplanin^{S}/ Linezolid^{S}	6/28 (24.00)
Vancomycin^{R}/Teicoplanin^{R}/ Linezolid^{S} (TRVRE)	0/28 (0.00)
Vancomycin^{R}/Teicoplanin^{R}/ Linezolid^{R} (TRLRVRE)	7/28 (25.00)
Vancomycin^{S}/Teicoplanin^{R}/ Linezolid^{R} (TRLRE)	0/28 (0.00)
Vancomycin^{S}/Teicoplanin^{S}/ Linezolid^{R}	3/28 (10.71)

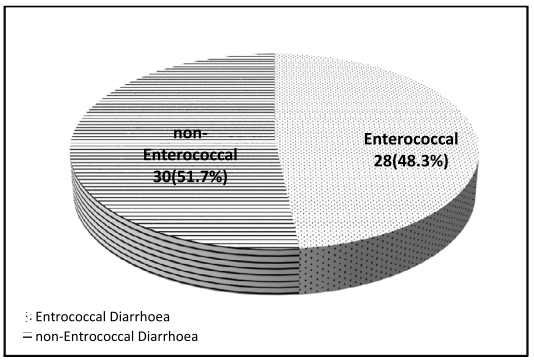

Fig. 1. Distribution of *Enterococcus* spp. among children diarrhea.

study publicized different resistance percentage as rifampin (92.86%), erythromycin (85.71%), nitrofurantion (64.29%), ciprofloxacin (60.71%), tetracyclin (57.14%), penicillin (53.57%), vancomycin (46.43%), linezolid (35.71%), teicoplanin (25%) and doxycyclin (17.86%) table (1).

Table (1) show the results for antibiotic resistance among Enterococcus spp. Our results in accordance with many Iraqi studies like Khalid (2016)[30], Chabuck et al., (2011)[31], Al-Marjani (2013)[32] and Al-Halaby AH, Al-Hashimy (2016)[33] who found (72-100%) of enterococci resistance to rifampin respectively. Many studies around the world also stated similar results, resistance to enterococci were (76-100%)[34-36]. The most common mechanism of resistance to rifampin is mutation in β subunit of RNA polymerase (encodes by *rpoB*)[37]. Resistance to erythromycin were (85.71%) and it is quite same those stated in another studies[30-33,38-40]. Co-existence of triple resistance to vancomycin/teicoplanin/linezolid were present in 7/13 (53.84%) of vancomycin resistant enterococci (VRE) table (2). Our results is the first who stated co-existence of resistance to vancomycin/teicoplanin/linezolid in Iraq while many studies in Iraq and neighboring countries not state such resistance[30-40]. The most common phenotypes of vancomycin resistance among VRE are VanA and VanB which related to *vanA* and *vanB* genotypes. Van A characterized by their co-resistance to both vancomycin and teicoplanin while VanB confer only resistance to vancomycin[41]. Our results stated both phenotypes, VanA in 7/13 (53.84%) while VanB in 6/13 (46.16%).

Resistance of enterococci to linezolid (LRE) were very rare and single cases documented around the world. Also the Co-existed resistance to vancomycin and linezolid (LRVRE) and vancomycin, teicoplanin and linezolid (TRLRVRE) were note documented yet in Iraq and this study seem the first to report TRLRVRE phenotypically. The results revealed that 7/28 (25%) of enterococci were TRLRVRE or LRVRE table (2). Linezolid resistance may be appear after treatment with linezolid while many cases reported the resistance in patients without prior use of linezolid[42-46].

Concern the resistance patterns, MDR, XDR and PDR, the results revealed that 1/28

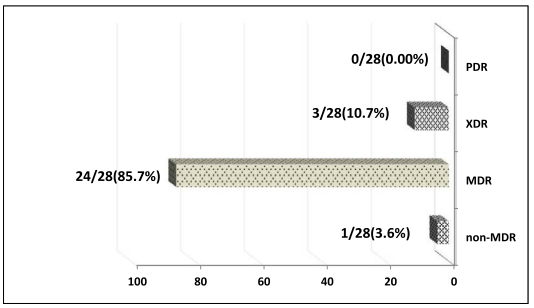

Fig. 2. Antibiotics resistance patterns among *Enterococcus* spp.

(3.6%), 24/28(85.7%), 3/28(10.7%) and 0/28 (0.00%) of enterococci isolates were non-MDR, MDR, XDR and PDR respectively.

Different percentage of MDR-enterococci were stated in many studies (28-63%)[47-50]. XDR-enterococci were also stated in many studies and compile (8-35%) of isolated enterococci. The resulted multidrug or extensive drug resistance, due to many factors such as antibiotic pressures or antibiotics abuse, can leads to costly, hard to cure, prolonged illness and high mortality infections[51,52].

CONCLUSION

The current study conclude that VanA and VanB phenotypes were common among MDR and XDR and although there is no using of linezolidbut the emergence of TRLRVRE isolates were stated.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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