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 (VanR/TecR) or VanB (VanR/Tecs). 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-negative facultative anaerobe, which normally dwell the 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. Enterococci, particularly relevant Enterococcus faecium and Enterococcus faecalis, have arise as objects of importance because of the distinctness of resistant strains of many drugs. 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. There are three main patterns of resistance: Multi-drug resistance (MDR) was indicated as acquired non-susceptibility to at least 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 classes of anti-microbials. 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. 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. Eflux pumps encoded by tetKand tetL were responsible for tetracyclines. 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 RNA by methylase. 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. Cultivation on m-EI chromogenic agar Agar chromogenic mEI is a chromogenetic agar for recovery and distinction of faecalis and faecium enterococci. It contain nutrients and cycloheximide for 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 using standard disk diffusion method upon Muller-Hinton agar after normalization of broth to 0.5 McFarland (1x10⁸ CFU/ml at OD=0.08).

Biosafety Aspects

The biosafety aspects include decontamination of swabs, broth, contaminated disposable and culture medium.

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 (GeE). 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.

Enterococcus spp. regards reservoir of intrinsic and inherently resistance to various antibiotics classes. The results of the current
studies published different resistance percentage as rifampin (92.86%), erythromycin (85.71%), nitrofurantoin (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 studies30-33,38-40. 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 resistance30-40. 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 vancomycin41. 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 (LVRRE) and vancomycin, teicoplanin and linezolid (TRLVRE) were note documented yet in Iraq and this study seem the first to report TRLVRE phenotypically. The results revealed that 7/28 (25%) of enterococci were TRLVRE or LVRRE table (2). Linezolid resistance may be appear after treatment with linezolid while many cases reported the resistance in patients without prior use of linezolid42-46.

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

### 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 total (VRE)   | 13/28 (46.42) |
| Teicoplanin total (TRE)  | 7/28 (25.00)  |
| Linezolid total (LRE)    | 10/28 (35.71) |
| Vancomycin/Teicoplanin/L | 6/28 (24.00)  |
| Vancomycin/Teicoplanin/L | 0/28 (0.00)   |
| Vancomycin/Teicoplanin/L | 7/28 (25.00)  |
| Vancomycin/Teicoplanin/L | 0/28 (0.00)   |
| Vancomycin/Teicoplanin/L | 3/28 (10.71)  |

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

Fig. 2. Antibiotics resistance patterns among Enterococcus spp.

(3.6%), 24/28(85.7%), 3/28(10.7%) and 0/28 (0.0%) 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 infections51,52.

CONCLUSION
The current study 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.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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