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In Vitro Effect Of Chitosan *Lactobacillus acidophilus* Nanoparticles on Vancomycin-Resistant Multidrug-Resistant *Enterococcus faecalis*

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Abstract

Multidrug-resistant (MDR) *Enterococcus faecalis* is involved in various illnesses. This research seeks to categorize and distinguish *E. faecalis* in various medical tests by VITEK and 16SRNA, particularly those with vancomycin-resistance. Identification of genes those are resistant to *VanA, VanB,* and *VanC.* And also use of Chitosan *L. acidophilus* nanoparticles serving like a normal remedy for vancomycin-resistant MDR (VRMDR) *E. faecalis* strains. *E. faecalis* became apparent in 85/145 body fluids, stool and urine samples (58.62%). 45/85 samples were found to be VRMDR *E. faecalis.* The outcome demonstrated that 66.66% of *E. faecalis* separates possessed the *VanA* gene, 53.33% harbored the *VanB* gene and 46.66% harbored the *VanC* gene, while 8.88% of them harbored the three genes. The effect of Chitosan *L. acidophilus* nanoparticles on VRMDR *E. faecalis* containing the three resistance genes that 20 µg/ml was the minimal bactericidal concentration, while 10µg/ml was the minimal inhibitory concentration. Consequently, as detected by TEM, Chitosan *L. acidophilus* nanoparticles could affect by damaging the cell wall, nuclear material and cytoplasmic structures. The IC50 of Chitosan *L. acidophilus* nanoparticles were 550.02±72.9ug/ml and the imipenem drug was 301.72±4.05ug/ml, showing that imipenem was more cytotoxic on human gastric epithelial cell line (GES1) than Chitosan *L. acidophilus* nanoparticles.

Keywords: *E. faecalis,* Nano *L. acidophilus,* Chitosan, Multidrug-resistant.

1. Introduction

Enterococcus faecalis is the gastrointestinal systems of several animals, having people, other primates, birds, reptites and insects are home to the gram-positive commensal, they also occur in nutrition, water, and soil (1).

The urinary system, surgical locations, respiratory system, digestive system, epidermis and smooth tissue are the usual disease sites. Additionally, the prevalence of illness brought on by isolates of the multidrug- renitent *enterococcus* is rising globally, causing severe clinical anti-infective therapy problems (2).

The use of various antibiotics in animal rearing contributes to the proliferation and dissemination of vancomycin- resistant *entercoccus* (VRE) in animals and the environment, which is likely produced by the co- selection of renitence genes within the typical mobile genetic elements (3). vancomycin resistant can either be inherited or gained by having one of eight different vancomycin renitent genes (*vanA, vanB, vanC*) (4) for treating systemic enterococcal diseases, the glycopeptide vancomycin is the initial substitute for the penicillin - aminoglycoside variation *Enterococcus* has been found to contain a rariety of vancomycin renitent genes. A number of enterococcal types have been shown to have *vanA* (top – grade resistant, *vanB*, *vanB2* and *vanD* with moderate to top grades of resistant and *VanC* (C1, C2, C3) resulting in low grade inherent renitence (5). In high of this, the capacity of *enterococcus* to develop renitence to antibiotics by a variety of mechanism (plasmids, transposons and chromosomal exchange or mutation) presents a significant treatment challenge (6).

Chitosan is a fascinating normal polysaccharide that has drowns considerable interest from the medical forming and study as a biological component and a medication delivery vehicle (7). Due to its simplicity, non - toxicity coagulation ability, smooth tissue compliance, innate immunity activity and inexpensive price. It is a suitable choice

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for encapsulating probitics (8). The best effect types of bifid bacteria are *lactobacillus acidophilus*. Because of its advantages, the study has revealed that it may be including in food that are fermented (9). However, for *lactobacillus acidophilus* to be effective as probiotics, it must adapt to the host's intestinal environment and be alive and physiologically active at the kindly pick in the host. Additionally they have to maintain their stability during transporting foodstuff, espicially everyday products, during processing, storage and product shelf-life (10).

This research sought to discover and separate vancomycin resistant MDR isolates of *E. faecalis* from clinical isolates from Egypt, find particular genes associated with different antibiotic resistance and assess the consequences of different concentrations of Chitosan *L. acidophilus* nanoparticles as a natural agent to prevent and control VRMDR *E. faecalis* strains. Moreover, the study has conducted the cytotoxicity effect of Chitosan *L. acidophilus* nanoparticles compared with the drug of choice on the human gastric epithelial cell line (GES1).

Materials and Methods:

Sample collection

145 distinct clinical specimens (Stool, Urine and body fluids specimens) were collected during May 2019 to March 2020. These specimens were collected from various hospitals in Egypt: 80 samples from Mbarret El-Asafra Hospital and 65 samples from El-Haram Hospital with research ethics approval (no. 20221215).

Bacterial isolation and identification

Enterococcus was specified depend on high protocol to research morphology, features that cause stains and biochemical reactions (11). Blood agar and Kenner-Faecal (K.F.) Streptococcal Medium agar (selective and differential media for *Enterococcus*) (Oxoid, England) was used to culture the samples, and then it was brood at 37°C for a day. All clinical specimens were identified as *Enterococcus* based on their colonial morphology and ability to grow esculin hydrolysis and tolerant to 6.5% sodium chloride in bile-esculin agar (12, 13).

Confirmation tests for *E. faecalis* by VITEK 2 system and 16SrRNA test

First, the bacterial identification was done by VITEK 2 updating system 9.02 (BioMerieux, USA) (14): the bacterium was inoculated into 0.85% NaCl solution, then inserted into the optical block of the DensiCheck. 145ul of the suspension was added to 3ml of sterile saline for preparing AST-GN cards. This tube was placed in the cassette with a susceptibility card. The cassette was transferred to the VITEK 2 cassette loading station within 10 minutes, then scanned cassette worksheet result is obtained after 5 to 8 hours.

The sequence match was done using Genbank database by 16SrRNA test; primer F: 5'AGAGTTTGATCCTGGCTCAG'3 & R: 5'GGCTACCTTGTTACGACTT'3 with 1500 bp was achieved utilizing the Basic Local Alignment Search Tool (BLAST) software and the sequence match facility in the GenBank database of the National Center for Biotechnology research (NCBI) (15).

Antibiotic susceptibility test (disc diffusion)

Ten different antibiotic from various group were used to test the isolated of *E. faecalis* isolates for their susceptibillity to various antibiotics including penicillins (ampicillin 10g), carbapenems (imipenem 100µg), macrolides (erythromcyin 5µg), cephalosporins (ceftriaxone 30µg and cephradine 5µg), monobactoms (aztreonam 15µg), aminoglycosides (streptomcyin 10µg), lincosamides (clindamcyin 150µg), nitrofurans (nitrofurantoin 300µg) and glycopeptides (vancomycin 30µg).

On Muller Hinton ager plates with 0.5 McFarland inoculum that had been up over night developed, antibiotic disc first were positioned. After that the plates incubated for a further 24 hours at 37°C. The inhibitory zone's diameter was evaluated and compared to requirements established by the clinical & lab institutional structure (16).

DNA extraction of *E. faecalis*

E. faecalis was freshly cultured on a 5ml nutrient broth medium at 37°C for a day, and the bacteria DNA fragment was extracted using a QIAamp DNAMini Kit (Cat. No. 51304 and 51306, QIAGEN, USA) (17).

Detection of *VanA, VanB,* and *VanC* genes

VanA, *VanB*, and *VanC* were detected using PCR analysis. Specific primers were used to amplify regions of *VanA*, *VanB* & *VanC* genes. (Metabion AG, Germany) as shown in table 1.

The Dream Taq Green PCR master mix (2X concentration), forward and reverse primers, template DNA, and water nuclease-free were added to a 25 µl Dream Taq Green PCR master mix for a total volume of 50 µl. Applied Biosystems Veriti 96well Thermal Cycler was utilised to establish the cycling conditions for gene detection (19).

Overlaying the reaction mixture with 50µl of performed PCR using the recommended thermal cycling condition was outlined below:

First denaturation takes place at 95°C for 5 minutes with 1 cycle. Denaturation for the 30s at 95°C, annealing for 30s at 56 °C, extension at 72°C for 30 seconds and number of cycles is 40; the last extension at 72°C for 10 min and number of cycles is 1. After that, the PCR products were loaded onto a 1.5 percent agarose gel (Biometra, Germany) and electrophoresed at 100V for around 30 minutes in 1

X TBE buffer. Next, 2µl of 10mg/ml ethidium bromide stained it (Sigma, USA). For data via software analysis, a gel documentation system (Geldoc-it, UVP, England) was utilized (ww.totallab.com, Ver.1.0.1). A gel of agarose was used to elute positive amplicons with 1500 bp. Micro spin filters were used to purify the resulting PCR products and quantities spectrophotometrically at wavelength 312nm (15).

Preparation of *Lactobacillius acidophilus* nanoparticle, which encapsulated in chitosan nanoparticles

According to the ionotropic gelation procedure, chitosan nanoparticles were created. A tripolyphosphate (TPP) aqueous solution was added to a chitosan solution to procedure blank nanoparticles. A homogenous solution was obtained by dissolving 1 g of chitosan powder in 200 ml of 1% acetic acid (pH = 4) and stirring for 6 hours. Thereafter, 150 ml of TPP 0.2% w/v was added dropwise. As chitosan nanoparticles formed, the onceclear fluid became murky. The suspension was then centrifuged three times for 30 minutes each at 9000rpm while adding distilled water (20, 21). As previously mentioned, the *lactobacillius acidophilus* was dissolved in a chitosan solution.

Chitosan nanoparticles were separated by centrifugation at a speed of 9,000 and a temperature of 4°C for 30 minutes to achieve the concentration of 10% w/w after tripolyphosphate was gradually added to the solution while magnetic stirring continued for 20 minutes (20, 21).

Characterization of Chitosan *Lactobacillius acidophilus* nanoparticles

TEM at an accelerating voltage of 200.0 kV was used (JEOL JEM-1400 series TEM, Japan) to study the morphological size of Chitosan *Lactobacillius acidophilus* nanoparticles. First, 1mg of Chitosan *L. acidophilus* nanoparticles was suspended in 10ml of distilled water and then 2 ul drops of nanoparticles were placed onto a parafilm and directly put on electron microscope (E.M.) grids. Finally, the filter paper was used to wick away specimen drop and placed in a petri dish.

Antibacterial screening of Chitosan *L. acidophilus* nanoparticles on VRMDR *E. faecalis*

Serial dilution of Chitosan *Lactobacillius acidophilus* nanoparticles was done VRMDR *E. faecalis* was subcultured overnight on Mueller Hinton agar in order to research their impact on it.

The minimal bactericidal concentration (MBC) and the minimal inhibitory concentration (MIC) were detected by using dilutions of Chitosan *Lactobacillius acidophilus* nanoparticles made in D.W. (1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/ml) (21). 95 ml of nutrient broth, 100 ml of successive chitosan *L. acidophilus* nanoparticle dilutions and 5 ml of microbial inoculum were used to fill the tubes.

Negative control tubes contain bacterial suspension without Chitosan *L. acidophilus* nanoparticle, whereas positive control tubes only contain Chitosan *L. acidophilus* nanoparticle suspension. For 24 hours, all tubes were incubated at 37°C. MIC and MBC values were calculated spectrophotometrically by measuring optical density at 600 nm on a spectrophotometer (T80 UV/VIS Spectrometer, United Kingdom) (22).

Detection of the effect of Chitosan *L. acidophilus* nanoparticles on VRMDR *E. faecalis* by using Transmission Electron Microscopy (TEM)

The effect of Chitosan *Lactobacillius acidophilus* nanoparticles on VRMDR *E. faecalis* was determined by TEM images (JEOL JEM-1400 series TEM, Japan) to study the morphology of *E. faecalis* as a control sample compared to standard McFarland inoculum of the treated one. First, bacterial suspension was cultured in 2 ml nutrient broth and incubated for 24 hrs at 37°C, then chitosan *L. acidophilus* nanoparticles were added the bacterial suspension then centrifuged and the palette was taken, then fixated in glutaraldehyde & osmium tetroxide, and dehydrated by adding alcohol.

The sample was then encased in epoxy resin. A microtome section was obtained with a thickness of between 500 and 1000 m. (Leica Ultracut UCT Ultramicrotome). The camera leica ICC50 HD was used to analyse thin sections that had been dyed with toluidine blue (1X).

Cytotoxicity assay of Chitosan *L. acidophilus* nanoparticles compared with Imipenem drug

Cells were cultivated using Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen/Life Technologies) supplemented with 10% Fetal Bovine Serum (FBS) (Hyclone), 10ug/ml of insulin, and 1% penicillinstreptomycin after being received from the American Type Culture Collection (Sigma). To remove all traces of serum that included trypsin inhibitor, the culture media was first removed from a centrifuge tube and the cell layer was rinsed with 0.25% (w/v) Trypsin 0.53 mM EDTA solution.

Cells were then put to the flask along with 2.0 ml of trypsin EDTA solution, and they were watched under a microscope until the cell layer was broken up. After adding 6.0 mL of complete growth media, cells were gently aspirated using a pipette, and then centrifuged at 125 x g for 5 min. After that, the cell pellet was suspended in a brand new growth media while the supernatant was discarded. The plates were then viewed using an inverted microscope after the cultures had been cultured at 37°C for 24 hours, and the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl 2Htetrazolium bromide) was then performed.

Multiwall plates worked well for the MTT in vitro cytotoxicity monitoring technique. Each MTT [M5655] vial was reconstituted in order to be used with 3 ml of medium, which was then added.

Reconstituted MTT was then added in an amount equal to 10% of the culture medium volume. Cultures were placed back in the incubator for 2 hours, depending on the type of cell and maximal cell density, after which the formazan crystals were broken down by adding MTT solubilization solution [M-8910] in a quantity equal to the volume of the original culture media. Different concentrations of cells were inoculated with Chitosan *Lactobacillus acidophilus* nanoparticles and, the most choice of antibiotics, Imipenem (100µg) (4.0, 16.0, 63.0, 250.0, 1000.0 ug/ml) dissolved in 10% FBS for 24 hrs, then by ROBONIK P2000 Eia reader device at 450 nm wavelength, the plates were measured.

Results

One hundred forty-five clinical specimens were collected from Mbarret ElAsafra Hospital and El-Haram Hospital from May 2019 to March 2020. Eighty-five isolates out of 145 (58.62%) were identified as *E. faecalis*. Four were isolated from body fluids, 30 from stool and 51 from urine samples. All isolates were identified as *E. faecalis* by the morphology of colonies, gram stain, growth in Azid Maltose Agar Media, non-fermentation of arabinose and positive results for esculin hydrolysis. While negative results for oxidase and catalase tests, then identified by using VITEK diagnostic systems and confirmed by 16-sRNA

Using the sequence match tool and BLAST program in the GenBank database at the National Center for Biotechnology Information (NCBI), each isolate's sequences were compared to type strains obtained from the Ribosomal Database Project (RDP) as shown in figure 1.

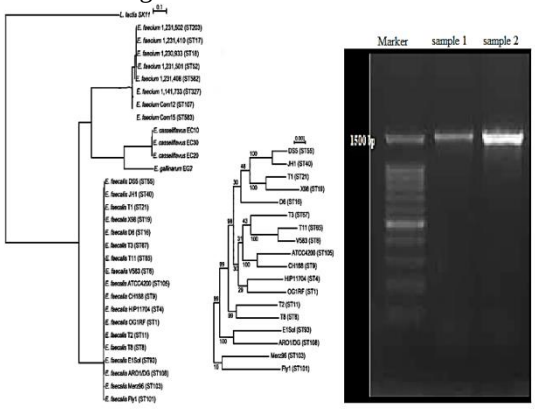

Fig. 1: Phylogenetic tree of the isolated *Enterococcus faecalis.* Sample 1 and 2 was confirmed test for identification of *E. faecalis*

Eighty-five *E. faecalis* samples were evaluated for antibiotic susceptibility using the disc diffusion plate method. 52.94% (45 out of 85) of them were found to be VRMDR *E. faecalis* strains (Table 2).

VanA (732 bp), *VanB* (647 bp) and *VanC* (822 bp) genes responsible for vancomycin-resistance in MDR *E. faecalis* strains. According to the results of the PCR amplification, 30 strains out of 45 (66.66%) harbored the *VanA* gene, 24 isolates (53.33%) harbored the *VanB* gene and 21 isolates (46.66%) harbored the *VanC* gene, while four isolates (8.88%) carried the three genes (Figures 2, 3, and 4).

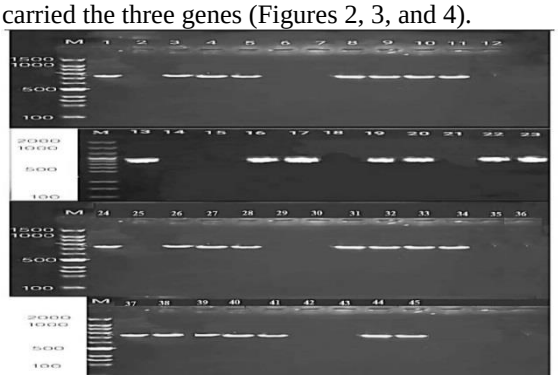

Fig. 2: Agarose gel electrophoresis show the amplified 732 bp DNA fragment *VanA* gene of VRMDR *E. faecalis*. M as Marker & 1, 2, 3, ….to 45 as no. of sample.

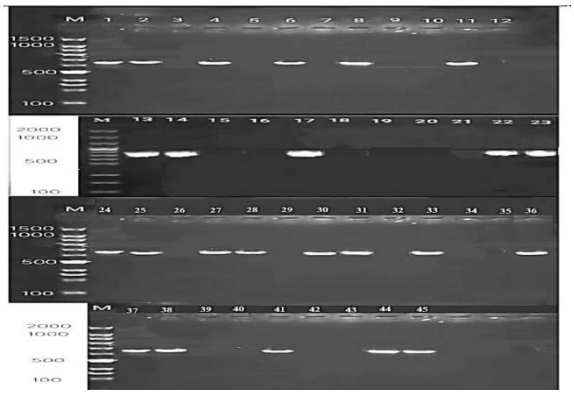

Fig. 3: Agarose gel electrophoresis show the amplified 647 bp DNA fragment *VanB* gene of VRMDR *E. faecalis*. M as Marker & 1, 2, 3, ….to 45 as no. of sample.

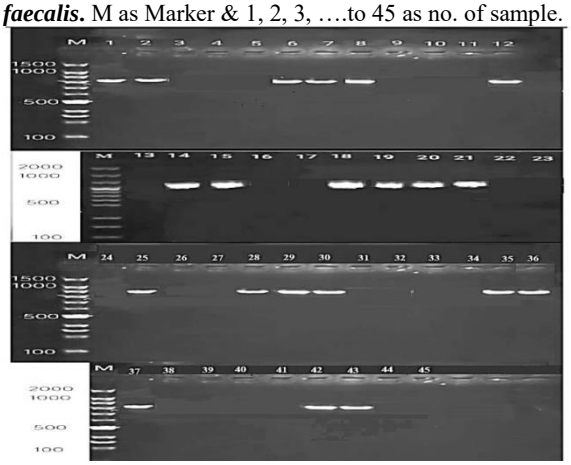

Fig. 4: Agarose gel electrophoresis show the amplified 822 bp DNA fragment *VanC* gene of VRMDR *E. faecalis*. M as Marker & 1, 2, 3, ….to 45 as no. of sample.

Characterization of Chitosan *L. acidophilus* nanoparticles was done

electron microscope transmission (TEM) at an accelerating voltage of 200.0 kV to study the morphology of the Chitosan *Lactobacillus acidophilus* nanoparticles (28.3 nm) (Figure 5).

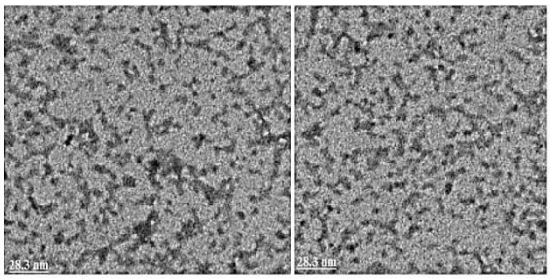

Fig. 5: Characterization of Chitosan *L. acidophilus* nanoparticles molecular size using TEM (nanoparticle size 28.3 nm).

The antibacterial activity of Chitosan *L. acidophilus* nanoparticles different dilutions on VRMDR *E. faecalis* strain isolates harboring *VanA* gene, *VanB* and *VanC* genes were assessed as optical density to calculate the MIC and MBC (Table 3).

The minimal inhibitory concentration (MIC) for the bacterial strains harboring the *VanA* or *VanC* genes was 20µg/ml nano chitosan *Lactobacillius acidophilus*, in the strains having *VanB* or the three resistant genes were 10 µg/ml nano chitosan *Lactobacillius acidophilus*. The minimal bactericidal concentration (MBC) for the strain that harboring the *VanA* or *VanC* genes was 30µg/ml, while the strain that harboring *VanB* or the three resistant genes were 20µg/ml.

The effect of Chitosan *L. acidophilus* nanoparticles on VRMDR *E. faecalis* strains harboring the three genes were determined by TEM images to study the changes in morphology *E. faecalis* compared to the control untreated *E. faecalis.* It was found that Chitosan *L. acidophilus* nanoparticles affected by the damaging the cell wall turning it ghost-like as a dead cell (the cell outline remains visible, but its nuclear material and cytoplasmic structures are not stainable formerly) (Figure 6).

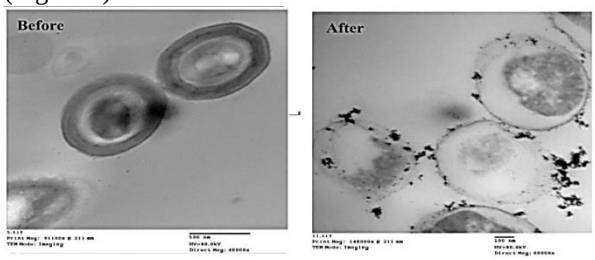

Fig. 6: TEM micrographs of untreated VRMDR *E. faecalis* and after adding Chitosan *L. acidophilus* nanoparticles as treated VRMDR *E. faecalis*.

Cytotoxicity was calculated using the original enzymatic reduction modification of MTT viability assay for Chitosan *L. acidophilus* nanoparticles compared to imipenem as the drug of choice. These results showed that the imipenem drug had a more potent inhibitory activity toward GES1 normal cells than Chitosan *L. acidophilus* nanoparticles.

The half-maximal inhibitory concentration (IC50) was used to measure the cytotoxicity of Chitosan *L. acidophilus* nanoparticles compared to imipenem. The results showed that Chitosan *L. acidophilus* nanoparticles were 550.02±72.9 ug/ml, and the imipenem drug was 301.72±4.05 ug/ml. Consequently, imipenem was more cytotoxic on GES1 normal cells than Chitosan *L. acidophilus* nanoparticles as shown in table 4.

Table 1: The sequences of the specific primers used to amplify regions *VanA, VanB* & *VanC* genes:

Primer	Sequence (5'-3')		Tm °C	Oligo ID	Product size (bp)	Ref.
VanA	Forward	GGGAAAACGACAATTGC	50.0	201109B029E01	732	**(17)**
	Reverse	GTACAATGCGGCCGTTA	50.0	201109B029F01		
VanB	Forward	ACGGAATGGGAAGCCGA	52.0	201109B029G01	647	**(17)**
	Reverse	TGCACCCGATTTCGTTC	52.0	201109B029H01		
VanC	Forward	ATGGATTGGTAYTKGTA	60.0	201109B029A01	822	**(17)**
	Reverse	TAGCGGGAGTGMCYMGTAA	60.0	201109B029B01		

Table 2: Percentage of susceptible (S), intermediate (I), and resistant (R) VAMDR *E. faecalis:*

Antibiotic susceptibility	Penicillin	Carbapenem	Monobactam	Cephalosporins		Macrolide	Lincosamide	Glycopeptide	Aminoglyoside	Nitrofuran
	AM*	IPM*	ATM*	CE*	CRO*	E*	DA*	VA*	S*	F*
% of susceptibility	26.89	89.12	30.00	28.68	35.66	30.00	31.71	00.00	30.90	30.00
% of intermediate	37.45	10.88	35.66	35.66	42.33	35.66	25.96	00.00	35.66	42.33
% of resistance	35.66	00.00	34.34	35.66	22.01	34.34	42.33	100.00	33.44	27.67

***AM (Ampicillin), IPM (Imipenem), ATM (Aztreonam), CE (Cephradine), CRO (Ceftriaxone), E (Erythromcyin), DA (Clindamcyin), S (Streptomcyin), VA (Vancomycin) and F (Nitrofurantoin).**

Table 3: Effect of different concentrations of Chitosan *L. acidophilus* nanoparticles on VRMDR *E. faecalis*:

Concentrations of nanoparticles (µg/ml)	Measuring on a spectrophotometer at 600 nm (optical density)				
	VRMDR *E. faecalis* have *VanA* gene treated with Chitosan *L. acidophilus* nanoparticles (mean value)	VRMDR *E. faecalis* have *VanB* gene treated with Chitosan *L. acidophilus* nanoparticles (mean value)	VRMDR *E. faecalis* have *VanC* gene treated with Chitosan *L. acidophilus* nanoparticles (mean value)	VRMDR *E. faecalis* have three genes treated with Chitosan *L. acidophilus* nanoparticles (mean value)	VRMDR *E. faecalis* have three genes treated with *L. acidophilus* nanoparticles (mean value)
1	0.093	0.090	0.089	0.059	0.078
10	0.077	0.088 (MIC)	0.065	0.057 (MIC)	0.069
20	0.065 (MIC)	0.099 (MBC)	0.045 (MIC)	0.060 (MBC)	0.066 (MIC)
30	0.100 (MBC)	0.110	0.095 (MBC)	0.079	0.082 (MBC)
40	0.116	0.129	0.111	0.085	0.098
50	0.228	0.133	0.224	0.099	0.110
60	0.350	0.131	0.322	0.095	0.104
70	0.400	0.180	0.198	0.105	0.115
80	0.408	0.222	0.420	0.118	0.125
90	0.422	0.352	0.533	0.255	0.298
100	0.430	0.455	0.598	0.302	0.369

Note: the Positive control for the Antibacterial Activity of Chitosan *L. acidophilus* nanoparticles is **0.040nm** and the Negative control for the Antibacterial Activity of Chitosan *L. acidophilus* nanoparticles is **1.660nm**,

Table 4: Results of GES1 normal cells with *Chitosan L. acidophilus* nanoparticles and Imipenem drug:

Sample	Nano *Chitosan L. acidophilus* / GES1					Imipenem / GES1				
Concentrations of dilutions (ug/ml)	**1000**	**250**	**63**	**16**	**4**	**1000**	**250**	**63**	**16**	**4**
Mean value	**0.21833**	**0.27267**	**0.31267**	**0.35867**	**0.4133**	**0.181**	**0.251**	**0.31233**	**0.35867**	**0.4223**
% Percentage	**44.423**	**55.7268**	**64.0485**	**73.6186**	**84.992**	**38.0252**	**52.7311**	**65.6162**	**75.3501**	**88.725**
Cytotoxicity IC50	**550.02±72.9 ug/ml**					**301.72±4.05 ug/ml**				

Discussion:

E. faecalis is opportunistic human pathogen that has frequently developed a resistance to numerous antibacterial substances. For healthy people, *Enterococcus* is usually not harmful, but it can also lead to endocarditis, bacteremia, urinary tract infections, wound infections, intra-abdominal and pelvic infections, and super-infections (23).

In the current study, 58.62% were identified as *E. faecalis*. 60.00% were detected from urine samples, 35.29% were from stool samples and 4.71% were from body fluids samples. Shridhar and Dhanashree conducted a similar investigation found that 150 samples were identified as *E. faecalis* (46.6%) from urine and (29.3%) from pus, followed by (11.3%) of blood and body fluids (24). In another finding, Maj et al., found that *E. faecalis* mainly were found in urine samples (57%), followed by pus (16%), blood (14%), tracheal aspirate (4%), and bile fluid (4%) (25).

Antibiotic resistance in microorganisms may increase due to using broad-spectrum antibiotics to treat infections (26). The population of *Enterococcus* may become resistant as a result of the use of antibiotics. In order to hasten the spread of resistance, horizontal gene transfer is crucial (27).

The current study showed that only 52.94% *E. faecalis* isolates recovered from the collected clinical specimens were confirmed VRMDR *E. faecalis*. VRMDR *E. faecalis* cases were highly resistant to the Glycopeptides group (Vancomycin 100%), Lincosamides group (Clindamcyin 42.33%), Penicillins group (Ampicillin35.66%), Aminoglycosides group (Streptomcyin 33.44%) and Cephalosporins group (Ceftriaxone 33.44% and Cephradine 33.33%). In another finding, Attia et al., found that VRMDR *E. faecalis* revealed 53.5% of the isolates (28). In a study done by Ali et al., sixteen (81.2%) MDR isolates were obtained from all samples. A high rate of resistance to tetracycline and erythromycin (81.2%) was observed in their MDR isolates (29).

The PCR product revealed that 66.66% carried the *VanA* gene, 53.33% carried the *VanB* gene and 46.66% carried the *VanC* gene, while 8.88% carried the three genes.

Regarding the detection rates of the *VanA* and *VanB* phenotypes, many investigations conducted in

various nations produced inconsistent findings. 90.9% of vancomycin resistant *E. faecalis* (VRE) isolates, according to a study by El-Shafei et al., had the *VanA* genotype (30). According to Ira et al., 2% of VRE isolates had the *VanB* genotype, while 87.5% of all VRE isolates had the *VanA* genotype (31). On the other hand, in the study of Surendra et al., all the isolates were positive for *the VanB* resistance genotype, and no *VanA* resistance genotype was detected. Both the *VanA* and *VanC* genes were detected in 2 isolates (32). El Shenawy et al. discovered (66.7%) resistance to the *VanA* gene in resistant isolates in a different investigation. They also discovered (21.05%) resistance to the *VanB* gene in the same isolates and (8.8%) resistance to the *VanC* gene in resistant isolates (33).

The effect of Chitosan *L. acidophilus* nanoparticles on VRMDR *E. faecalis* containing the three genes by using different concentrations was determined. 20µg/ml was the minimal bactericidal concentration (MBC), while 10µg/ml was the minimal inhibitory concentration (MIC). Consequently, as detected by TEM, Chitosan *L. acidophilus* nanoparticles could affect by damaging the cell wall, nuclear material, and cytoplasmic structures.

In the current study that Nano chitosan *L. acidophilus* was chosen to see its effect by TEM on VRMDR *E. faecalis* as it has the higher effect than chitosan only. In another study conducted by Hassan et al., *Enterococcus faecalis* was resistant strain to chitosan nanoparticles (34). In another study by Ebrahimnejad et al., the encapsulation process (Chitosan *L. acidophilus*) increased the viability of bacteria in the gastric acid condition (10).

Cytotoxicity was done to show the viability of cells by a reduction assay of MTT. Results showed that Chitosan *L. acidophilus* nanoparticles had more significant cell viability toward GES1 normal cells than imipenem drug.

Conclusion:

The results of the current study demonstrated the high efficacy of chitosan *L. acidophilus* nanoparticles against VRMDR *E. faecalis* that contain (*VanA*, *VanB*, and *VanC*) resistance genes. The minimal bactericidal concentration (MBC) was 20% and the minimal inhibitory concentration (MIC) was 10%. Imipenem is more cytotoxic to GES1 normal cells than Chitosan *L. acidophilus* nanoparticles (the most effective drug of choice on VRMDR *E. faecalis*).

Competing interests

There are no stated conflicts of interest by the authors .

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