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, reptiles 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- resistant 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 enterococcus (VRE) in animals and the environment, which is likely produced by the co-selection of resistance genes within the typical mobile genetic elements (3). vancomycin resistant can either be inherited or gained by having one of eight different vancomycin resistant 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 variety of vancomycin resistant 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 resistance (5). In high of this, the capacity of enterococcus to develop resistance 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 probiotics (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-Asaafra 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'AGAGTTTGATCCTGCTCAG'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 susceptibility to various antibiotics including penicillins (ampicillin 10g), carbapenems (imipenem 100μg), macrolides (erythromycin 5μg), cephalosporins (ceftaxime 30μg and cephradine 5μg), monobactoms (aztreonam 15μg), aminoglycosides (streptomycin 10μg), limcosamides (clindamycin 150μg), nitrofurans (nitrofurantoin 300μg) and glycopeptides (vancomycin 30μg).

On Muller Hinton agar 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.totalab.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 312 nm (15).

Preparation of Lactobacillus acidophilus nanoparticles, 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 produce 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 lactobacillus 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 Lactobacillus 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 Lactobacillus 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 Lactobacillus 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 Lactobacillus 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 Lactobacillus 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 nm. (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), 10μg/ml of insulin, and 1% penicillin-streptomycin 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-srRNA

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](image1)

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](image2)

![Fig. 3: Agarose gel electrophoresis show the amplified 647 bp DNA fragment VanB gene of VRMDR E. faecalis](image3)

![Fig. 4: Agarose gel electrophoresis show the amplified 822 bp DNA fragment VanC gene of VRMDR E. faecalis](image4)
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).

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 Lactobacillus acidophilus, in the strains harboring VanB or the three resistant genes were 10 µg/ml nano chitosan Lactobacillus acidophilus. The minimal bactericidal concentration (MBC) for the strain that harboring the VanA or VanC genes were 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).

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Tm °C</th>
<th>Oligo ID</th>
<th>Product size (bp)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VanA Forward</td>
<td>GGGAAAACGACAATTCGC</td>
<td>50.0</td>
<td>201109B029E01</td>
<td>732</td>
<td>(17)</td>
</tr>
<tr>
<td>VanA Reverse</td>
<td>GTACATGCACCGCTTTA</td>
<td>50.0</td>
<td>201109B029F01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VanB Forward</td>
<td>ACCGGATGGGAAAGCCGA</td>
<td>52.0</td>
<td>201109B029G01</td>
<td>647</td>
<td>(17)</td>
</tr>
<tr>
<td>VanB Reverse</td>
<td>TGCCACCCGATTCTTC</td>
<td>52.0</td>
<td>201109B029H01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VanC Forward</td>
<td>ATGGATCTGCTAYTGTGA</td>
<td>60.0</td>
<td>201109B029A01</td>
<td>822</td>
<td>(17)</td>
</tr>
<tr>
<td>VanC Reverse</td>
<td>TAGEGAGAGTGYMTGTA</td>
<td>60.0</td>
<td>201109B029B01</td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antibiotic susceptibility</th>
<th>Penicillin</th>
<th>Carbapenem</th>
<th>Monobactam</th>
<th>Cephalosporins</th>
<th>Macrolide</th>
<th>Lincosamide</th>
<th>Glycopeptide</th>
<th>Aminoglycoside</th>
<th>Nitrofurantoin</th>
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</thead>
<tbody>
<tr>
<td>AM*</td>
<td>26.89</td>
<td>89.12</td>
<td>30.00</td>
<td>28.68</td>
<td>35.66</td>
<td>30.00</td>
<td>31.71</td>
<td>0.00</td>
<td>30.90</td>
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<tr>
<td>IPM*</td>
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<td>ATM*</td>
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<td>CE*</td>
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<td>CRO*</td>
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<td>E*</td>
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<td>DA*</td>
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<td>VA*</td>
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<td>S*</td>
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<tr>
<td>F*</td>
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</table>

% of susceptibility 26.89 89.12 30.00 28.68 35.66 30.00 31.71 0.00 30.90 30.00
% of intermediate 37.45 10.88 35.66 35.66 42.33 35.66 25.96 0.00 35.66 42.33
% of resistance 35.66 0.00 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 (Cefttriaxone), E (Erythromycin), DA (Clindamycin), S (Streptomycin), VA (Vancomycin) and F (Nitrofurantoin).

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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 (Clindamycin 42.33%), Penicillins group (Ampicillin 35.66%), Aminoglycosides group (Vancomycin 100%), and Cephalosporins group (Ceftriaxone 33.44%) and Cephaparins 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% of the samples were highly resistant to the *VanA* gene, 53.33% carried the *VanB* gene and 46.66% carried the *VanC* gene, while 8.88% carried the three genes. In another finding, Maj et al., found that VRMDR *E. faecalis* revealed 53.5% of the isolates (28).

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

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

<table>
<thead>
<tr>
<th>Concentrations of nanoparticles (µg/ml)</th>
<th>Measuring on a spectrophotometer at 600 nm (optical density)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRMDR <em>E. faecalis</em> have VanA gene treated with Chitosan L. acidophilus nanoparticles (mean value)</td>
<td>VRMDR <em>E. faecalis</em> have VanB gene treated with Chitosan L. acidophilus nanoparticles (mean value)</td>
</tr>
<tr>
<td>1</td>
<td>0.093</td>
</tr>
<tr>
<td>10</td>
<td>0.077</td>
</tr>
<tr>
<td>20</td>
<td>0.065</td>
</tr>
<tr>
<td>30</td>
<td>0.100</td>
</tr>
<tr>
<td>40</td>
<td>0.090</td>
</tr>
<tr>
<td>50</td>
<td>0.079</td>
</tr>
<tr>
<td>60</td>
<td>0.067</td>
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<tr>
<td>70</td>
<td>0.060</td>
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<tr>
<td>80</td>
<td>0.050</td>
</tr>
<tr>
<td>90</td>
<td>0.040</td>
</tr>
<tr>
<td>100</td>
<td>0.030</td>
</tr>
</tbody>
</table>

**Note**: The Positive control for the Antibacterial Activity of Chitosan L. acidophilus nanoparticles is 0.040mm and the Negative control for the Antibacterial Activity of Chitosan L. acidophilus nanoparticles is 1.600nm.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentrations of dilutions (ug/ml)</th>
<th>Nano Chitosan L. acidophilus / GES1</th>
<th>Imipenem / GES1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value</td>
<td>1000</td>
<td>250</td>
</tr>
<tr>
<td>% Percentage</td>
<td>44.423</td>
<td>55.7268</td>
<td>64.0485</td>
</tr>
<tr>
<td>Cytotoxicity IC50</td>
<td>550.02±72.9 ug/ml</td>
<td>301.72±4.05 ug/ml</td>
<td>301.72±4.05 ug/ml</td>
</tr>
</tbody>
</table>

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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