Enhancement of Gentamicin Sensitivity in *Enterococcus faecalis* using Antidiabetic Molecule Gliclazide

**ABSTRACT**

Enterococci, a low-grade pathogen, emerged as a potent nosocomial agent and have recently drawn the global attention because of resistance issues. To deal with this serious threat and reversal of drug sensitivity pattern, we made an attempt to sensitize the cells of *Enterococcus faecalis* with an oral hypoglycemic molecule gliclazide belonging to the class sulfonylurea. Interestingly, it was observed that results were quite encouraging as it was able to enhance gentamicin sensitivity by reducing the minimum inhibitory concentration (MIC). The decrease in MIC of gentamicin to *E. faecalis* is an indicator of reversibility of drug resistance. The findings have confirmed the concept that prior course or combination therapy of oral hypoglycemic drug with antibiotic gentamicin can be effective against Enterococci strains. However, auxiliary tests still need to be carried out further to understand the exact mechanism of the enhancement procured by gliclazide. The results have sowed the seeds of the concept of using gliclazide as a drug-resistant reversal molecule.

**Keywords:** *Enterococcus faecalis*, Gentamicin resistance, Gliclazide.


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**Conflict of interest:** None

**INTRODUCTION**

Enterococci, traditionally regarded as a low-grade pathogen, have recently drawn the global attention by being increasingly associated with nosocomial infections worldwide. They are generally found to be associated with urinary tract infections, soft tissue infections, bacteremia, endocarditis, neonatal septicemia, and rarely meningitis. In the last decade, Enterococcus had stood out to be the second most common organism responsible for nosocomial infections after *Staphylococcus aureus*. The reason behind the establishment of Enterococci as a prominent cause of hospital-associated infections is the organism’s intrinsic resistance to all currently available cephalosporins and aminoglycosides. Its capability of acquiring and exchanging genes encoding antimicrobial agent resistance also adds on to worsen the situation. Enterococci isolated from clinical specimens were initially sensitive to treatment with aminoglycosides but, over the years they have started demonstrating high levels of resistance to both gentamicin and streptomycin generally mediated by aminoglycoside-modifying enzymes. A common regime for serious Enterococcal infection like septicemia is the combination of cell wall inhibitors such as penicillin with aminoglycoside such as gentamicin. The combination therapy of cell wall active agents and aminoglycoside usually results in the synergic killing of the organism. Agents interfering with the cell wall synthesis increase the uptake of aminoglycoside acting on the proteins involved in electron transport. The facultative anaerobic metabolism of *Enterococcus* imparts them a low-level resistance to all aminoglycosides. However, an increased resistance to gentamicin and streptomycin has started posing a threat of leaving us with no other combination of antimicrobial agents. Thus, it has now become an absolute necessity to deal with the problem of multidrug resistance which has been neglected enough. With an effort to overcome the burning issue of antibiotic resistance, we made an attempt to sensitize the cells of *Enterococcus faecalis* with an oral hypoglycemic molecule gliclazide belonging to the class sulfonylurea. Gliclazide is known to binds to adenosine triphosphate (ATP)-dependent K+ channel on the cell membrane which inhibits a tonic, hyperpolarizing efflux of potassium, it causes the electric potential over the membrane to become more positive and thus depolarizing the cell membrane. The use of gliclazide would enhance the cell membrane permeability of the Enterococcal cells which consequently will have an effect on its sensitivity to the antimicrobial agent gentamicin.

**SHORT COMMUNICATION**

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**Corresponding Author:** Raman P Yadav, Professor and Technical Director, Department of Medical Biotechnology, MGM Institute’s University Department of Biomedical Sciences, MGM Institute’s OMICS Research Center, MGM Central Research Laboratory, MGM Medical College and Hospital, MGM Institute of Health Sciences, Kamothe, Navi Mumbai-410209, Maharashtra, India, Phone: +919987036408, e-mail: raman.yadav@mgmmumbai.ac.in
MATERIALS AND METHODS

Gentamicin was chosen as it is the most widely used aminoglycoside for the treatment of serious Enterococcal infections, normally in combination with cell wall active agents. Enterococcus strain used in the study was isolated from blood culture of a patient with bacteremia hospitalized at a tertiary care center. The isolated strain was identified as E. faecalis with the aid of traditional biochemical methods according to the scheme given by Facklam and Collins.12 The isolate speciated to be E. faecalis was subjected to susceptibility testing by Kirby Bauer’s disk diffusion method to the commonly used antibiotics at our hospital settings.13 Gentamicin was the antibiotic which was chosen to be used further in our study. The minimum inhibitory concentration (MIC) of gentamicin was determined using high-level gentamicin strip (Hi-Media, Mumbai) by Epsilometer test (e-test) method. After the MIC value for gentamicin was obtained, strains were then sensitized using gliclazide (Tokyo Chemical Industry Co., Limited, Tokyo, Japan). The concentration of the gliclazide used was 0.05 mg/10 ml. A total of 10 ml of brain heart infusion (BHI; Hi-Media, Mumbai) broth was inoculated with the E. faecalis strain. To this BHI broth 0.05 mg of gliclazide was added and incubated at 37°C for 4 hours. The turbidity after 4 hours was matched with 0.5 McFarland’s standard solution containing 10^5 colony-forming units/ml organisms. These sensitized (4 hours) cells were then subjected to E-test to determine whether there is any reduction in the MIC value. The same experiments were performed using 0.86% normal physiological saline containing 0.05 mg/ml gliclazide to check whether there is any difference in the sensitization on the cells of E. faecalis in reference to BHI. The controls contained BHI broth and 0.86% normal physiological saline inoculated with E. faecalis without gliclazide.

RESULTS

An attempt to check the effect of the drug on the cell membrane permeability of the bacterial cells and its consequent effect on its sensitivity to the antimicrobial agent gentamicin was carried out. Satisfactory findings have been obtained in this initial attempt. The initial (before sensitization) MIC of gentamicin with E. faecalis was found to be 24 µg/ml of BHI on high-level gentamicin strip (Hi-Media, Mumbai) under Epsilometer test (Fig. 1). After the sensitization of the microbial cells with gliclazide at the concentration of 0.05 mg/10 ml at 37°C for 4 hours, interestingly it was observed that MIC value of gentamicin for E. faecalis was dropped to 12 µg/ml of BHI (Fig. 2) on high-level gentamicin strip under Epsilometer test. Additional incubation of the cells with gliclazide at 37°C in 0.86% normal physiological saline for 4 hours promoted the drop of MIC further up to 8 µg/ml by Epsilometer test (Fig. 3). In sum, orally active antidiabetic molecule gliclazide has displayed as a potent molecule for enhancement of the activity of antibiotic gentamicin during killing of E. faecalis.

DISCUSSION

A major reason behind survival of Enterococci in the hospital settings is its intrinsic resistance to the commonly used antibiotics and its ability to acquire resistance
to several others. To deal with this serious threat and reversal of drug sensitivity pattern, an attempt was made to enhance the drug sensitivity of *E. faecalis* for gentamicin by using an oral hypoglycemic drug gliclazide. The findings have confirmed the concept that prior course or combination therapy of oral hypoglycemic drug with antibiotic gentamicin can be effective against *E. faecalis*. However, auxiliary tests still need to be carried out further (undertaken in our laboratory) to understand the exact mechanism of the enhancement procured by gliclazide. It will be interesting to determine whether gliclazide can enhance the sensitivity of other antibiotics too. It is submitted that on an extensive literature search, such studies could not be found related to the present study. Though Sakharkar et al in their study used antibiotic combinations to enhance antibacterial efficacy and to prevent the development of resistance, natural products have been used for enhancing the sensitivity of a few antibiotics. The *in vitro* activities of antibiotic and physicochemical combinations against *Pseudomonas aeruginosa* were evaluated using the checker board assay and time-kill curve methods. There was synergism between gentamicin and caffeic acid and the MIC of gentamicin was 2 µg/ml. When gentamicin was combined with one-quarter of the MIC of caffeic acid, the MIC of gentamicin was reduced 4-fold. These results indicate the potential efficacy of phytochemicals in combination with antibiotics for enhancing total biological activity.14 Reuk-ngam et al studied the synergistic effect of coronarian D (from the rhizomes of *Hedychium coronarium*) and different antibiotics on different bacteria. They saw best synergistic effect from the combination of coronarian D with gentamicin and coronarian D with oxacillin against *E. faecalis*. A concentration at 0.5 MIC coronarian decreased the MIC of oxacillin and gentamicin in a range 16 to 260-fold.15 Chaves et al found that the combination of ethanol extract of *Nasutitermes corniger* and gentamicin in *Escherichia coli* and erythromycin in *S. aureus* helped in reducing the MIC of the respective antibiotics.16 Recently, Kristen et al reported the isolation of 3,4-dibromopyrrole-2,5-dione from Enterobacteriaceae and *P. aeruginosa*, from the marine bacterium *Pseudalteromonas piscicida* and used for antibiotic activity enhancement.17 3,4-Dibromopyrrole-2,5-dione which represents an inhibitor of Resistance-Nodulation-Division transporters decreased the MICs of two fluoroquinolones, an aminoglycoside, a macrolide, a beta-lactam, tetracycline, and chloramphenicol. Our results, i.e., effect of the oral hypoglycemic molecule gliclazide as prior treatment from gentamicin action on *E. faecalis in vitro*, were quite encouraging. It is able to enhance drug sensitivity probably through modulation of ATP-dependent K+ channel on the cell membrane. The decrease in MIC of gentamicin to *E. faecalis in vitro* is an indicator of reversibility of drug resistance. The results have sowed the seeds of concept of using gliclazide as a drug resistance reversal molecule. Infections in diabetic patients are generally difficult to tackle. But the ability of oral hypoglycemic molecule gliclazide to help decrease MIC of gentamicin to *E. faecalis in vitro* is a unique observation. This concept may greatly benefit diabetic patients with *E. faecalis* infection.

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