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Evaluation of antibacterial activity of vitamin b1 encapsulated chitosan nanoparticle against some human clinical pathogens

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Abstract

In recent years, resistance to commercially available antimicrobial agents by pathogenic bacteria has become a serious problem. Hence, there is a need for environmentally safe drug to use as antimicrobial agent. Nanotechnology is the fast-growing science with commercial applications in many sectors, including therapeutics. This study aims to evaluate vitamin B1 encapsulated chitosan nanoparticle (VCNP) for its antibacterial activity. Antibacterial activity of the VCNP was performed by disk diffusion method against two strains of gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and three strains of gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, and Klebsiella pneumonia*). VCNP exhibited the highest antimicrobials activity against *E. coli* (20 ± 0.63mm), followed by *K. pneumonia* (18 ± 0.42mm), *S. typhimurium* (14.6 ± 0.75mm), *B. subtilis* (14.3 ± 0.55mm), *S. aureus* (13 ± 0.48mm). The present study suggest that VCNPs can be used as potential antibiotic drug.

Keywords: chitosan nanoparticle: encapsulation: antimicrobial activity: disk diffusion assay: inhibition

Introduction

Antibiotics are powerful drugs that have alternatives to health care around the world and saving millions of lives [1]. Bacterial resistance to antibiotics has a severe public health problem worldwide [2]. In the United States, antibiotic-resistant bacteria cause more than 2.8 million cases of severe illnesses and 35,000 deaths [3, 5]. Therefore, alternative control methods are needed for managing the bacterial pathogens.

Nanotechnology has widely used in many fields, such as food protection, textiles, cosmetics, agriculture, and biomedical [6, 10]. The use of nanoparticle is gaining important in the present century as they possess defined chemical, optical, and mechanical properties. Nanoparticles (NPs) have provided an alternative strategy to target bacterial infection, and NP can act as antimicrobial agents due to their ability to interact with microorganisms [11, 12]. Silver nanoparticles (AgNPs) and Cu-chitosan NPs are the most effective nanoparticle against bacteria [13, 14], but other metal NPs such as AuNPs, TiONPs, and CuONPs, have also demonstrated bactericidal effects [15, 16]. Though the metallic nanoparticles are most promising, its possible risk factor restricts its use. Therefore, NP prepared from environmentally safe compound to use as antibiotic gains more attention.

In recent decades, the importance of thiamine (vitamin B1) and riboflavin as a nutrient and act as an antimicrobial drug target for control of fungi, bacteria and virus diseases has been reported [17, 20]. Thiamine is a colourless and water-soluble vitamin [21]. It has the antiberibery factor first derived from rice bran [22]. The active coenzyme form is thiamine pyrophosphate (TPP) [23]. However, the major challenge of using vitamins to control microbe is its high sensitivity and low stability in aqueous media.

Chitosan is a biopolymer and it is made up of β - (1→4) linked D-glucosamine and N-acetyl D- glucosamine units [24]. Chitosan is biocompatible, biodegradable and non-toxic natural polymer having excellent antimicrobial properties against gram-positive and gram-negative bacteria [25, 31]. The development of chitosan based nanoparticles as drugs improved the pharmacokinetics and therapeutic index of antimicrobial drugs [32].

The mechanism of nanoparticles and their antibacterial effects are as follows: direct interaction with the bacterial cell wall (disruption of the bacterial cell membrane), triggering of innate as well as adaptive host immune responses, generation of ROS, etc [11, 33].

There are several reports on antibacterial activity of polymeric nanoparticles, metal nanoparticles, and plant based nanoparticles [34, 36]. In this study, we have used vitamin B1 encapsulated chitosan nanoparticle to evaluate against the growth inhibition of gram-positive and gram-negative bacteria.

Chitosan nanoparticle loaded with vitamin B1 had been prepared and characterized [37]. The aim of the present study was to investigate antibacterial effect of chitosan nanoparticle loaded with thiamine against gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Salmonella typhimurium, and Klebsiella pneumonia*) bacteria to use as novel antibiotic drug.

Materials and Methods

Bacterial strain obtained from Microbial Type Culture Collection, India was used to evaluate the antibacterial activity *in vitro*. Nutrient broth (NB) and nutrient agar (Hi-media, India) were used as growth media. Sterile disk and Ampicillin disk (Hi- media, India) were used to determine inhibition zone.

Preparation of chitosan nanoparticle encapsulated with vitamin B1

The preparation of chitosan nanoparticle loaded with vitamin B1 (VCNP) was done by the method of [37]. VCNP

(1mg/ml) was suspended in sterile dis.H_2O and used to evaluate antibacterial activity.

Determination of antibacterial activity

The synthesized VCNPs were tested for their antimicrobial activity by disk diffusion method against human pathogenic Organisms gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, and Klebsiella pneumonia*). The pure cultures of each test strain was subcultured on nutrient broth and kept at 37 ºC on orbital shaking (200 rpm) incubator (Scigenics Biotech, India) o/N. Each bacteria (100 µl) swabbed on separate nutrient agar (NA) plates using L-rod. Sterile discs of 6 mm diameter were separately impregnated with 100 µl of VCNP. Ampicillin was used as positive control, while sterile dis.H_2O served as negative control. All the plates were incubated at 37 ºC for 24h. The diameter of the inhibition zones were measured in millimeters (mm), and all the experiments were performed in triplicate.

Statistical analysis

Data were analysed by one way ANOVA followed by the Duncan`s Multiple Range Test.

Result and Discussion

VCNPs were investigated to evaluate their antibacterial activity against gram-positive (*B. subtilis, S. aureus*) and gram-negative bacteria (*E. coli, S. typhimurium, and K. pneumonia*) using disk diffusion method (Fig 1). The highest zone of inhibition was observed in *E. coli* (20±0.63mm). And the lowest zone of inhibition was observed in *S. aureus* (13±0.75mm). The inhibition of bacteria by VCNP is in the order of *E. Coli*>, *K. Pneumonia*>, *S. typhimurium*>, *B. subtilis*>, and *S. aureus*.

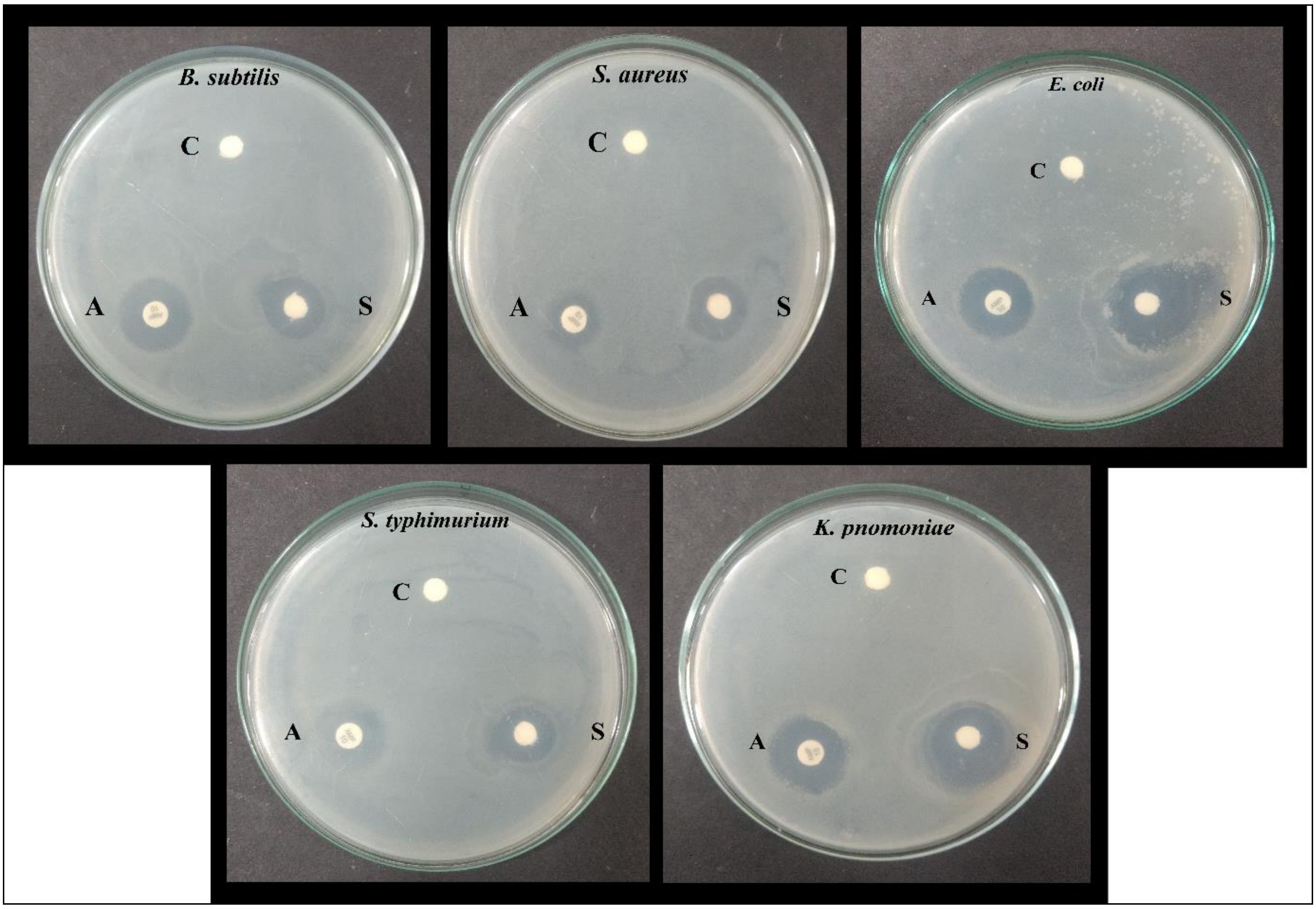

Fig 1: Antibacterial activity of thiamine loaded chitosan nanoparticle against gram-positive and gram-negative bacteria by disk diffusion method **C -** negative control, **A -** positive control, and **S -** VCNP.

Gram-negative bacteria are more susceptible when compared to the Gram-positive bacteria (Fig 2). This may be due to the surface charge of the VCNP with very high positive charge. Due to this, the VCNP may be easily attached to the cell surface of gram-negative bacteria and thereby degrade the cell wall causing disruption of the structure and inactivate the bacteria through lysis. The gram-positive bacteria having thick layers of peptidoglycan when compared to the gram-negative bacteria and hence least activity was observed [38, 39]. All these results indicates that the VCNP can be used as antibiotic drug to control gram-negative bacteria. To our knowledge, this is the first report on antibacterial activity of VCNP.

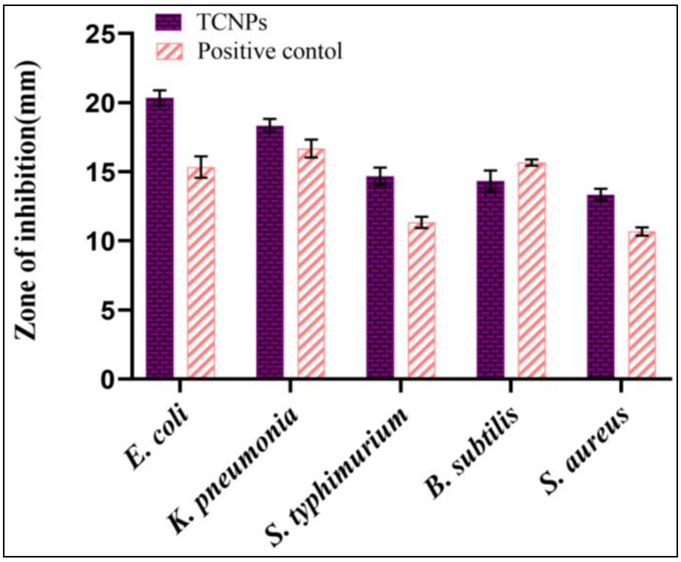

Fig 2: Zone of inhibition (mm) of vitamin encapsulated chitosan nanoparticle and positive control (ampicillin) against various human pathogenic bacteria.

Conclusion

In conclusion, the present investigation reveals that the VCNP inhibits the growth of all the tested pathogenic gram-positive and gram-negative bacteria, which indicates that the chitosan loaded with vitamin B1 may contain a broad range of antibacterial activity. This biopolymer based nanoparticle can be used as biocompatible, effective therapeutic drug against bacterial infections.

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