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Chitosan-Cefixime as Personalized Antibacterial Agent Against E. coli O157:H7

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ABSTRACT

Antibiotics are widely accessible. Nevertheless, food-borne bacteria exhibit a vast array of resistance. Utilizing natural ingredients like chitosan and chitosan-cefixime nanoparticles, which have potent antibacterial qualities, in conjunction with innovative technologies like chitosan loaded with antibiotics, the present research seeks to combat germs that are resistant to many drugs. Five strains of Escherichia coli O157:H7 were utilized to determine antibiotic resistance. The antibacterial properties of free cefixime and chitosan-cefixime nanoparticles were evaluated against strains of harmful bacteria. The findings demonstrated that E. coli O157:H7 comparatively had significant resistance to many antibiotics.

On the other hand, c chitosan-cefixime nanoparticles showed strong antibacterial activity against E. coli O157:H7, but free cefixime did not demonstrate any inhibitory zone. When compared to strains 1, 2, 3, 4, and 5 of E. coli O157:H7, the inhibition zones of chitosan-cefixime nanoparticles were 23.3 mm, 19.8 mm, 16.9 mm, 18.2 mm, and 22.4 mm, respectively. According to the results, chitosan-cefixime nanoparticles have better antibacterial action against dangerous pathogens than free cefixime. Therefore, using chitosan-cefixime nanoparticles for food preservation could be suggested.

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INTRODUCTION

The food industry strives to generate safe and high-quality food items because food safety is an important issue for many nations these days (1). Bacillus, Campylobacter, Clostridium, Escherichia, Shigella, Staphylococcus, and Yersinia are the species of bacteria that cause potential health dangers (2, 3). Staphylococcus and Listeria monocytogenes cause fatal diseases like meningoencephalitis and listeriosis, as well as serious illnesses like bacteremia and listeriosis (4). E. Coli is one of the 12 bacterial genera that the World Health Organization has identified as the most dangerous to human health. Interestingly, E. Coli's resistance to antibiotics has been steadily increasing (5).

Bacteria are becoming increasingly resistant to drugs. Using antibiotics excessively or incorrectly will worsen this resistance. The usage of some natural compounds that are generally accepted as safe (GRAS) has increased due to consumers' inclination to avoid meals that contain chemicals that may negatively impact their health (6).

Antibiotics have been utilized for many years in the medical and cosmetic industries, as well as antimicrobial agents, fungicides, and antiparasitic compounds (7). Cefixime is prescribed for the treatment of bacterial infections of the ears, throat, tonsils, and urinary system, as well as bronchitis (infection of the respiratory tubes going to the lungs) and gonorrhea (a sexually transmitted illness). Cefixime belongs to

a group of drugs known as cephalosporin antibiotics (8). When Gram-negative bacteria become resistant to aminopenicillins, cefaclor, and sulfonamides, cefixime, a third-generation cephalosporin, may be utilized as a second line of therapy (8, 9). More than 90% of E. Coli strains were shown to be sensitive to cefixime (9). In the last decade, resistance to this antibiotic has been reported in E. coli strains. One of the solutions to deal with antibiotic resistance is the use of polymer nanoparticles such as chitosan (10).

Because of its great biological properties and natural source, chitosan is a suitable biomaterial for the preservation of food (11). A naturally occurring bioactive substance, nano-chitosan inhibits harmful bacteria, including Listeria monocytogenes and Staphylococcus aureus (12). In addition to being used as carriers of antibiotics, edible coatings and films derived from chitosan offer high promise for use in food product preservation. Combining antibiotics with chitosan films improves their antimicrobial efficiency and their ability to combat food-borne bacteria and postharvest fungus in food items when compared to pure films and coatings (13).

This in vitro investigation's purpose was to assess the antibacterial efficacy of a cefixime solution containing chitosan and cefixime against E. coli O157:H7, which is resistant to many drugs.

MATERIALS AND METHODS

Pathogenic bacterial strains

The ZistYar Sanaat Microbiological Resource Center (ZYS CO., Iran) provided us with four pathogenic strains of E. coli O157:H7. For subsequent tests, the test bacteria were maintained at 4 °C after being cultivated on Mueller Hinton agar (MHA) and subsequently in tryptic soy broth (TSB) at 37 °C for 24 hours. A 100 ml flask filled with 50 ml of tryptic soy broth was contaminated with a loopful of each evaluated pathogenic bacterium (10^6 CFU/ml), as measured by the plate count experiment. The flask was then incubated for 24 hours at 37 °C and 150 rpm in a shaker incubator.

Antibiotics

Table 1 lists five popular antibiotics utilized in medical treatment that belong to various categories (Oxoid, UK). Sterilized Petri plates with MHA were streaked with one milliliter of each bacterial inoculum (10^6 CFU/ml). After placing the 20 antibiotic disks (Table 1) in the middle of the infected plates, they were incubated for 24 hours at 37 °C. The Clinical Laboratory Standard Committee (14) classified the examined bacteria's response findings to various antibiotics as sensitive, intermediate, and resistant.

Preparation of chitosan-cefixime nanoparticle

One milliliter of pure water, one milliliter of hydrogen chloride (pH=4.5), and one gram of chitosan powder (Sigma-Aldrich, USA) were combined and stirred for an hour at 800 rpm utilizing a magnetic stirrer. After that, it was centrifuged for five minutes at 1000 rpm. One milliliter of ethanol was used to dissolve 50 mg of myristic acid and 100 mg of N-hydroxysuccinimide (NHS). The resultant solution was then applied drop-wise to chitosan for 24 hours. After that, chitosan nanogels (pH=8.5) were precipitated by raising the pH of the process with diluted sodium hydroxide (0.1 M). After dissolving the chitosan nanogel with diluted hydrochloric acid, the liquid was sonicated for half an hour utilizing an ultrasonic homogenizer. Nine milliliters of PBS were mixed with one gram of cefixime powder. After adding 10 milliliters of

Table 1. E. Coli O157:H7›s susceptibility to several antibiotics.

Antibiotics	E. Coli O157:H7				
	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Ampicillin	15.5mm/ I	17.8mm/S	17.3mm/S	9.8mm/R	6.2mm/R
Cephalexin	11.4mm/R	13.1 mm/R	12.8mm/R	15.3mm/I	11.2mm/R
Amikacin	9.8mm/R	15.7mm/I	13.9mm/I	7.9mm/R	9.2mm/R
Rifampicin	16.4 mm/I	16.2mm/I	19.3mm/S	18.9mm/W	12.1mm/R
cefixime	9.8mm/R	10.3mm/R	6.4mm/R	8.8mm/R	11.2mm/R

R: Resistant; I: Intermediate; S: Sensitive;

CLSI 2017: Clinical Laboratory Standards Institute 2017

cefixime solution to the chitosan solution, sonication was performed. Cefixime was encapsulated in chitosan by continuing the sonication for fifteen minutes.

Antibacterial activity of chitosan-cefixime nanoparticle using agar well diffusion assay

Sterile MHA was covered with one milliliter of the E. coli O157:H7 inoculum. 100 µl of chitosan-cefixime nanoparticle was added to each well after the 9-mm diameter well was cut out of the agar utilizing a sterilized cork borer. After one hour at room temperature, the plates underwent incubation for twenty-four hours at 37 °C. The positive control was a cefixime disk (30 µg) purchased commercially. Millimeters were used to measure the inhibitory zone.

STATISTICAL ANALYSIS

SPSS software version 16 was used to compute the statistical evaluation for this research, and one-way analysis of variance (ANOVA) was performed on the data. Additionally, Tukey's HSD post-statistical procedure was used to quantify the difference in target gene expression between the control and treatment samples.

RESULTS

Sensitivity of pathogenic bacterial strains to different antibiotics

As Table 1 shows, E. coli O157:H7 strain 3 exhibited more resistance than strains 1, 2, 4, and 5. The studied antibiotic cefixime caused resistance in E. Coli O157:H7 strains 1, 2, 3, 4, and 5 of 9.8mm, 10.3mm, 6.4mm, 8.8mm, and 11.2mm, respectively. Based on the findings, the five E. Coli O157:H7 strains may be categorized as multi-drug-resistant bacteria.

Morphology of the chitosan-cefixime nanoparticle

Chitosan-cefixime nanoparticles had spherical shapes with an average size of 77.31±4.2 nm. Utilizing dynamic light scattering (DLS), the average diameters of the chitosan-cefixime nanoparticles were 162.7 ± 3.13 nm (Table 2). In this investigation, the DLS-measured nanoparticle sizes were larger than the SEM-measured ones.

Antibacterial activity of chitosan-cefixime nanoparticle

Cefixime and chitosan-cefixime nanoparticles significantly reduced the development of the investigated bacterial strains, as seen in Table 3. Nevertheless, distinct inhibitory zones were noted depending on the kind of pathogenic bacteria and the solution employed at a 2% concentration. Cefixime did not exhibit antibacterial activity against the various strains of E. coli O157:H7; the inhibition zones for strains 1, 2, 3, 4, and 5 were 11.5 mm, 9.3 mm, 6.9 mm, 8.8 mm, and 10.2 mm, respectively. The inhibition zones of chitosan-cefixime nanoparticles against strains 1, 2, 3, 4, and 5 of E. coli O157:H7 were 23.3 mm, 19.8 mm, 16.9 mm, 18.2 mm, and 22.4 mm,

Table2. Chitosan-cefixime nanoparticle synthesis and characterization. Mean ± SD, n = 3, is used to represent the data.

Nanomaterials	Polydispersity index (PDI)	Surface charge (mv)	Ty-CsNG size (nm) (SEM)	Hydrodynamic diameter (nm)
Chitosan-cefixime	0.178± 0.012	3.44 ± 1.21	77.31±4.2	162.7 ± 3.13

Table 3. E. Coli O157:H7›s susceptibility to chitosan-cefixime nanoparticle.

Antibiotics	E. Coli O157:H7				
	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
chitosan-cefixime nanoparticle	23.3 mm/S	19.8 mm/S	16.9 mm/S	18.2 mm/S	22.4 mm/S
cefixime	11.5 mm/R	9.3 mm/R	6.9 mm/R	8.8 mm/R	10.2 mm/R

R: Resistant; I: Intermediate; S: Sensitive;

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respectively.

DISCUSSION

One of the most important problems facing public health worldwide today is bacterial multidrug resistance. According to prior research, the strains of E. coli O157:H7 may be categorized as multi-drug-resistant bacteria (15). This might be explained by the lipopolysaccharides found in E. coli O157:H7's cell wall, which serve as a potent barrier against antibiotics and make bacteria resistant to a number of them. Furthermore, by hydrolyzing the b-lactam ring in the antibiotics, members of the Enterobacteriaceae family may create b-lactamases, which can make the bacteria resistant to b-lactam antibiotics (15, 16).

Furthermore, every strain of E. coli O157:H7 exhibited multidrug resistance. These findings are consistent with those of prior research (16). Innate and acquired resistance are the two forms of resistance that E. coli O157:H7 strains exhibit, which may be the cause of their multidrug resistance. A wide range of antibiotics, including the majority of cephalosporins and other b-lactams, are naturally resistant to E. coli O157:H7 (17).

Since cefixime was unable to stop the development of the investigated bacterial strains, new and improved antibacterials are required to overcome this problem (18). On the other hand, the inhibitory zone of the chitosan-cefixime nanoparticle was larger. These findings concur with those previously published (19). The bioactive volatile components of chitosan-cefixime nanoparticles may be responsible for their antibacterial action (20). Furthermore, compared to E. coli O157:H7 Strain 3, E. coli O157:H7 Strain 1 showed greater sensitivity to chitosan-cefixime nanoparticles. Numerous factors, such as the greater resistance of certain strains of Gram-negative bacteria, may account for this.

The chitosan-cefixime nanoparticle has shown encouraging antibacterial efficacy against the pathogenic bacterium E. coli O157:H7. Compared to free cefixime, the chitosan-cefixime nanoparticle displayed a larger inhibitory zone against E. coli O157:H7. These findings are consistent with earlier reports (21). Nano-chitosan demonstrated a larger inhibition zone than chitosan at the same dosage, despite the fact that 2% chitosan demonstrated antibacterial activity against the bacterial strains that were investigated. The characteristics of nano-chitosan might explain this.

CONCLUSION

One of the most important problems facing public health worldwide today is bacterial multidrug resistance. Therefore, to overcome this obstacle, new and improved antibacterials are required. The findings obtained suggest that the antibacterial activity of chitosan-cefixime nanoparticles is superior to that of free cefixime against harmful microorganisms. Consequently, it may be advised to employ chitosan-cefixime nanoparticles for food preservation.

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Author contributions

Conceptualization, Romina Hosseinzadeh; writing and editing: Reyhaneh Sadat Moosavi-Kohnehsari All authors reviewed the manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

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