Design and Manufacture of Nanomedicine for Aflax Pump Inhibitors in Clinical Isolates of Methicillin-Resistant Staphylococcus Aureus (MRSA) Bacteria

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Abstract

The consequences of this bacterium can be severe. Methicillin is one of these drugs, this drug, like other beta-lactams, inhibits Trans peptidases by binding to penicillin-binding protein (PBP). It prevents the formation of peptide glycans in the bacterial cell wall and destroys the bacterial cell wall. Chemical structure modification has made it resistant to the beta-lactamase enzyme produced by some bacteria. It was made in 1959. Today, it has been removed from the list of drugs and better alternatives such as cloxacillin and nafsilin are available. It has a lower spectrum than penicillin but it works well against beta-lactamase-producing organisms such as Staphylococcus aureus. Design and manufacture of nanoparticles for Aflax pump inhibitors in methicillin-resistant Staphylococcus aureus bacteria Preparation of BSA-meticillin nano-drug: This was done using the desolvation method and after ensuring the formation of nanodars using the XRD and FT-IR spectra, Microdiagnostic antibiogram testing was performed on methicillin-resistant Staphylococcus aureus and the phenotypic pump activity of all isolates was performed by agar technique containing Ethidium bromide by vil card method. According to a study, the Nano medicine albumin-methicillin has the highest inhibition of methylcellulose-resistant Staphylococcus aureus Aflax pump.

Keywords: *Albumin nanoparticle, Methicillin, Study of Aflax pump activity, Methicillin-resistant Staphylococcus aureus*

Introduction

Since the discovery of bacteria, humans have always sought to find an effective cure for their infections; Bacteria have also developed effective mechanisms to kill antibiotics. Today, with the emergence of drug resistance among pathogenic bacteria, the treatment of these infectious diseases has faced many problems. In recent decades, Staphylococcus aureus has been the leading cause of nosocomial infection. One of the problems with staphylococcal infections is that they do not respond to methicillin. After the advent of methicillin resistance, resistant antibiotics, including vancomycin, were introduced to treat staphylococcal infections. The first resistant strains were identified in 1960 and by 1980, resistance was rapidly emerging among bacterial strains and now there are endemic resistant strains in various medical centers around the world. Up to 70% of nosocomial staphylococcal infections have been reported.[1]

Ranjbar et al. (2018) conducted a study to determine the pattern of antibiotic resistance in strains of Staphylococcus aureus isolated from clinical samples of Imam Reza Hospital in Kermanshah. The results of this study showed high antibiotic resistance on the hospital side of Staphylococcus aureus. Therefore, in order to prevent an increase in resistance to common antibiotics, over-the-counter prescribing and unnecessary use of

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available antibiotics should be avoided.[2]

Garcia et al. (2019) to investigate the molecular characteristics of Staphylococcus aureus isolated from patients and army hospital staff: Methicillin resistance was investigated. The results showed that using MLRFT method with low power dissipation and high reproducibility, we are able to identify different bacterial strains. 63% of the clusters (24 strains) are double in the clusters which indicate limited infection transmission. In the study of resistance to methicillin in 90% of resistant strains with total isolated strains (55%), it can be concluded that; Methicillin-resistant strains are easily transported.[3]

Henwood et al. (2017) The aim was to investigate the high prevalence of methicillin-resistant Staphylococcus aureus and Vancomycin-resistant Staphylococcus aureus (VRSA) by MIC by E-Test method. The results showed that; Due to the prevalence of methicillin resistance in nosocomial infections, the use of cloxacillin does not appear to be effective in initiating empiric anticonvulsant therapy. Conversely, in infections obtained from the community, the use of cloxacillin in the treatment of lichen is recommended due to its lower resistance. On the other hand, it seems that due to the high cost of the anti-biogram method with E-test, it is possible to trust the results of valid disks in the anti-biogram.[4]

Jamulitrat et al. (2017) the goal was to examine Staphylococcus aureus nose carriers and determine the resistance of its antibiotic pattern among clinical staff at Ali Ibn Abitaleb Hospital in Rafsanjan. The results showed that; According to the impressive statistics of Staphylococcus aureus nasal carriers in hospital staff, especially methicillin-resistant cases, in order to prevent nosocomial infections, oxacillin and penicillin are recommended to identify the carriers of the microbial in all hospital staff and to treat it in the next step.[5]

Curtis (2018) the aim was to evaluate the drug resistance pattern of negative staphylococcus aureus in children. The results showed that; Staphylococcus aureus drug resistance and drug resistance pattern is unpredictable and multiple resistances are common. Vancomycin is the drug of choice for septicemia because only 26% of cases were resistant to it.[6]

Prashanth and Badrinath. (2016) conducted a study of the abundance of methicillin resistance gene (mec-A) in different strains of Staphylococcus aureus with PCR and determining its antibiotic sensitivity. The findings of this PCR study showed that; Of the 70 isolates obtained, 50% of the strains (35 cases) show methicillin resistance genes. However, in the study of the antibiotic resistance pattern by the agar-diffusion disc method, only 31.4% of the strains (22 cases) showed resistance to methicillin / oxacillin.[7]

Unfortunately, increasing the resistance of bacteria to stronger antibiotics threatens to increase the risk of human infectious diseases.[8]

As a result of this study, the aim was to make nanoparticles effective on methylphenyl-resistant Staphylococcus aureus Aflax pump and thus to treat infections caused by antibiotic resistance of this bacterium. (If the Staphylococcus aureus bacterium is linked to methicillin-resistant Staphylococcus aureus infections).

Materials and Methods

Nanoparticle Preparation Method (BSA)

This nanoparticle is made by simple massification.

In the next step, we dissolve the serum albumin cow with two values of 0.1 gr and 0.01 gr in two separate human beings in cc 2 of 10 mM solution of sodium chloride, Then, using 0.1 N normal sodium hydroxide solution, the solution is brought to 9, by adding a drop of sodium hydroxide solution and measuring the pH each time using a PH meter. At this step we add 8 ml of ethanol as an anti-solvent drop by drop to the protein solution, after the nanoparticles are formed; about 2 to 3 drops are stabilized by the addition of glutaraldehyde (a homogeneous double cross-linking agent). The resulting solution is stirred at room temperature for 24 hours by a magnetic stirrer.

Method of measuring MIC by microbroth dilution

The method of determining the minimum inhibitory concentration was performed by microbroth using Mueller Hinton's culture medium and antibiotic powders and McFarland's 0.5 suspensions according

to the International Committee on Clinical Laboratory Standards (NCClS).

MICs were determined based on the recommended CLSI method, and the lowest concentration of antibiotics that inhibited bacterial growth was recorded as MIC.

In determining the MIC using the Microbroth dilution test method used in our studies, the materials used are:

· Antimicrobial agent and preparation of suitable diluents

· Suitable environment for the growth of microorganisms

· Microbial suspension

In this method, 18-24-hour cultures of Staphylococcus aureus bacteria were used to control the effect of single-walled carbon nanotubes attached to Methicillin (as an antimicrobial drug).

A) Antimicrobial drugs

The antibiotic powder used was prepared with potency (µg / mg specification, production date, expiration date and instructions for storage). Table M100-S4 was selected to determine the general sensitivity to ampicillin according to NCCLS standards.

In general, antibiotics have a standard unit of activity that may vary with the actual weight of the powder. Therefore, we must standardize the antimicrobial solution based on the amount of antibiotic powder used.

The following formula was used to determine the amount of powder or solvent volume:

Preparation of methicillin and albumin-methicillin nanoparticles

To prepare the desired dilutions using the mentioned formula, calculate the desired volume for the preparation of dilutions of 1280µg / ml and then the rest of the dilutions are prepared. The potency of ampicillin powder is 1000. Based on this, we weigh 0.01 g, equivalent to 10 mg of antibiotic powder, with a digital scale and increase its volume to 10 ml with phosphate buffer. The above solution has a dilution of 10 mg / ml (Figure 1).

Figure 1. Sterilizing solutions

Now, with a syringe filter, filter 0.45 micrometers 5 ml of each solution under completely sterile conditions to be free of any microorganisms.

Then, from this solution, prepare dilutions with final concentration (1280-160-20-5.5 2g / ml). After that, the next concentrations, starting at 512µg / ml and finally ending at 0625/0µg / ml, are prepared. (All steps are done inside sterile microtubes.)

Results and Discussion

Results of albumin nanoparticle binding to methicillin results

Diffusion disc

This method was performed to assess the

susceptibility of methicillin-resistant Staphylococcus aureus strain, albumin-Methicillin nanoparticles, albumin nanoparticles and 9 plant extracts(Blue and alcoholic extracts of savory, blue and alcoholic dill, blue stevia, thyme blue, Saveh pomegranate juice, forest pomegranate peel and Saveh pomegranate peel).

Result: Growth aura with a diameter of 9 mm around the disc impregnated with alcohol extract of safflower (dilution of 10 mg per ml) and a growth spurt with a diameter of more than 20 mm around the disc impregnated with methicillin, A growth spurt with a diameter of 14 mm was observed around the disk impregnated with the nano-drug methicillin (Figure 2,3).

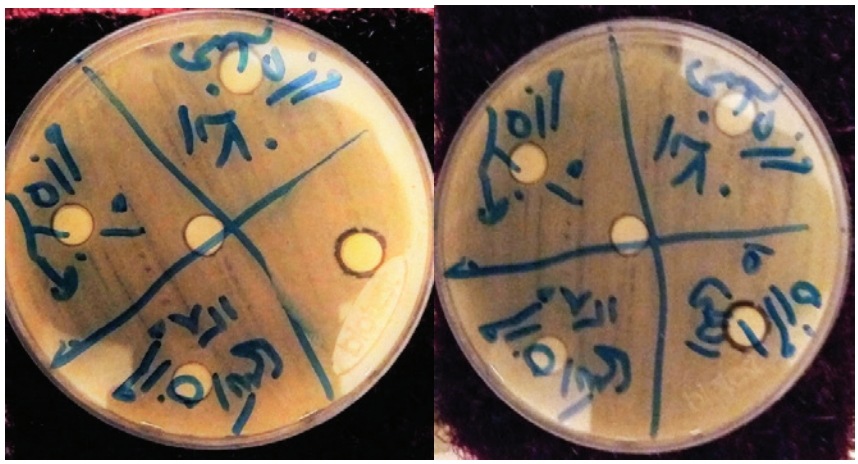

Figure 2. Testing the diffusion disc of savory alcoholic extract

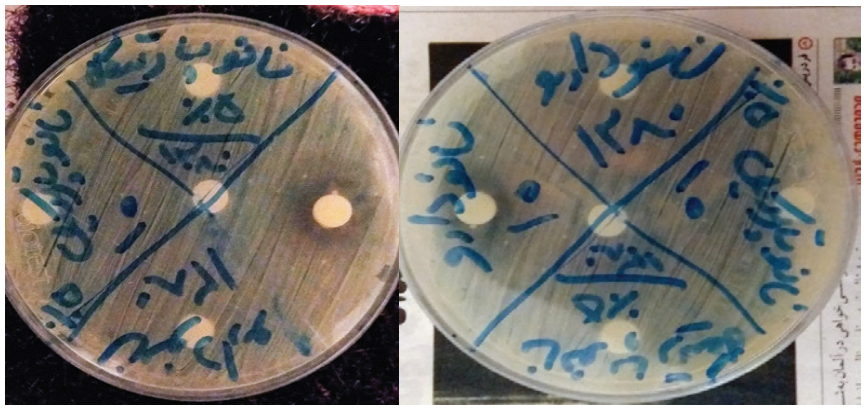

Figure 3. Nanofibrin diffusive disc test

Microbial results

Pipe MIC results

The experiment was performed only for 10 dilutions of pomegranate juice, and after 18-24 hours of incubation at 37, turbidity (bacterial growth) was observed visually.

Result: Turbidity was seen in pipe number 3 and above, and pipes number 2, 1 and 3 were completely transparent. (MIC = 0.25 mg / ml)

MIC results by microbroth dilation method

The MIC test was performed for Methicillin, albumin-Methicillin nanoparticles, albumin nanoparticles and in two wavelength ranges (405-450) and (492-620) and their antibacterial activity was tested in In vitro.

As can be seen in the tables and diagrams related to MIC results, the minimum inhibitory concentration of albumin-Methicillin sample is equal to 512 µg / ml dilution, and in the free Methicillin sample the minimum inhibitory concentration is observed on 16 µg / ml dilution. Nano medicine production efficiency is 0.77%. This indicates that the nano-drug produced has better antimicrobial activity than the methicillin drug. In fact, encapsulating methicillin on nanoparticles has

increased the antibacterial effect of methicillin-resistant Staphylococcus aureus.

Phonetic examination without antibacterial agent

In low concentrations of Ethidium bromide, the bacterial Aflax pump was active, thus removing Ethidium bromide and no red color was seen on the sides. Gradually, with the increase in the amount of Ethidium bromide, the Aflax pump was unable to remove this amount of Ethidium bromide from the bacterium. As a result, red was seen on the sides.

Bacterial strains are divided into three categories based on fossil fluorescence:

1. No active Aflax system potential: Types of fluorescence emission at an error of 0-1 mg / l of Ethidium bromide. None of the study subjects fell into this category.

2. Aflax system with medium activity potential: Types of fluorescence emission at an error of 1-2 mg / l of Ethidium bromide. Numbers 3 or, in other words, 20% of the subjects studied were in this category.

3. With the potential of active Aflax system: The strains that show fluorescence at a maximum concentration of Ethidium bromide 2-2.5 mg / l. Numbers 2 and Numbers 1, 4, and 5, or 80% of the subjects studied, fall into this category (Figure 4).

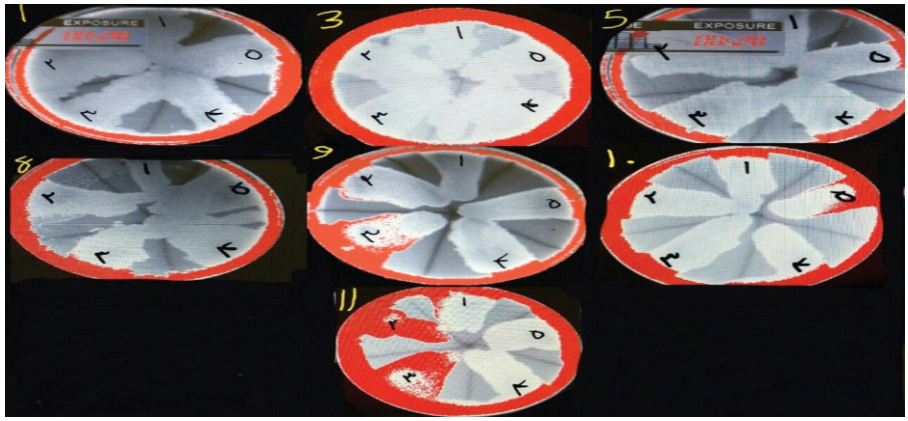

Figure 4. Images of Staphylococcus aureus Aflax pump activity

Phonetic examination with antimicrobial agent

1- Methicillin (pictured) (Figure 5): Inhibition of the 20% side effects of Aflax pump at 2 mg / l Ethidium bromide showed.

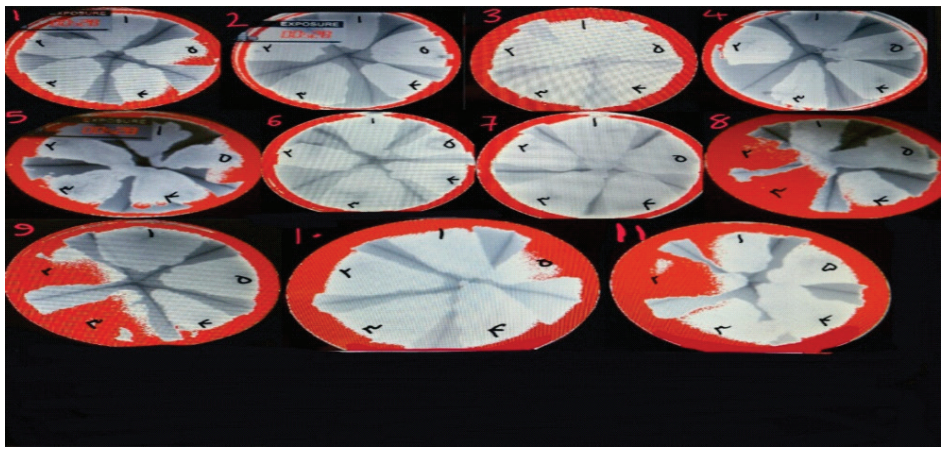

Figure 5. Pictures of the activity of Oreos Staphylaxis Aflax pump + methicillin

2- Albumin nanoparticles (0.5 g) (Figure 6): (pictured): Inhibition of 20% of the falafel pump showed side effects at 2 mg / l Ethidium bromide.

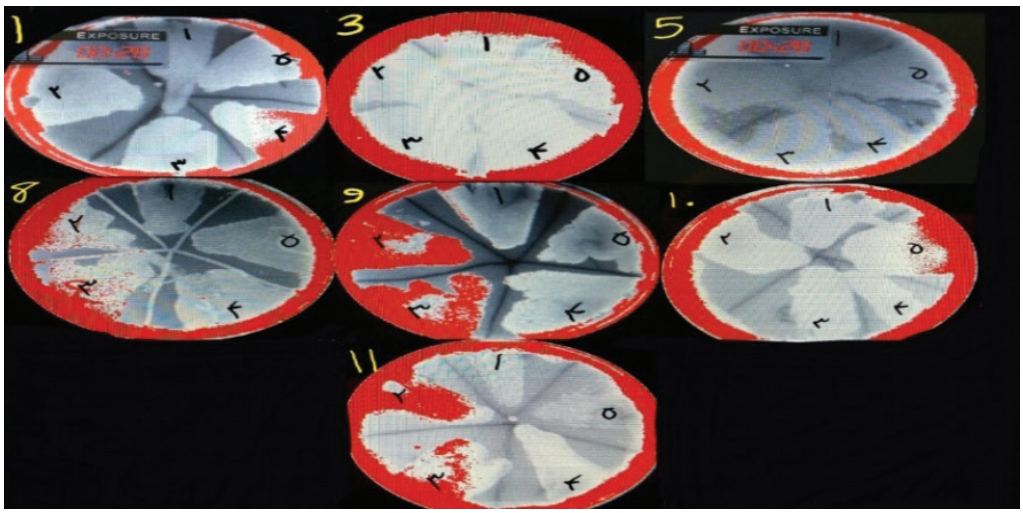

Figure 6. Images of the activity of Staphylococcus aureus + Nanoparticle albumin pump (0.5g)

In all images of the gel dock device, the numbers 6-1 on the number plate of the strains and the numbers on the left of the atomic concentration of bromide indicate each plate according to the table below.

Table1. Ethidium bromide concentrations of TSA culture medium

Number	1	2	3	4	5	6	7	8	9	10	11
Ethidium bromide concentration (mg / l)	0	0.25	0.5	0.75	1	1.25	15	1,75	2	2.25	2.5

Conclusion

Albumin nanoparticles (0.5 g) had 125g / ml = 125 / MIC = according to the Micro Broth Dilution method, it had a inhibitory effect on Methicillin -resistant Staphylococcus aureus bacteria and inhibition of the 20% side effects of Aflax pump at a concentration of 2 l / mg of Ethidium bromide.

Lagamayo. (2015) examined the microbial resistance, phenotypic and genetic evaluation of the norlax Aflax pump at methicillin-resistant Staphylococcus aureus (MRSA) and ciprofloxacin. There is a link between the Staphylococcus aureus strain and the resistance to ciprofloxacin on the Staphylococcus aureus strain. Thus, the development of Aflax pump inhibitors may be useful in controlling ciprofloxacin-resistant strains.[10]

Martins et al. (2013) investigated the antibacterial effect of silver nanoparticles with antibiotic inhibitors that inhibit protein synthesis on staphylococcus aureus isolated from cases of bovine mastitis. The results of this study show that; The resistance of Staphylococcus aureus isolates to erythromycin, gentamicin, streptomycin, and doxycycline was 100, 22, 100, and 8%, respectively. Eight percent of the isolates were sensitive to a concentration of 25 micrograms per milliliter of silver nanoparticles. Growth of 98% of the samples was inhibited at concentrations between 50 and 100 µg / ml.[11]

Simhon et al. (2010) were conducted to evaluate the carriers of Staphylococcus aureus and its antibiotic resistance pattern and to compare the results of the medical staff of Sina Hospital in Hamadan and the administrative staff of Hamadan University of Medical

Sciences. The results showed a higher rate of Carreier staphylococcus aureus in medical personnel than in the comparison group. It was also shown that there was no significant difference between the Carreier rate of Staphylococcus aureus nose with age, sex, activity section and duration of hospitalization. All insulated strains were resistant to penicillin.[12]

Podnos et al. (2011), the antifungal effect of silver nanoparticles in acrylic resins. The results of this study show that the anti-fungal effect increased in acrylic resin containing silver nanoparticles with increasing contact time and concentration of silver nanoparticles.[13]

Wales (2011) examined nanoparticles in the diagnosis and treatment of hepatitis C infection.[14] According to the results, one of the best nanoparticles in the diagnosis of this infection was gold nanoparticles. If the right dose of this nanoparticle is used, no toxicity will be applied to the various cells and tissues of the body. In addition, gold nanoparticles, due to their resonant surface pulmonary resonance properties, change the color of the sample solution from red to blue if they bind to the target genome and accumulate. Then, using the colorimetry method, it can be determined whether there is a virus genome in the patient sample. The time required to perform this experiment was very short, in addition, due to the use of small amounts of this nanoparticle in this method, the test will be cost-effective.

Aflax's contribution to the pharmacological resistance of a number of clinical strains is one of the factors that should be considered in the design of new antibiotics or any other active compound. The main challenge is to discover a compound that, as a barrier to Aflax in a non-specific way, targets a wide range of Aflax systems of different bacterial species.

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Ethical Clearance: Obtained from the Institutional Ethics Committee of Islamic Azad University of Iran.

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