Kufa Journal for Veterinary Medical Sciences Vol.5 No.1 (2014) 137-145

Kufa Journal for Veterinary Medical Sciences
www.vet.kufauniv.com

Antibacterial Activity Of Meropenem loaded to Chitosan Matrix

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Abstract:

The antimicrobial activity of Meropenem released from chitosan matrix against gram positive and gram negative bacteria were studied. The inhibition zone diameter were determined After(24,48)hrs of incubation using agar diffusion assay . The results showed that both matrices were very active to deliver the antibiotic .there are significant increasing $p<0.05$ in inhibition zone after 48 hrs compared with 24 hrs of incubation. In 100 and 200mg of chitosan loaded with meropenem Also there is significant $p<0.05$ increasing in the antibiotic delivery in 200mg chitosan matrix. This study suggest to use such matrices in drug delivery system for local bioavailability of compound antibiotic against gram positive and gram negative bacteria at the same time which is very important in the treatment of some bacterial infections.

Key word: meropenem, chitosan.

الفعالية الضد مايكروبية للنكومايسين والجنتا مايسين المتحررة من سبائك الكيتوسان والكيتوسان جلاتين

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الخلاصة:

درست الفعالية الضد ميكروبية المتحررمن سبيكة الكيتوسان والكيتوسان جلاتين ضد البكتريا الموجبة والسالبة لصبغة غرام.استخدمت طريقة الانتشار بالاكار وحدد قطر منطقة التثبيط بعد (24و48) ساعة من الحضن . اظهرت النتائج ان كلا السبيكتين كانت فعالة في تحرر المضادات الحيوية . وظهرت زيادة معنوية في تحررکلا المضادين الحيويين ضمن مستوى احتمال $p < 0.05$ بعد 48 ساعة من الحضن مقارنة ب 24 ساعة من الحضن. ولوحظ وجود زيادة غير معنوية في تحرر المضادين في سبيكة الكيتوسان جلاتين. تقترح هده الدراسة استخدام مثل هده السبائك في انظمة التحرر الدوائي للوفرة الحيويةالموقعية (الموجهة) للمضادات الحيوية المركبة ضد البكتريا السالبة والموجبة لصبغة غرام والتي تكون مهمة في معالجة بعض الاصابات البكتيرية.

Introduction:

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biological properties. Therefore developing a drug delivery system that optimizes the pharmaceutical

action of drug while reducing its toxic side effects in vivo is a challenging risk. One of the approaches is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles.(1)The use of polymer in drug delivery systems is widely applied in pharmaceutical studies .Since the discovery of mucoadhesive polymer the research in the drug delivery of these systems was increased. such polymer can be designed for drug delivery in nose,mouth ,vagina,stomach,intestine and rectum(2). Most of the polymers prepared from water insoluble polymers are involved heat,organic solvent or high shear force that can be harmful to the drug stability. Moreover, some preparation methods such a emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming.(3) In contrast, water soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force. Among water soluble polymers available chitosan is one of the most extensively studied. Furthermore it posses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. Because of the biocompatibility and specifity, of chitosan is widely used in pharmaceutical applications such as drug delivery system(4,5,6,) .

Chitosan is linear polysaccharide polymer of d-glucos-amine [(1- 4)-2-amino-2-deoxy-β-D-glucan].. Several drug delivery systems based on chitosan for other routes of administration are also being investigated. the good muco adhesive properties of chitosan make it a promising candidate for development of intestinal delivery system(7).The biocompatible chitosan was used as potential delivery system for the controlled and localized release of endothelial cell growth factor which is stimulate visualization(8)..The present study deals with Meropenem. it is a β-lactam antibiotic used to treat a wide variety of infections. It is belongs to the subgroup of carbapenem(9,10),structurally it is, 3-[5-(dimethylcarbamoyl) pyrrolidin-2-yl] sulfanyl-6- (1-hydroxyethyl)-4-methyl-7-oxo- 1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid. Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections, including meningitis and pneumonia. The spectrum of action includes many Gram-positive and Gram-negative bacteria (including *Pseudomonas*) and anaerobic bacteria. The overall spectrum is similar to imipenem although meropenem is more active against *Enterobacteriaceae* and less active against Gram-positive bacteria. It is also very resistant to extended-spectrum beta lactamases but may be more susceptible to metallo-beta-lactamases(11). Meropenem is frequently given in the treatment of febrile neutropenia. This condition frequently occurs in patients with hematological malignancies and cancer patients receiving anticancer drugs that cause bone marrow suppression. It is approved for Complicated skin and skin structure infections, Complicated intra-abdominal infections and Bacterial meningitis. There are many studies for develop novel drug localized antibiotic delivery. Drug delivery systems could be designed to deliver drugs locally in the oral cavity, stomach small and large intestine and the rectum, Stomach-specific antibiotic drug delivery, for instance, would be highly beneficial in the treatment of gastrointestinal infection (12). The aim of this study was try to ensure an in vitro long term delivery of Meropenem loaded to chitosan matrix.

Materials and Methods:

1-preparation of merpenem-chitosan matrices

Chitosan solution was prepared by dissolving (2% w/v) of chitosan powder

from(Fluka, switzweland) in 100 ml of 0.1N acetic acid with stirring. Then 250 mg of meropenem antibiotic brought from the local market were added with stirring for 1hr at room temperature. Gluteral aldelyde were then added to the mixture in the ratio 1ml/100ml ascross liking agent(12). 100ml of 0.1 M of sodium hydroxide was added to the mixture . The mixture was filtered and washed in distilled water until pH changed to 7 then dried using air dried technique by leaving the matrix in dried hood (13).

3- Antimicrobial activity (antibacterial testing).

Five species of bacteri *Escherichia.coli, Pseudomonas aerogenosa , ,Staphylococus aureus,Staphylococus epidermidis,Salmonella sp,* were used in this study obtained from the Department of Microbiology,Medicine colloge,kufa university. The bacterial species were identified according to(14),and maintain on nuteriet agar slants and recovered for testing by sub-culturing in nutrient broth for 24hrs .the antimicrobial activity tests were then carried out by agar diffusion assay (15), 100 mg and 200mg discs of chitosan matrix loaded with the antibiotic were impregnated in spreaded agar with test organisms .standard chitosan and standard antibiotic were used as control group . (for each species). Then the plats were incubated at 37C°.Antimicrobial activity was evaluated by measuring the inhibition zones diameter after 24 and 48 hrs of incubation (each assay in this experiment was repeated triple times) the analysis of variance ANOVAusing spss program microsoft company were used for the statistical analysis of the results .

Results and Discussion:

The results in figures(1,2) showed the comparison between the standard antibiotic meropenem and standard chitosan after(24,48hr)of incubation. These figures showed significant $p<0.05$ differences in the antibacterial activity between standard antibiotic and standard chitosan after (24,48hr) of incubation against tested bacteria. the result in the figure (3) showed the comparison in the antibacterial activity between standard meropenem and the matrix of chitosan loaded with This antibiotic after 24hr of incubation this figure showed no significant diferences in the antibacterial activity of both the matrix and standard meropenem against most tested bacteria specially against *E. coli , salmonella* and *pseudomonas* species while there is significant $p<0.05$ increasing in the antibacterial activity in the meropenem antibiotic compared with the matrix loaded with the same antibiotic after 24hr of the incubation. The figure (4) showed the comparison in the antibacterial activity between chitosan matrix and the standard antibiotic after 48hr of incubation.it appears that there are no significant $p<0.05$ in the antibacterial activity between the standard meropenem and this matrix against most tested bacteria specially in the 200 mg of chitosan matrix which is loaded with this antbiotic while there is significant $p<0.05$ increasing in standard meropenem compared with (100 mg)of the matrix against *S aureus* and *S. epidermis* . The figure (5) . showed the comparison between (100mg) and (200mg) of chitosan loaded with meropenem after 24hr of incubation these results showed no significant in the antibacterial activity between both matrices against the tested bacteria. the figure (6) . Showed the comparison between (100mg) and (200mg) of chitosan loaded with meropenem after 48hr of incubation these results showed significant $p<0.05$ in the antibacterial activity in (200mg)matrix compared with (100 mg) of chitosan while there is no significant differences between both matrices in salmonella species. The figures (1,2) indicates that all tested bacteria are highly sensitive against meropenem antibiotic compared with standard chitosan . specially the *E. coli*

bacteria. The result may reflect the activity of the antibiotic against either gram positive or gram negative bacteria Meropenem is bactericidal. It inhibits bacterial wall synthesis like other beta-lactam antibiotics. But In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases. Resistance generally arises due to mutations in penicillin binding proteins, production of metallo-beta-lactamases, or resistance to diffusion across the bacterial outer membrane (9,10). The result in figure (3) may reflect the small amount release of the antibiotic because of the gradual releases of this antibiotic from the matrix. The released may be due to the higher swelling rate of both matrices, which lead to increasing the antibiotic releases because of increasing the distance between the polymer chains (13,15,16).Figures.(3,4 ,5,6,7) appeared a significant increasing $p<0.05$ in inhibition zone diameter for both matrices after 48 hrs of incubation compared with 24hrs.These increasing in the inhibition zones may related to the higher released of antibiotic from these matrices which may form hydrogel compound when absorbed the water from the culture media (17,18,19,20) . also the increasing of inhibition zone after 48 hrs may be due to the continuous delivery of both antibiotics from the matrices(21,22)).Also The release of drug from chitosan based dosage form depends upon the morphology, size, density and extent of cross-linking of the particulate system, physicochemical properties of the drug as well as the polymer characteristics such as either it is hydrophilic or hydrophobic, gel formation ability, swelling capacity, muco-adhesive or bioadhesive properties (7) The release of drug from chitosan particulate systems involves three different mechanisms: erosion, by diffusion and (c) release from the surface of particle. The release of drug mostly follows more than one type of mechanism. In case of release from the surface, adsorbed drug dissolves rapidly and it leads to burst effect when it comes in contact with the release medium. (23). on the other hand the differences in inhibition zone diameter between two matrices may also be due to the percentage of chitosan in the matrix (24) found that the drug release rate was dependent on the molecular weight of chitosan and particle size of the microspheres. The microspheres prepared from high molecular weight chitosan have shown slow release of drug as compared to those prepared from low molecular weight chitosan has lower solubility and formation of the high viscosity gel layer around the drug particles upon contact with the medium (25).Its indicats from figures (5,6) that both matrices were released the antibiotics successfully which may be useful for treatment of some bacterial infection which needs to the presence of gram positive and negative antibiotics simultaneously.

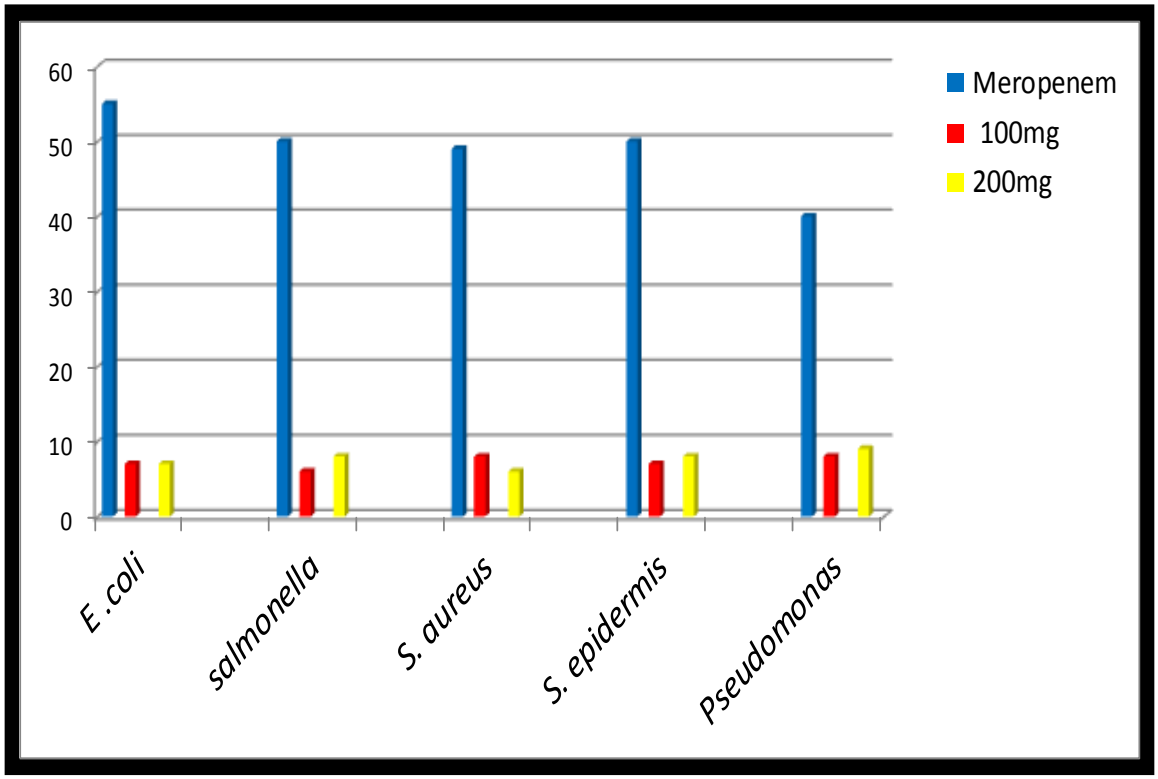


Figure (1) Comparison between standard meropenem and 100mg and 200mg of standard chitosan matrix after 24hrs of incubation

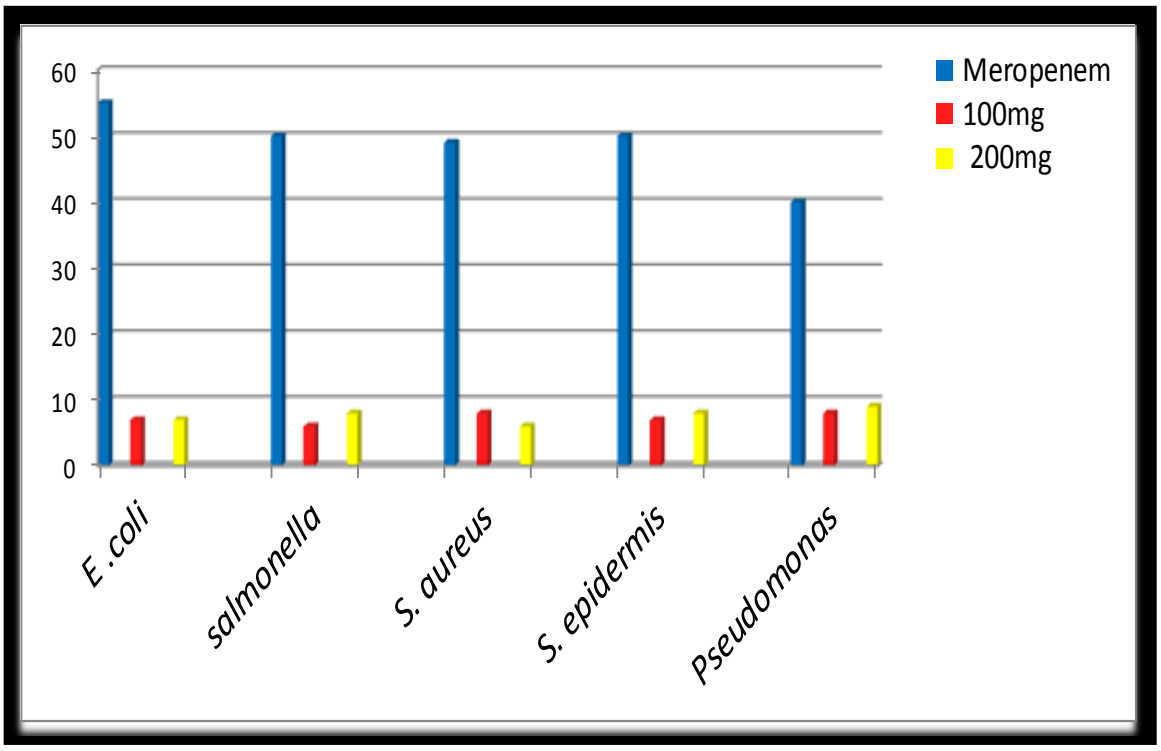


Figure (2) Comparison between standard meropenem and 100mg and 200mg of standard chitosan matrix after 48 hrs of incubation

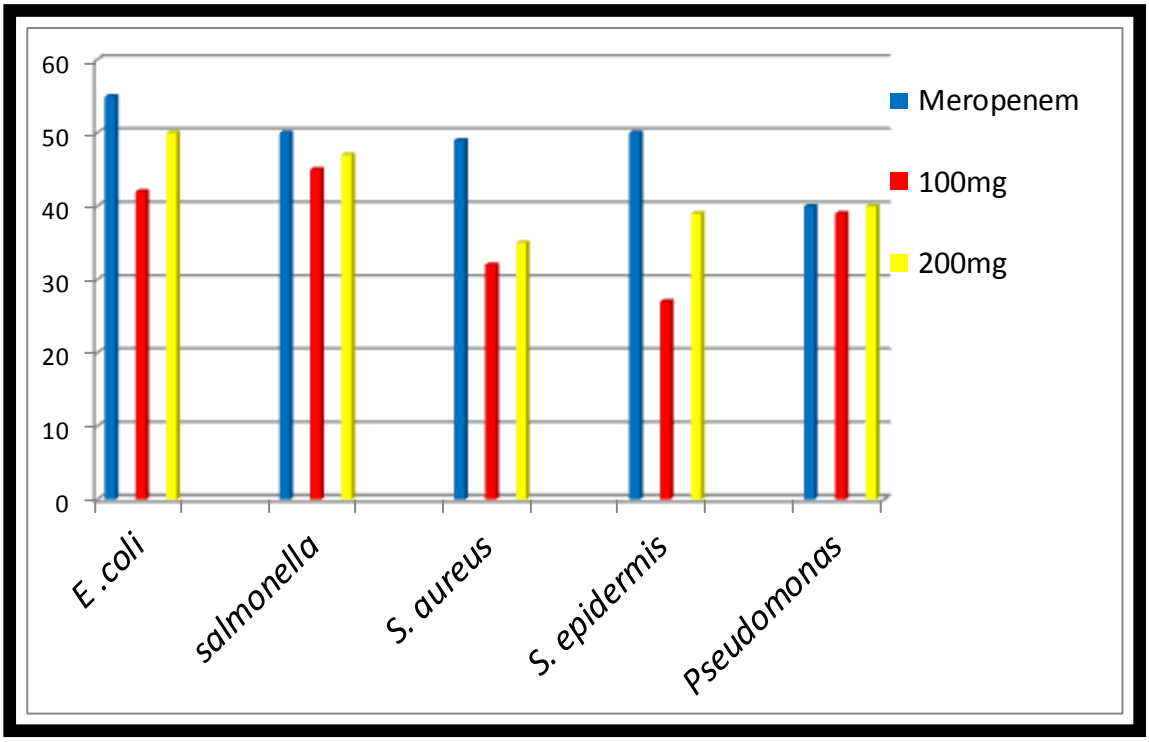


Figure (3) Comparison between standard meropenem and (100,200)mg of chitosan matrix loaded with meropenem after 24hrs of incubation

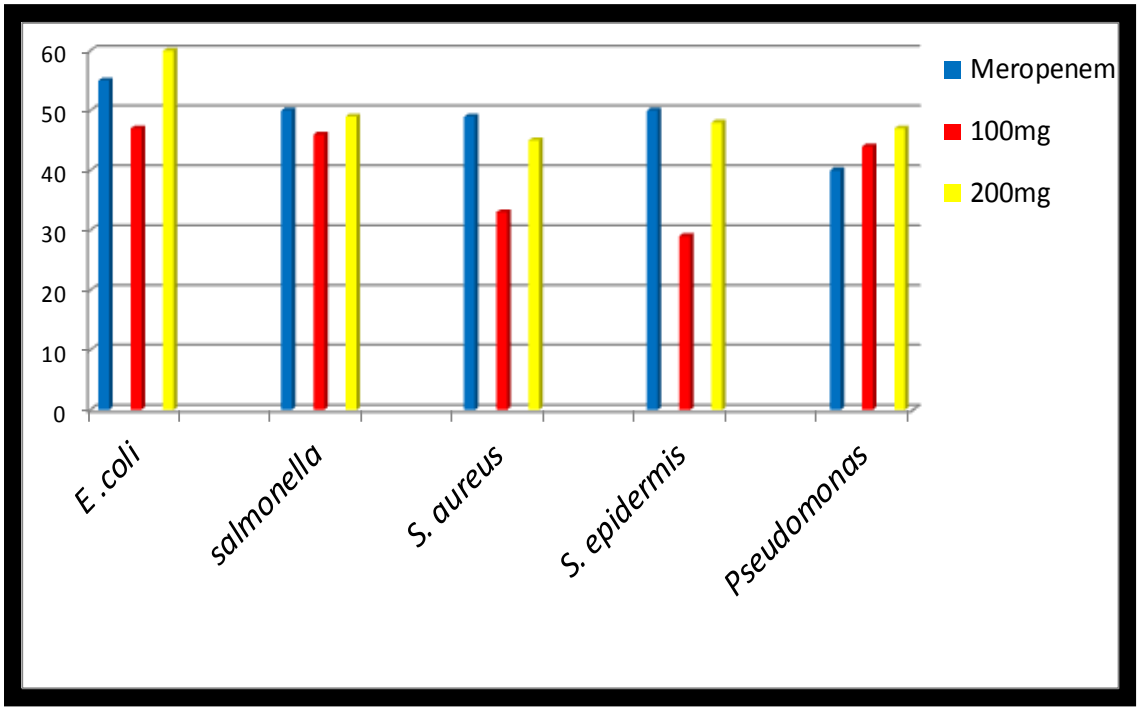


Figure (4) Comparison between standard meropenem and (100 , 200) mg of chitosan matrix loaded with meropenem after 48 hrs of incubation

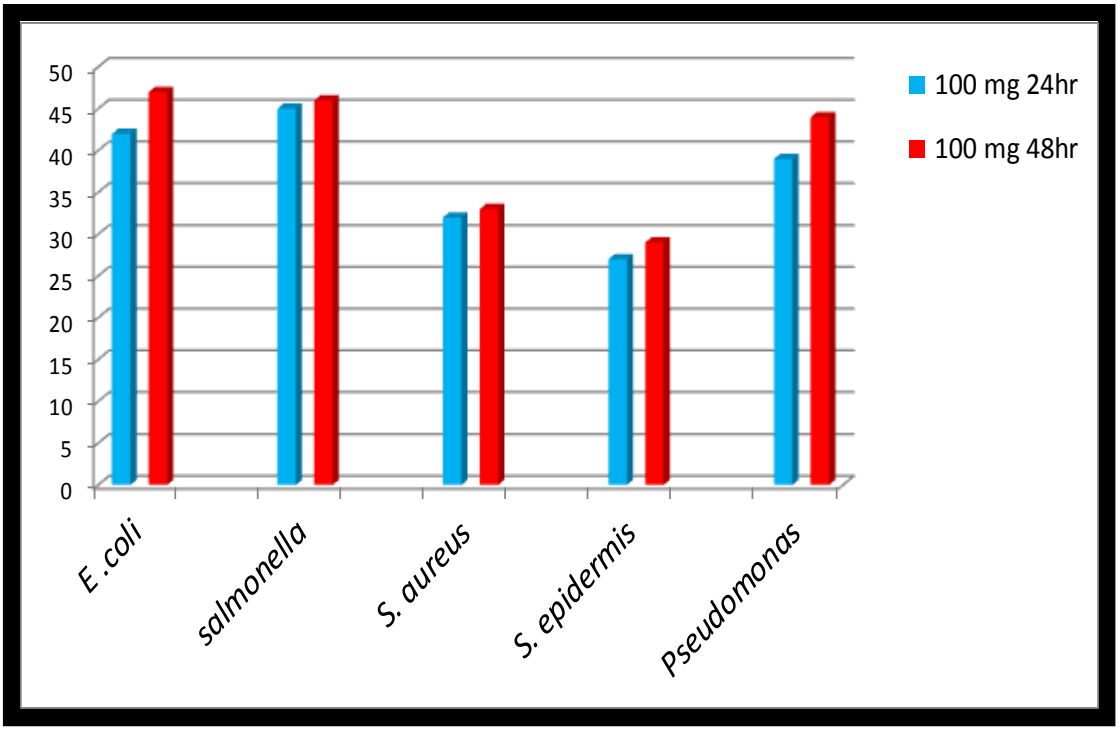


Figure (5) Comparison between (100and 200) mg of chitosan matrix loaded with meropenem after 24hrs of incubation

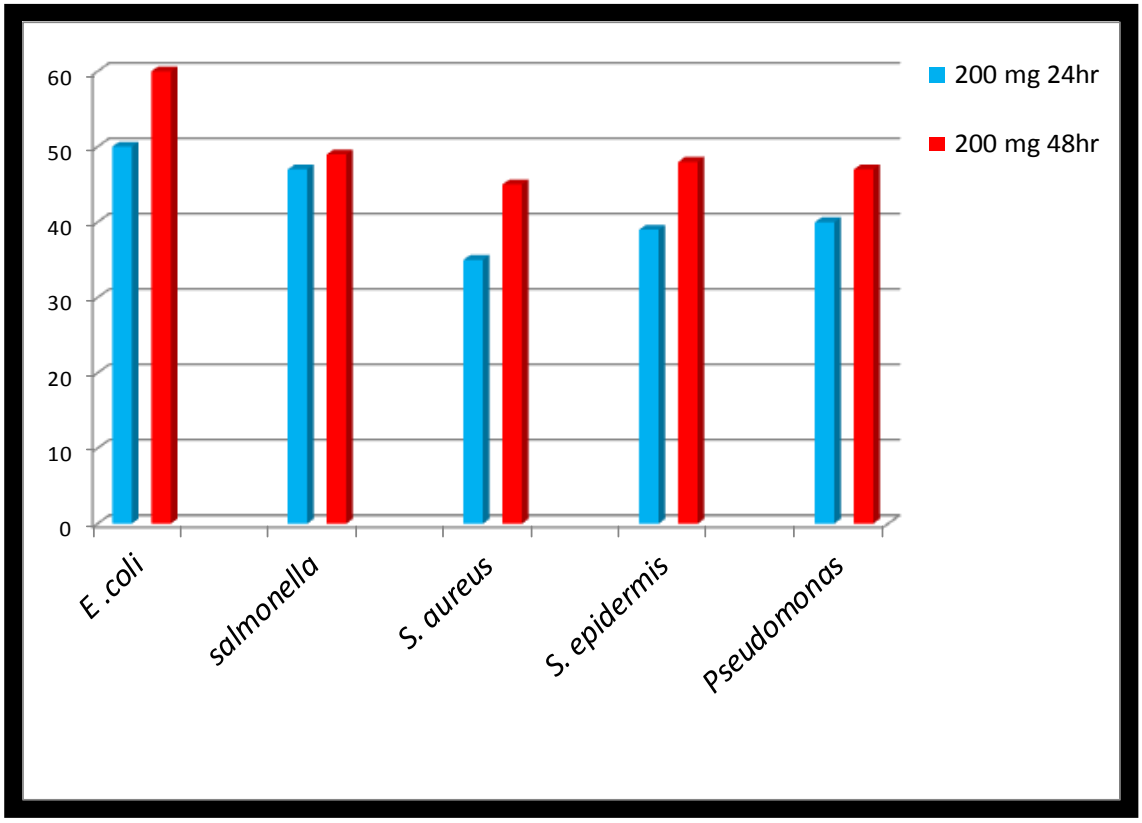
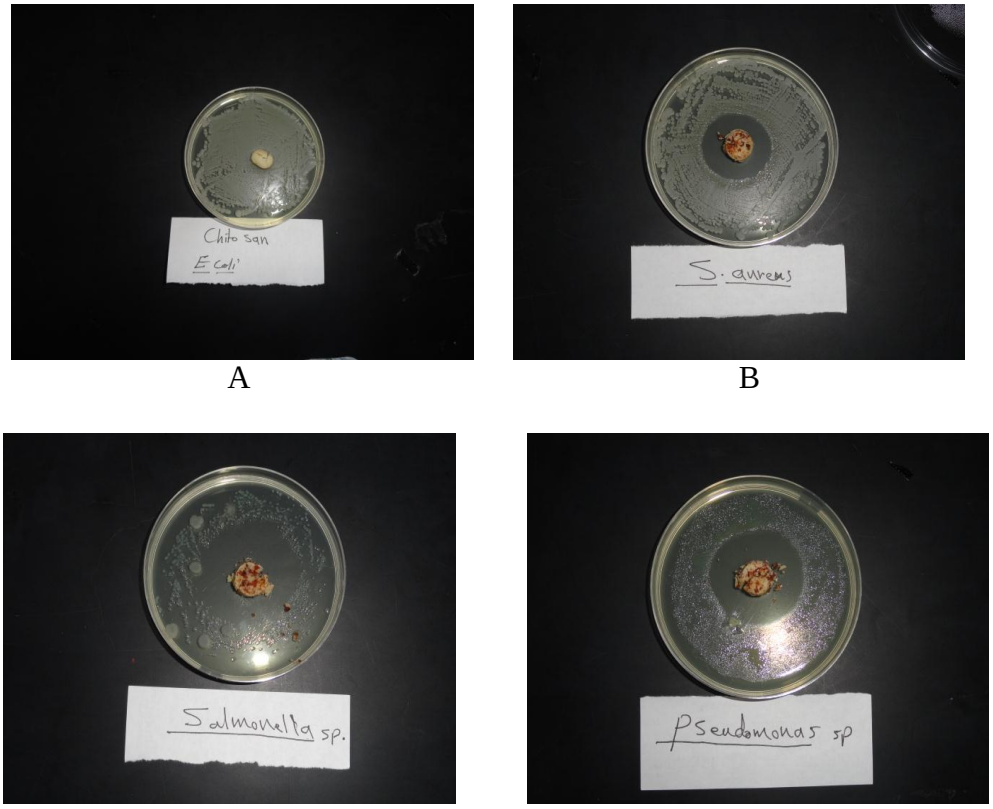


Figure (5) Comparison between (100and 200) mg of chitosan matrix loaded with meropenem after 48hrs of incubation



A B

C D

Figure(7) Antibacterial activity of the standard chitosan and chitosan loaded with meropenem against gram positive and negative bacterial species.

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