Original Research Article

Effectiveness of Chitosan Coating Catheter in Preventing Catheter- Associated Urinary Tract Infection (CAUTI)

Abstract:

Catheter-associated urinary tract infection (CAUTI) is a serious health issue that develops in hospitalized patients. The number of CAUTI cases could be reduced by preventing microbial adhesion and biofilm formation. Chitosan demonstrates antimicrobial activity against a variety of microorganisms. Coating the surfaces of silicon catheters has been shown to minimize or prevent bacterial adhesion and biofilm formation. In this study, we investigated the efficacy of chitosan, in different molecular sizes, as a polymer-coated silicon catheter. Chitosan of a high (HMw), medium (MMw), and low (LMw) molecular weight, at a pH value of 5.0-6.0, used to coat catheters, was examined using two methods, namely the crystal violet and sonication methods. The first method showed that clinical *Klebsiella pneumoniae* had a significantly ($p<0.05$) lower level of bacterial adhesion with MMw chitosan, and *Klebsiella pneumoniae* ATCC 13883 with LMw of chitosan. Using the sonication method, the MMw chitosan showed a decrease in bacterial viability for the *Escherichia coli* clinical isolate, *Klebsiella pneumoniae* reference strain, and *Escherichia coli* reference strain at to a greater extent than the HMw and LMw. Chitosan with three molecular weights (Mw) showed some antibacterial activity when the time-kill assay was applied. However, overall, HMw chitosan grades were not found to be effective as an antibacterial coating agent, although the MMw and LMw presented a potential antiadhesive polymer material to reduce CAUTI.

Keywords: CAUTI, Chitosan, molecular weight, antibacterial, anti-adhesion

1. Introduction

Urinary catheters are common medical devices that have been used to control urine drainage in different clinical situations for several years. The mishandling of the catheter increases the likelihood of hospitalized patients developing catheter-associated urinary tract infection (CAUTI), which is a significant cause of hospital-acquired infections (HAI) and limits long-term usage of these catheters. To reduce the CAUTI risk, two specific practices prove to be effective. One of these is to decrease the risk by reducing the duration of catheterization as soon as possible by terminating the catheter if unnecessary [1, 2]. These approaches include using sterile equipment and aseptic procedures during the insertion of the catheter [3]. Another effective approach is to use a catheter containing antimicrobial agents and/ or antifouling coatings to inhibit the adherence of bacterial cells inside the catheter inner lining [4]. The urinary catheter should preferably be made of biocompatible, antimicrobial, and antifouling materials. The incidence of HAI urinary tract infections may increase when using non-ideal materials in the manufacture of urinary catheters [5].

Biofilm comprises microorganisms bound to each other's surface, with the presence of an extracellular matrix containing the microorganisms' components and/or the organisms' secreted products [6]. Cells within the biofilm could be irreversibly attached to the surface and to each other through the adhesive substance that they secrete [6]. The development of untreated CAUTI due to biofilm formation, as a consequence of long-term catheter use, is likely [7, 8]. The first and most important step in the creation of biofilms is the adherence of bacteria to a surface. Limiting the adhesion of bacterial cells is a fundamental step that could potentially minimize or prevent the development of biofilms on medical equipment, thereby reducing the risk of infections associated with biofilms. Antibiotic therapy can eradicate planktonic bacteria, but once antibiotic therapy ends, the infection may recur [9].

Several materials and compounds that are used with catheter coating can help to eliminate the chance of developing biofilm, and can help to reduce the chance of CAUTI with hospitalized patients [10]. These materials have mainly been investigated in pre-clinical environments, but some have been tested further in a clinical setup. In clinical trials and in vitro studies, the use of antifouling strategies—based on the antiadhesive surface coating approach to prevent the initial attachment of the pathogen cells—was believed to be best suited for use against biofilm development on the catheter material [10].

Chitosan is a polysaccharide composed of glucosamine (2-amino2-deoxy-d-glucose) and N-acetyl glucosamine (2-acetamido-2-deoxy-d-glucose) bonded by β-(1–4). Chitosan has been shown to have many biological properties, and it is commonly used as a widely recognized safe excipient in various fields, e.g., pharmaceuticals. This is the only natural cationic polysaccharide that is protonated at a pH below that of pKa (~6.3) and interacts with negatively charged compounds due to its amino groups [9]. Chitosan is a film-forming polymer that demonstrates a certain level of antimicrobial activity against some microorganisms. When used as a coating on surfaces, it has been shown to effectively minimize or prevent bacterial adhesion and biofilm formation [11]. The interaction that occurs between the catheter matrix and chitosan polymers during the impregnating method could play a role in reducing the adhesion of the bacterial cells and formation of biofilm. The impregnation of chitosan with a different molecular weight to a silicon catheter produces an antifouling effect. This antifouling effect may be due to physical adsorption or anti-adhesive and surface-initiated immobilization, which reduce the attachment of the biological negative charge to the bacterial cell membrane. The antibacterial mechanism of chitosan's effect on planktonic cells was investigated; however, the mechanism of chitosan as an anti-adhesive coating on a catheter to prevent the accumulation of microbial cells and the formation of biofilm, is not yet understood.

This study aims to examine the antibacterial and antiadhesive activity of different molecular weights of chitosan and their effect on preventing the growth and adhesion capability of Gram-negative bacteria on silicon indwelling urinary catheters.

2. Materials and Methods

2.1. Chitosan Solution.

A total of 5g of each of the following products was dissolved in 500 ml of a 1% acetic acid solution, to produce a chitosan solution with a concentration of 1% (w/v): high molecular weight chitosan (HMw), medium molecular weight chitosan (MMw) and low molecular weight chitosan (LMw) . The chitosan solution was placed in a magnetic stirrer and underwent gentle stirring at 50 °C until the solution dissolved, and it was left overnight at room temperature. The pH values (5.0- 6.0) were modified to the human pH, in order to test the antibacterial activity. Then, the solution was sterilized by autoclave at 121 °C for 15 minutes and maintained it at room temperature until use.

2.2. Bacterial Strains.

In this study, two bacterial strains with ESBL; *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E.coli*) that had been isolated from patients with urinary tract infections (UTI), were identified using VITEK® 2 (Biomerieux-diagnostics, France) and the reference strains (*K. pneumoniae* ATCC13883 and *E.coli* ATCC 25922). The bacterial strains were prepared and standardized to be equal 10^8 CFU/ml in the nutrient broth and maintained at 37 °C.

2.3. Artificial Urine Medium.

An artificial urine medium (AUM) formula was performed as described by Campana et al. (2017). Briefly, the solution contained 25.0 g/L of urea, 9.0 g/L of sodium chloride, 3.0 g/L of ammonium chloride, 3.0 g/L of hydrated sodium sulfite, 2.5 g/L of anhydrous disodium hydrogen orthophosphate, 2.5 g/L of potassium dihydrogen ortho- phosphate, and 2.0 g/L of creatinine,

which was placed in 1 liter of distilled water and maintained at pH 6, after which it was sterilized by autoclave at 121°C for 15 minutes.

2.4. Time-kill assay of the bacterial strain with the chitosan

This assay was performed as described by Campana et al. (2017). The assay was performed to identify the antimicrobial activity of HMw, MMw and LMw of chitosan on the isolates (clinical isolates of *K. pneumoniae* and *E.coli* and the standard *K. pneumoniae* ATCC 13883 and *E.coli* ATCC 25922. These isolates were grown overnight on MacConkey agar (pre-prepared by SPML, Dammam), then inoculated into a 10 ml nutrients broth (Oxoid). After incubation for 18h at 37°C, each suspension was centrifuged (4000 rpm; 10 minutes at room temperature) and resuspended in the same medium. Following this, 500 µl of bacterial suspension (10^8 cfu/ml) was added to the well of the microplate with 24 wells (SPL Life Sciences, Gyeonggi, Korea) with 500 µl of chitosan (2 wells of HMw, MMw and LMw) and incubated at 37 °C. One aliquot from each well that contained the bacterial suspension from each isolate and each chitosan (HMw, MMw and LMw) at time 0h, 2, 4, 6 and 12h were serially diluted in saline and plated on Muller-Hinton agar (pre-prepared by SPML, Dammam) and incubated for 24 h. In each plate, colony forming was united and enumerated. This assay was repeated in triplicate across three different weeks.

2.5. Chitosan coating.

Foley catheter (silicon foley, 2-way catheter purchased from Jamjoom Medical Industries, Jeddah, Saudi Arabia) were cut into segments of 1 cm length, rinsed with 70% ethanol then left to dry in sterile microplates with 24 wells. In the two microplates with 24 wells, 15 of the wells were filled with 2 ml of chitosan, HMw, MMw and LMw, after which 1cm catheter segments were immersed and 500 µl of bacterial suspension (10^8 cfu/ml) was added to each of these wells. Three of the wells were used as a negative control, in which chitosan (HMw, MMw and LMw) was added without any bacterial isolate. The positive controls (each tested strains) only included 1cm catheter segments without chitosan in 2 wells of both of the 24-well microplates. These microplates were incubated at 37°C for 24 hrs.

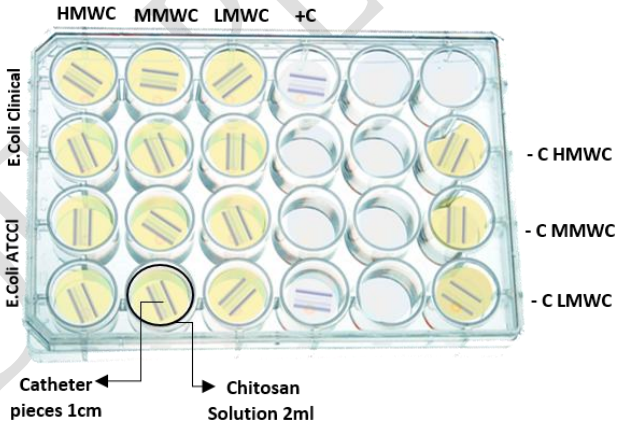


Figure 1: Diagram of the position of the catheter segments inside the 24-well microplates, including the chitosan solutions.

2.6. Sonication Method to Assess Adhesion of Bacterial Cell on Coated Catheter.

After 24 hours of incubation, the catheter segments were washed with phosphate-buffered solution (PBS). Then, they were prepared for sonication by immersing it in 1 ml normal saline. The catheter segments sonicated for 2 minutes at 40 kHz to release the colonized bacteria on the catheter wall. The residual solution from sonication was diluted with normal saline to 1:1000 and then incubated after being cultured for 24 hours at 37 °C. Following this, the colony forming units were counted for each test. This procedure was repeated three times on different days.

2.7. Crystal violet method to assess adhesion of bacterial cells into the chitosan coated catheter

From the prepared inoculum, with 10^8 CFU/ml of nutrient broth, 2 ml of the chitosan was added to a 24-well microplate that included the 1 cm segments of sterilized catheter, as described in Section 2.5. After the incubation of the catheter segments, with each grade of chitosan for 24 h, each bacterial strain-prepared inoculum was added in duplicate and incubated for 18 h to investigate the effect of chitosan in the growth of each bacteria. After incubation, each of the catheter segments were washed with PBS and transferred to fresh plates, after which they were stained with 2ml of crystal violet (Innovating Science, New York, USA) for 15 minutes. After staining with crystal violet, the segments were washed with distilled water, after which 1.5 ml of 90% ethanol was added to solubilize the stain.

The ethanol contained the amount of crystal violet that stained the bacteria inside the catheter segments, which was then transferred into 96 new microplate wells so as to measure the optical density at 570 nm using the HumaReader HS microplate reader (HUMAN, Wiesbaden, Germany). This optical density corresponded to the quantity of bacteria that adhered to the catheter segments and helped to measure the effect of bacterial growth and adhesion on chitosan inhibition. These procedures were repeated three times on different days.

2.8. Fourier Transform Infrared Spectroscopy (FTIR) characterization of the catheter's molecular structure chitosan coating

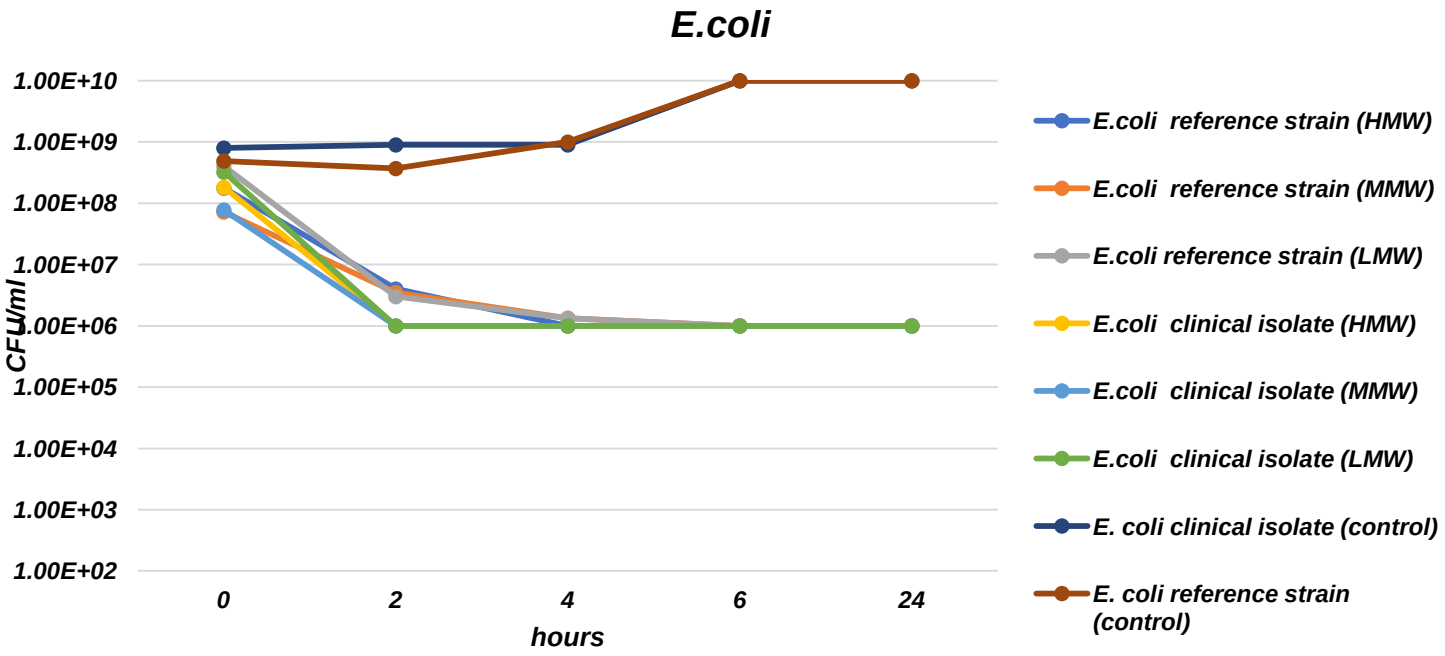
The silicon catheter matrix was assessed using an FTIR analysis of the catheter after chitosan coating. After the coating step, which was described earlier in this paper, the silicon catheter segment and silicon with chitosan (high, medium and low) molecular weight were analyzed using FTIR measurements. The FTIR measurements were carried out on IRAffinity-1S class 1 FTIR spectrophotometer (Shimadzu,) equipped with attenuated total reflectance (ATR) with a diamond crystal plate. The spectra were recorded in the spectral range of 4000-400 cm^{-1} at a 4 cm^{-1} spectral resolution, using LabSolution IR control software (Analytical data system for FTIR, USA) for a 40 sample/background. The chitosan-coated catheter segments (high, medium and low molecular weight) were recorded and compared to the silicon (uncoated) catheter and the chitosan powder.

3. Result

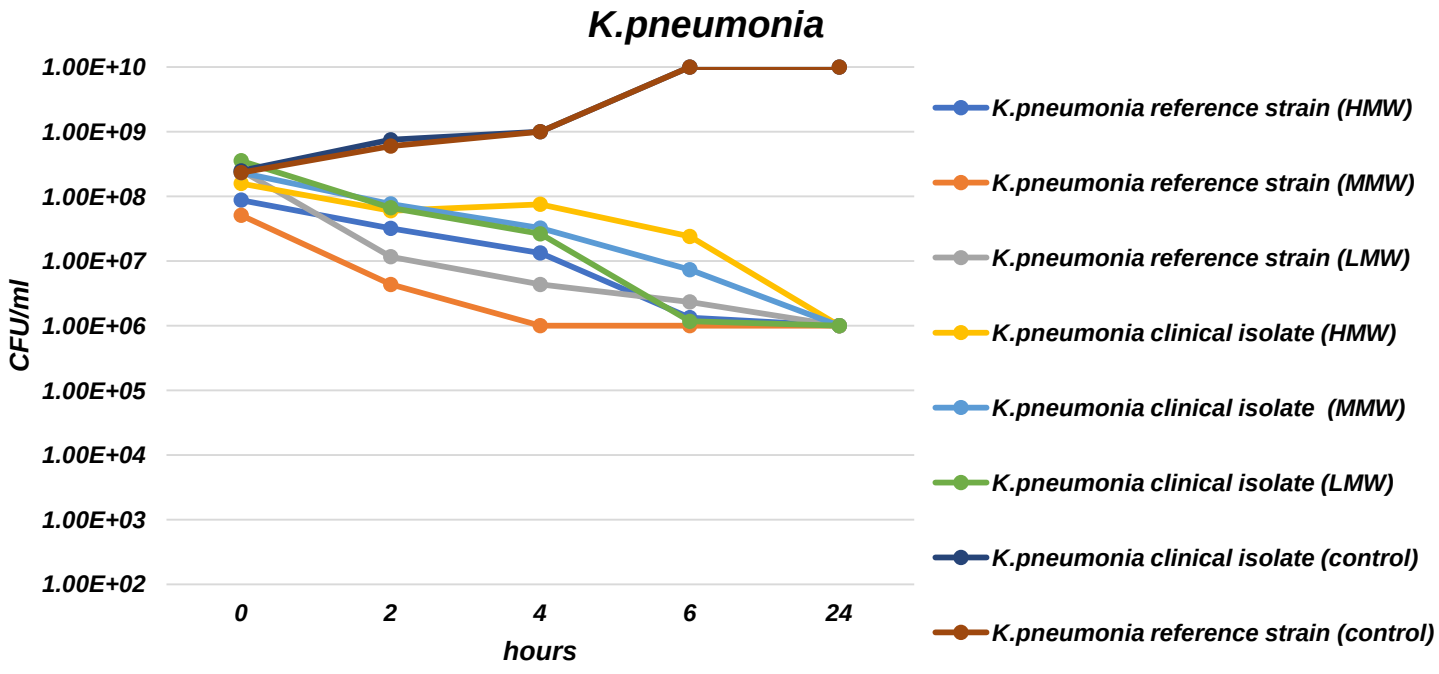
3.1. Time-kill assay of the chitosan on the bacterial isolates

Chitosan of different grades (HMw, MMw and LMw) were incubated with the two tested *E. coli* isolates and the colony-forming units (CFU) per milliliter were measured, which showed a dramatic decrease after two hours of incubation. As presented in Figure 2 a., the *E. coli* clinical isolates decreased dramatically to the base line, which is equal to the starting inoculum (10^6) and showed that no colony formed when incubated with HMw, MMw and LMw chitosan after two hours of incubation. The other isolates (*E. coli* reference strain) decreased to the baseline after four hours of incubation with HMw, MMw and LMw chitosan. A statistically significant decrease in the CFU/ ml values was observed for all tested strains. The control strains showed no statistically significant growth over the incubation time.

K. pneumoniae showed a different growth inhibition, presented as CFU/ml in Figure 2 b., by showing variability of growth with different chitosan grades. Only two inoculums from the *K. pneumoniae* reference strain isolate were less or equal to half of the baseline after two hours when incubated with MMw and LMw chitosan. After four hours, only one inoculum (*K. pneumoniae* reference strain) with the MMw chitosan reached the baseline of the CFU/ml. Colonies were not observed for any of the isolates after 24 hours incubation with the three grades of chitosan. In comparison, the control strains shows no statistical significance and the cfu/ml increased over time.



(a)



(b)

Figure 2: (a) the effect of each chitosan grade over time (0,2,4,6 and 24 h) on the growth of *E.coli*. (b) the effect of each chitosan grades over time (0,2,4,6 and 24 h) on the growth of *K. pneumoniae.*

3.2. Effectiveness of chitosan catheter coating by sonication method

Using the sonication method, the number of bacteria colony-forming units per milliliter (CFU/ml) was calculated for each grade of chitosan and each bacterial isolate used in this method.

The variability of bacterial colonization was illustrated, as the *E. coli* reference strain had a CFU/ml value ($4.59*10^8$ CFU/ml) of lower than $P<0.05$, whereas MMw was used compared to a positive control ($8.00*10^8$ CFU/ml) ($P>0.05$), and the other strains appear to have increased in CFU/ml value. The clinical isolate *K. pneumoniae* showed a significant increase in bacterial adhesion ($8.43*10^9$ CFU/ml) compared to the positive control ($1.71*10^9$ CFU/ml) ($p<0.05$). The *K. pneumoniae* reference strain also showed a significant increase ($6.77*10^9$ CFU/ml) when compared to the positive control ($2.10*10^8$ CFU/ml).

The MMwC showed a greater decrease in bacterial viability for the *E.coli* clinical isolate, *K. pneumoniae* reference strain, and *E.coli* reference strain compared to HMw and LMw (Figure 3). On the other hand, when using LMw, the *K. pneumoniae* clinical isolate showed lower bacterial viability compared to the other molecular weights of chitosan (Figure 3).

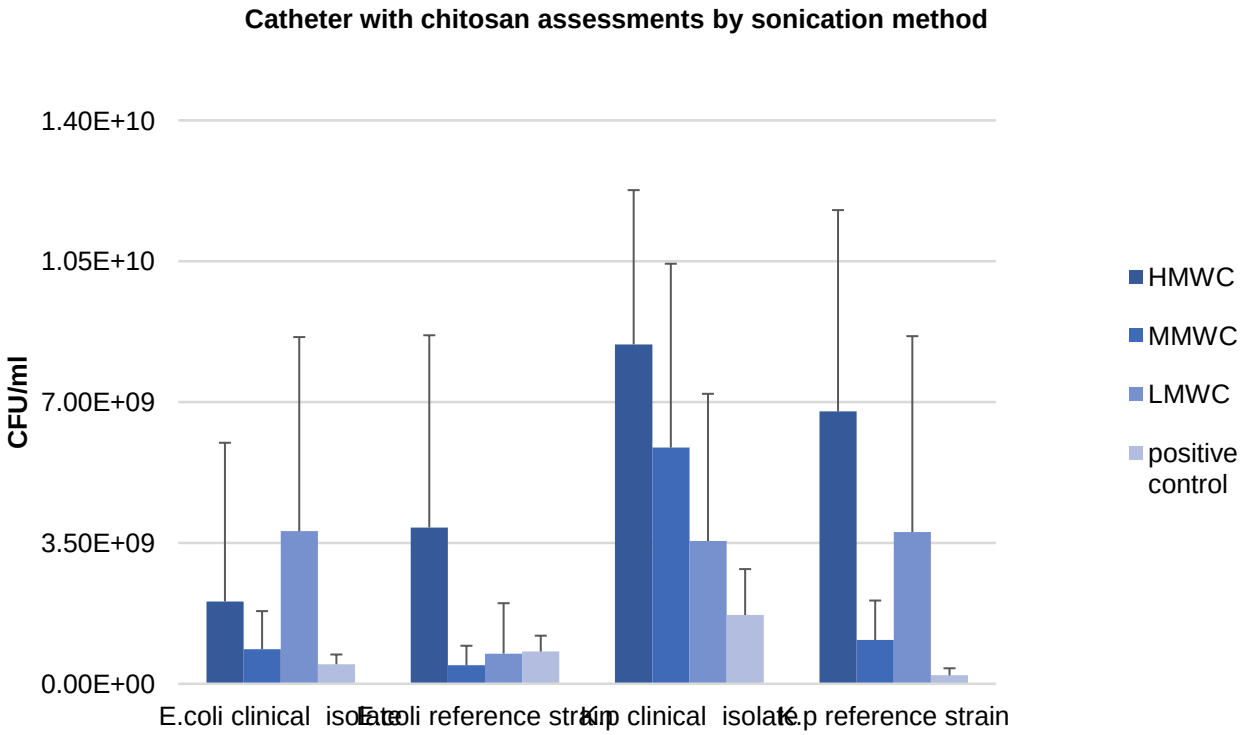


Figure 3: The catheter with chitosan by sonication method. Positive control: catheter segments without chitosan. HMWC: high molecular weight of chitosan. MMWC: medium molecular weight of chitosan. LMWC: low molecular weight of chitosan. *E. coli*: *Escherichia coli*. *K.p*: *Klebsiella pneumoniae*.

3.4. Effectiveness of chitosan catheter coating by crystal violet method

In the crystal violet method (Figure 4), the results show that the *K. pneumoniae* clinical isolate had the highest occurrence of biofilm formation on catheters without chitosan, while the *K. pneumoniae* clinical isolate coated with chitosan shows a better effect in lowering the biofilm formation of bacteria on the catheter (Figure 5). The LMw chitosan may decrease the adhesion of

the bacterial biofilm to the chitosan-coated catheter in the *E.coli* clinical isolate, *E.coli* reference strain, and *K. pneumoniae* reference strain. On the other hand, MMw in the clinical isolate *K. pneumoniae* showed lower bacterial adhesion than the other molecular weights of chitosan. The *K. pneumoniae* had better inhibition compared with *E.coli*., particularly the clinical isolate *K. pneumoniae*, which showed a significantly lower amount of bacterial biofilm when MMw was used, and the same was observed for the *K. pneumoniae* reference strain when LMw was used ($p < 0.05$) . However, the other strains had non-significant levels ($P > 0.05$).

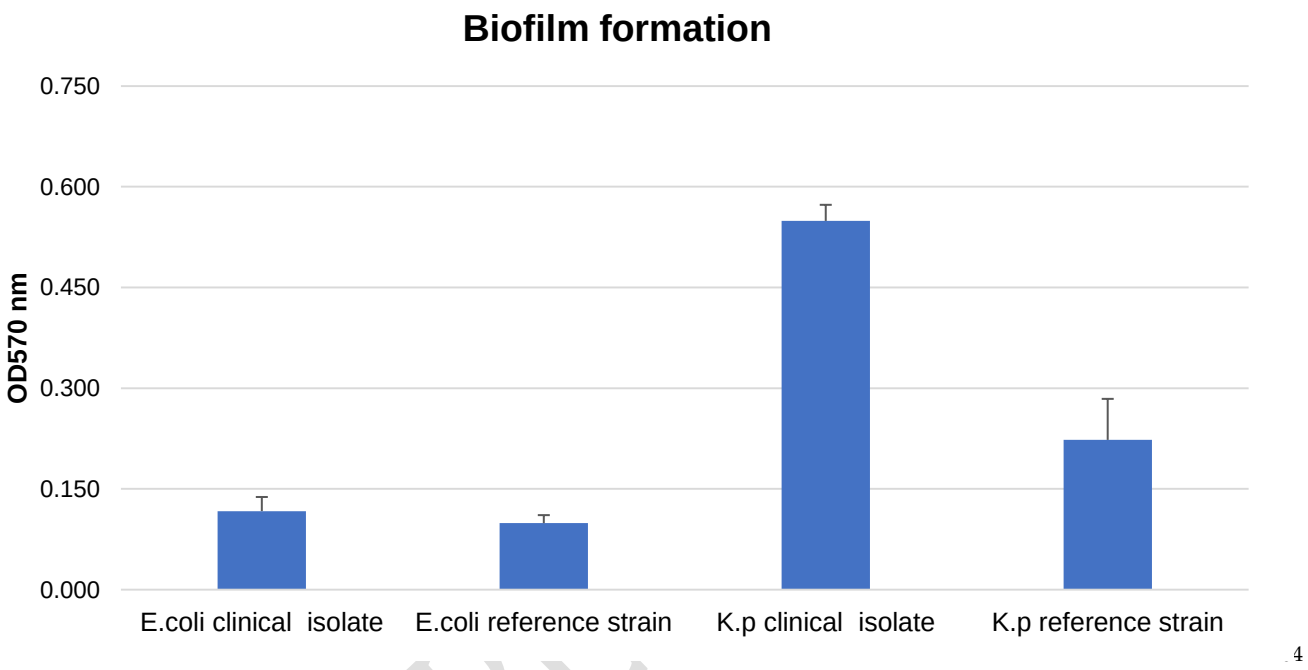


Figure 4: The biofilm ability measurement for each bacterial strain without chitosan and from inside the microplate wells without the catheter. *E. coli; Escherichia coli*. *K.p; Klebsiella pneumoniae*.

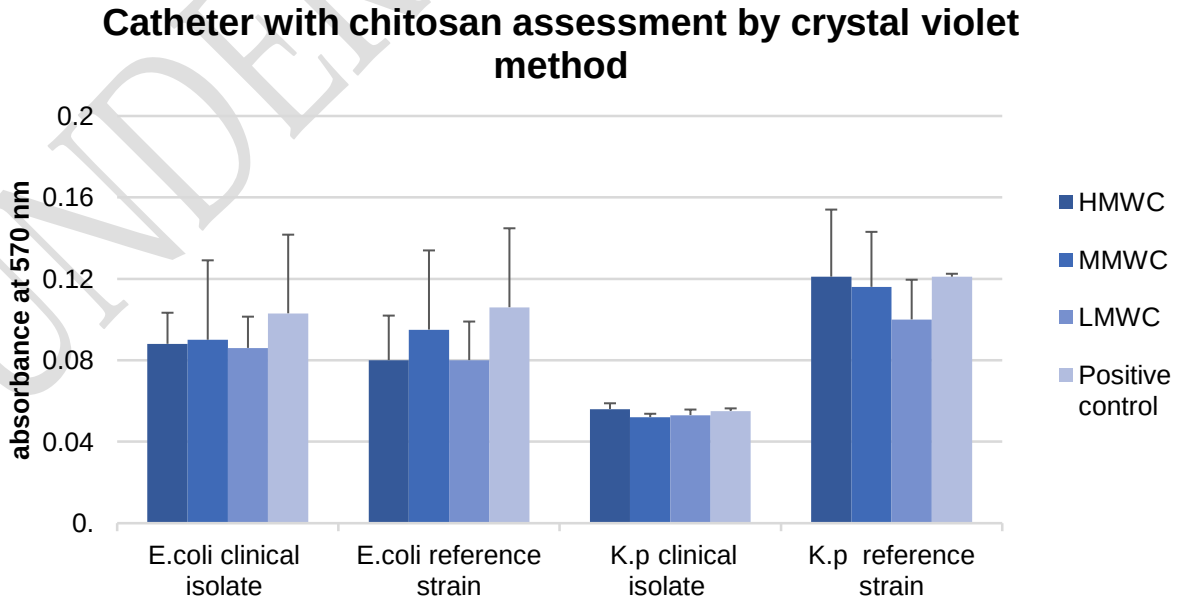


Figure 5: Catheter with chitosan using the crystal violet method for each bacterial isolate. These bars present the stained material absorbance at 620 nm after incubation of the catheters coated with three Mw grades and the bacterial isolates. Positive control bars represent the crystal violet-stained biofilm, formed by the isolates inside the catheter incubated without the chitosan. Positive control: catheter segments without chitosan. HMWC: high molecular weight of chitosan. MMWC: medium molecular weight of chitosan. LMWC: low molecular weight of chitosan. *E. coli*: *Escherichia coli*. *K.p*: *Klebsiella pneumoniae*.

3.5 Analysis of chitosan coated catheter by FTIR

3.5.1 HMw chitosan, MMw chitosan and LMw chitosan

The FTIR spectra show similar absorption bands for high molecular weight chitosan, medium molecular weight, and low molecular weight, all in powder form. As shown in Table 1, fingerprint peaks ranging between 600-1400 cm-1 are attributed to the out-of-plane of the bending vibration of C-H, which peaked at 771.53 cm-1 for all of the Chitosan samples of the different molecular weights.

At the 1600-1800 cm-1 range, an increase in C=O stretch in COOH was observed at 1554.63, 1653.00, 1683.86, and 1734.01 cm-1, with NO2 stretch at 1554.63 cm-1. The clear peak observed at 1683.86 cm-1 can be linked to the stretching variation of C=C bond. The peaks on 3400-4000 cm-1 range showed axial O-H stretching and N-H bonds at 3853.77 cm-1. As for the MMW chitosan, an increase in C=O stretch in the COOH was observed at 1558.48, 1653.00, and 1734.01 cm-1, with NO2 stretch at 1558.4 cm-1. The clear peak at 1653 cm-1 can also be linked to the stretching variation of C=C bond. Axial O-H stretching in the N-H bond is attributed to peaks that appeared after the 3000 cm-1 range. Increases in the C=O stretch in COOH were observed at 1506.41, 1554.63, 1653, and 1683.86 cm-1 with NO2 stretch at 1506.41-1554.63 cm-1 and stretching variations in the C=C bond at 1653-1683.86, on the LMW chitosan. A weak OH stretch on the NH bond was detected in the regions above 3000 cm-1.

Table 1. Summary of FTIR spectra for chitosan in different grades (high, medium and low), silicon catheter coated with the high, medium and low chitosan and silicon catheter without coating.

Peaks of chitosan HMw	**Peaks of chitosan MMw**	**Peaks of chitosan LMw**	**Peaks of silicon with chitosan HMw**	**Peaks of silicon with chitosan MMw**	**Peaks of silicon with chitosan LMw**	**Peaks of silicon**
Wavelength (cm^{-1}) and functional group						
C-H bend: 771.53	C-H bend: 771.53	C-H bend: 771.53	C-H bend: 650.66-785.03	C-H bend: 650-770	C-H bend of chitosan: Absent	C-H bend: 650.66-785.03
C=O: 1600-1730	C=O:1734.01	C=O:1500-1600	Si-O-Si: 1000-1260	Si-O-Si: 1000-1260	Si-O-Si: 1000-1260	Si-O-Si: 1000-1260
C=C stretch: 1683.86	C=C stretch: 1500-1653	C=C stretch: 1653-1683.86	C=O stretch: 1653-1683.68	C=O stretch: 1653-1683.86	C=O stretch: 1676.14	C=O stretch: absent
Si-C stretch**:** absent	Si-C stretch: absent	Si-C stretch: absent	Si-C stretch: 788.89	Si-C stretch: 788.89	Si-C stretch: 785.03	Si-C stretch: 785.03

C-H stretch**:** absent	C-H stretch: absent	C-H stretch: absent	C-H stretch: 2900	C-H stretch: 2962.66	C-H stretch: 2962.66	C-H stretch: 2962.66
OH stretch on NH: 3853.77	axial O-H stretch on N-H bond: <3000	weak O-H stretch on N-H bond: <3000	weak O-H stretch on N-H bond: <3000	weak O-H stretch on N-H bond: <3000	O-H stretch on N-H bond: <3000	O-H stretch on N-H bond: absent

3.5.2. FTIR spectra for the silicone catheter coated with chitosan (HMw, MMw and LMw)

The FTIR analysis of the silicon catheter coated with chitosan of low, medium, and high molecular weights shows the absorption bands of the chitosan and silicone combined. The peaks in the range between 1000 to 1260 cm-1 belong to Si–O–Si bonds of silicone, the curves showed an increase in intensity in the peaks of C-H stretching of CH3 with a peak at 2962.66 cm-1. Furthermore, the absorption bands at 1554.63–1700 cm-1 belong to the C=O bond of chitosan, as well as the peaks shown on the range of 650-870 cm-1, which are attributed to the bending vibration of the C-H bond of the chitosan, with the peak at 788.89 cm-1 belonging to the Si-C stretching of silicone.

4. Discussion

Biofilm formation to the catheter surface begins with bacterial adhesion onto the catheter's inner surface. The occurrence of CAUTI could be reduced by preventing bacterial adhesion and then biofilm formation. The use of silicon catheter and silicon coated with noble metal led to a reduction in the bacterial biofilm formation compared to other types of catheter used previously, such as latex [12-14]. One way to prevent bacterial adhesion is by coating the catheter's inner surfaces with antimicrobial/antiadhesive material. Chitosan is a film-forming polymer that demonstrates some antimicrobial activity against a wide variety of microorganisms that form biofilm [15]. When coated to the surfaces of silicon catheters, chitosan was found to minimize or prevent bacterial adhesion and biofilm formation either as an antimicrobial or anti-adhesive agent.

The current study shows three different chitosan, HMw, MMw, and LMw with antimicrobial activity. This activity of the chitosan was dependent on their molecular weight, with a pH value of 6. A variability of results was detected when using two different assessments of coating methods on biofilm, namely the sonicating and crystal violet methods. The chitosan prepared for coating was also tested in vitro before coating the silicon's inner surface in the time-kill study. Khan et al. (2020) studied chitosan and its derivatives closely, and analyzed its ability to kill bacteria and inhibit biofilm formation as well as the benefit of using it as a drug carrier. They mentioned that LMW chitosan was more effective than HMw chitosan on antibiofilm properties, which is in agreement with the results presented in the current study. It is believed that chitosan, and its derivatives, have the ability to diffuse into biofilm membrane and facilitate an interaction between positively charged chitosan and negatively charged biofilm constituents matrix [11]. In the current study, the more effective coated catheters were formed by the LMw chitosan and MMw chitosan, but the effect varied for the *E. coli* clinical isolate and the reference strain. Figure 5 shows that *E. coli* colonization was reduced by chitosan coating compared to the positive isolate (the colonized plain silicon catheter without coating). Despite the reduced CFU/ml of *E. coli* with chitosan after 2 hours of incubation in the time-kill assay, and after 6 hours with *K. pneumoniae*, the difference in the CV assay showed that the clinical isolate, the *K. pneumoniae* reference strain, and the *E.coli* reference strain decreased most dramatically with the MMw chitosan coating, and that the *K. pneumoniae* clinical isolate reduced with LMw chitosan. The sonication method showed a discrepancy in the results recorded, based on the number of viable counts in the CFU of the recovered cells. We believe that the antiadhesive properties of the chitosan were more pronounced in the CV assay more than in the sonication assay. There is a difference between the antifouling (antiadhesive) mechanisms and biocidal mechanisms for

the chitosan in the catheter material [16]. The chitosan, as a polymer attached to the silicon, was believed to apply steric and electrostatic repulsion, which are considered to be antifouling mechanisms, and to prevent the adhesion of bacterial cells to the silicon inner surfaces [5, 10]. This antifouling activity was observed clearly with the CV assay used in our study on both *K. pneumoniae* and *E. coli*. The biofilm, which was formed after coating with chitosan in the CV assay, showed a reduction ($P>0.05$) for both *K. pneumoniae* and *E. coli* compared to the CV assay of the strains themselves (Figure 4). However, the sonication assay exhibited less bactericidal activity of the chitosan compared to the CV staining method. Khan et al. (2020) suggested that chitosan is usually functional in an acidic environment, but in our current study, the pH was adjusted to be close to the pH of the urine (pH≈6,), which is an important condition to mimic the environment in which urinary catheters are installed and used. Campana et al. (2017) also found that the most distinct effects were seen in acidic condition (pH 5) of more than (pH 6), and chitosan with LMW of 50 kDa were shown to be more antibacterially active than the chitosan with HMw of 150 kDa for both *K. pneumoniae* and *E. coli*. The chitosan is essential for the functioning of inhibitory activity, either as a bactericidal or fungicidal or antifouling material in a media with a pH closer to the urine pH in the patient. It is for this reason that the current study investigated the activity of chitosan in a pH that reflected the urine condition, thereby explaining the use of the artificial urine media.

Our study also investigated the effect of chitosan on both *K. pneumoniae* and *E. coli*, which displayed sensitivity to chitosan in a time-dependent way, as represented by the time-kill assay. Conversely, the CV method shows a variability in the reduction of bacterial adhesion by different molecular weights within 24 hours. The efficacy of the chitosan coating in reducing the bacterial adhesion was found to be dependent on the type of microorganism, molecular weight and the pH of the media. These isolates were varied in the biofilm formation that occurred without the catheter, as presented in Figure 4. The variation in biofilm formation depends on the bacterial strain, which showed that *E. coli* isolates have less biofilm formation ability than *K. pneumoniae.* After incubating these isolates with catheters, which may behave differently, the biofilm formation increased, thus reducing the gap between high-biofilm producers [8]. Therefore, *E.coli* and *K. pneumoniae* could better produce a biofilm using a catheter. In the current study, Figure 5 shows that chitosan has an effect on the production of biofilm for *K. pneumoniae* clinical isolates compared to the biofilm without the catheter. Both *E. coli* isolates showed a small inhibition of biofilm formation after incubation with the chitosan- coated catheter, but not as pronounced as the reduction of biofilm for *K. pneumoniae.* This illustrates the effect of the type of isolate and the ability to form biofilm in vitro on chitosan effectiveness.

The FTIR characterization on the coated silicon proved that the addition of amino groups (NH and stretching of OH) from chitosan to the silicon material could contribute to the antiadhesive effect. It was found that the amino group and polysaccharide in chitosan strongly relate to the antibacterial activity [5, 17, 18]. Many Gram-negative bacteria produce the urease enzyme that catalyzes urine during catheter implementation in the patient. The urease breakdown the urine and increase ammonia, thereby inducing the formation of crystals, which enhance the ability of bacterial cells to attach to silicon material [5]. The presence of polysaccharides and the amino group attached to the silicon could reduce the potential of encrustation. We believe that both steric repulsion and electrostatic repulsion could be the mechanism of preventing the attachment of bacterial cells in the amino group with N-H and OH stretching, and the polysaccharide group in the chitosan. It could prevent the formation of biofilm by preventing the adhesion effect and by intensifying the steric repulsion of the chitosan molecules [19].

There are a limited number of studies that investigate the effect of Mw of chitosan in the bactericidal activity. One study found Mw did not act against *E. coli* and *Bacillus subtilis* [20]. Some studies reported that the increase in chitosan Mw reduced the bactericidal activity of chitosan [17]. In the current study, we found that chitosan Mw had a bactericidal effect, presented by the time-kill results for *K. pneumoniae* after 4 hours exposure, and for *E. coli* after 2 hours exposure in pH 6. However, all Mw degrees had a reduced effect of biofilm formation on *K. pneumoniae* and *E. coli*, except where LMw reduced the *E. coli* clinical isolate biofilm in the CV assay and the *K. pneumoniae* reference strain, compared to the positive control ($p<0.05$).

Conclusion

In conclusion, chitosan was found to have some efficacy in reducing bacterial adhesion to urinary catheters, indicating that it could be used as a polymer against microbial biofilms by reducing the adhesion of bacterial cells dependent on the pH condition. Our findings indicate the potential ability of chitosan, dependent on the molecular wight, to act as a non-antibiotics antiadhesion and preventive agent in biofilm-related infections, specifically for the urinary tract multi-drug resistant bacterial species. The limitation of this study was the short time period for testing chitosan coating with the bacterial cells, as we only allowed the bacteria to grow for 24 hours.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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