Research Article

Investigating the Anti-Biofilm Ability of Synergistic Drug Coated Urinary Catheters: *In vitro* Study

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ABSTRACT

Aim: Coating the surface of urinary catheter using two different synergistic drug combinations to prevent bacterial adhesion was considered as the primary objective of the study. **Materials and Methods:** Coatings were carried out using two steps like seeding and crystallization with Polyethylene Glycol (PEG) as binding agent. Coated samples were analysed for FESEM analysis and FTIR analysis. FTIR spectrum of the synergistic drugs and carrier coated silicone was analysed for detecting the chemical interactions among them. Finally, anti-biofilm assay using minimal biofilm eradication concentration was determined to check the efficacy of coated samples. **Findings:** Topographical analysis of coated samples revealed as large uniform and continuous layer of parallelogram shapes on the catheter surface. From FTIR spectrum it was observed that addition of drugs and carriers did not altered the functional group of silicone. C-H vibration for methyl groups of silicone, terminal C-H stretch of drugs and asymmetric methyl groups of carriers was evident from some of the common peaks. The obtained peak showed that the functional group of silicone was not altered. **Conclusion:** These *in vitro* outcomes suggest that the antimicrobial combinations used in the present research (Cefixime+Ciprofloxacin; and Ofloxacin and Ornidazole) with specific carriers (tocopherol acetate and beta cyclodextrin) can possibly use to combat bacterial colonization and catheter-associated infections.

Keywords: Cefixime, Ciprofloxacin, Ofloxacin, Ornidazole, Tocopherol acetate, Beta cyclodextrin.

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INTRODUCTION

A goldsmith named Bernard created the first known malleable urinary catheter in 1779, although Greek, Egyptian, and Chinese people have been using them since the third century B.C. Originally utilised either unmodified or with hydrogel or Teflon coatings, latex was not the best material due to its inappropriate qualities, which included poor adhesion, low resistance to chemicals and UV light, and potential allergic reactions.[1] As silicone avoids many of the issues latex catheters have, it is increasingly more frequently utilised as the base catheter material.[2]

Urinary catheter use has surpassed other foreign body insertions as the second most common reason for CAUTIs. Urinary tract infections, particularly those in patients receiving catheterization, account for more than 40% of nosocomial infections.[3] Long-term (28-day) catheter insertions have a markedly increased risk of urinary tract infections; in these patients, the rate of infected catheters approaches 100%. One major issue that contributes to the failure of standard therapies is the increased resistance of biofilm bacteria to antibiotics. Antibiotic resistance in biofilm is primarily due to the extracellular polysaccharide matrix.[4] The biofilm microflora exhibits greater diversity the longer the patient has had the catheter. 99.9% of microorganisms can build biofilms on a variety of surfaces, including inert and biological surfaces.[5]

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Gram-negative rods (*Escherichia coli, Klebsiella pneumoniae, Enterobacter* sp., *Pseudomonas aeruginosa, Proteus mirabilis*, etc.,) and *Enterococci* (particularly *Enterococcus faecalis*) are the most common cause of urinary tract catheter infections.[6]

The cell surface of *E. coli* contains high molecular weight compounds called capsules. *In vitro*, it can also produce biofilm.[7,8] It forms biofilm with great propensity. Multiple drug-resistant bacteria called *Staphylococcus aureus* is the source of some nosocomial illnesses. It forms biofilm on catheters and long-term wounds.[9] Immunocompromised patients are more vulnerable to infections caused by the well-known opportunistic pathogen *S. epidermidis*. Because *S. epidermidis* can develop biofilms, there is an increased risk of contamination after surgical polymeric device installation.[10]

The majority of recent research has focused on antimicrobials, which have become the most preferred coating due to their multifaceted capacity to attack bacteria in five main functions: (1) targeting cell membrane sterols; (2) inhibiting the production of proteins; (3) inhibiting the synthesis of nucleic acids; (4) inhibiting the synthesis of cell walls; and (5) inhibiting certain metabolic pathways.[11]

Broad-spectrum antibiotics called fluoroquinolones work by attaching themselves to bacterial DNA gyrase and preventing the synthesis of new bacterial DNA.[12] According to Collins *et al.* (2011), DNA gyrase is a crucial bacterial enzyme that causes double-stranded DNA to undergo ATP-dependent negative supercoiling.[13] A broad-spectrum antibiotic that works against both Gram-positive and Gram-negative bacteria is ciprofloxacin.[14] It works by blocking the enzymes DNA gyrase, a type II topoisomerase, and topoisomerase IV, which are required to separate the DNA of bacteria, thereby preventing cell division.[15] Due to the advent of more costly and sophisticated medication combinations, the usage of ornidazole for the treatment of mixed aerobic and anaerobic infections has decreased recently. When compared to all other antibiotics, ornidazole is still regarded as the "gold standard" antibiotic.[16]

In order to avoid the formation of biofilms surrounding implant-associated tissues, Tocopherol Acetate (TA) offers a sustained release of anti-infective chemicals.[17] This study used it as a coating material in urinary catheters for the prolonged release of synergistic antibacterial medicines because of its qualities, which include resistance to oxidation, suppression of platelet adhesion and aggregation, and biocompatibility.[18]

The main goal of the study was to coat the urinary catheter's surface with two distinct synergistic chemical combinations to inhibit bacterial adhesion. The coating process involved two stages, namely seeding and crystallisation, use Polyethylene Glycol (PEG) as the binding agent. Coated samples underwent FTIR and FESEM analyses. The objective of the analysis was to identify any chemical interactions between the synergistic medicines and carrier coated silicone using the FTIR spectrum. Ultimately, a minimal biofilm eradication concentration anti-biofilm assay was developed to evaluate the effectiveness of coated samples.

MATERIALS AND METHODS

Selection of Synergistic antibacterial drugs and Drug carriers

As per literature surveys,[19,20] the oral antibiotic drugs (Cefixime and Ofloxacin) were selected in the present study. To investigate its synergistic activity two fluoroquinolone drugs (Ornidazole and Ciprofloxacin) was selected. Ornidazole was used as combinational therapy by adding with Ofloxacin and Ciprofloxacin was added with Cefixime. Two drug carriers Tocopherol acetate and beta cyclodextrin was used to add it with above mentioned two drug combinations.

Coating the catheter surface with synergistic drugs using standard Two Dip-coating method[21]

Catheter samples were coated with synergistic drugs using the method described by Boccaccini *et al.*, (2003).[21] In brief, the samples were coated using two-dip coating slurry method. Using Polyethlyene glycol and synergistic-drugs the sample were coated and used for analysing antibacterial activity, anti-biofilm activity and other physical characterization techniques.

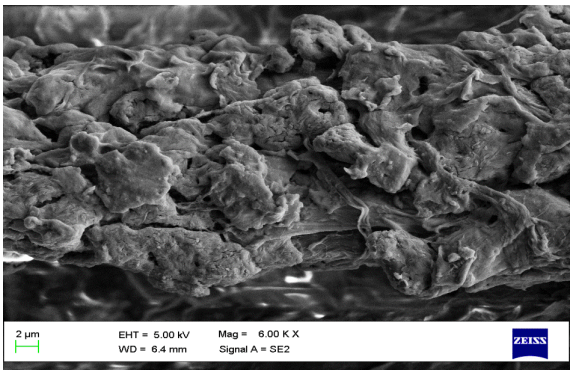
Surface characterization of synergistic drug coated urinary catheter samples

FESEM analysis-Topographical analysis of coated Catheters[22]

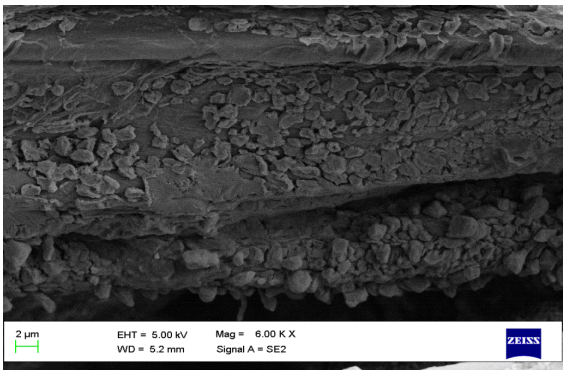
The synergistic drugs+carrier present in the surface of catheters was observed under FESEM apparatus. The surface was visualized to check the coated drugs in the form of crystals on samples.

Fourier Transform Infra-Red spectroscopic analysis of coated catheters[23]

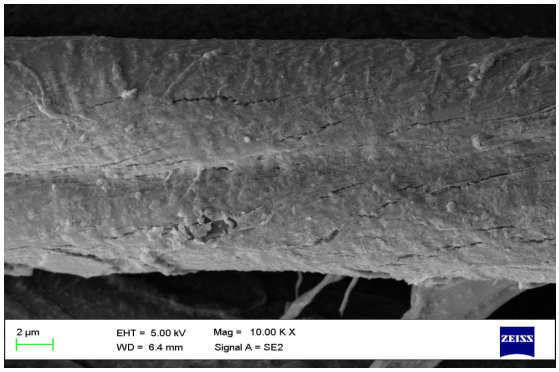
The FTIR absorption spectra of the catheter (silicone), synergistic drugs (Cefixime+Ciprofloxacin), and carriers (tocopherol acetate) were recorded in the range of 400-4000 cm^{-1} by KBr disc method. Similar experimental



Coated-Cefixime+Ciprofloxacin



Coated-Ofloxacin+Ornidazole



Uncoated samples

Figure 1: Scanning Electron Microscopic analysis of coated and uncoated catheter samples.

set up was done for another drug and carrier combination (Ofloxacin+Ornidazole with beta cyclodextrin as carrier).

Anti-biofilm assay using minimal biofilm eradication concentration[24]

Anti-biofilm of the coated catheter samples were analysed using minimal biofilm eradication concentration by method described by Perumal and Mahmud (2013).[24] Briefly, microbial growth was developed in microtitre plate and its minimal-biofilm eradication was determined. The bioassay was performed in triplicates.

RESULTS

Scanning Electron Microscopic analysis of coated and uncoated catheter samples

Topographical analysis of coated samples was carried out using FESEM analysis. The analysis exhibited drug molecules on the catheter surface. Drug coatings as large uniform and continuous layer of parallelogram shapes on the catheters were observed. In Figure 1 the difference between the coated and uncoated catheter samples were presented.

FTIR analysis of coated and uncoated catheter samples

FTIR analysis was used to detect the chemical interaction among the synergistic drugs, carrier, biomedical materials and *drug-carrier coated* (*dcc*) materials. In Figure 2 the uncoated samples were compared with Cefixime+ciprofloxacin coated catheter samples; similarly in Figure 3, the uncoated samples were compared with Ofloxacin+Ornidazole coated catheter samples. FTIR spectrum separately for the plain catheter samples, synergistic drugs (Cefixime, ciprofloxacin, Ofloxacin and Ornidazole), drug carriers (tocopherol acetate and beta cyclodextrin) and drug-carrier coated catheter samples were explained separately below.

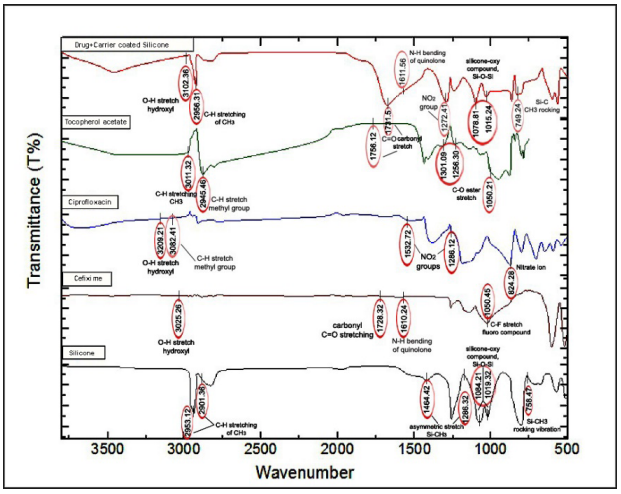


Figure 2: FTIR spectrum of coated and uncoated catheter samples (Cefixime+ciprofloxacin+Tocopherol acetate).

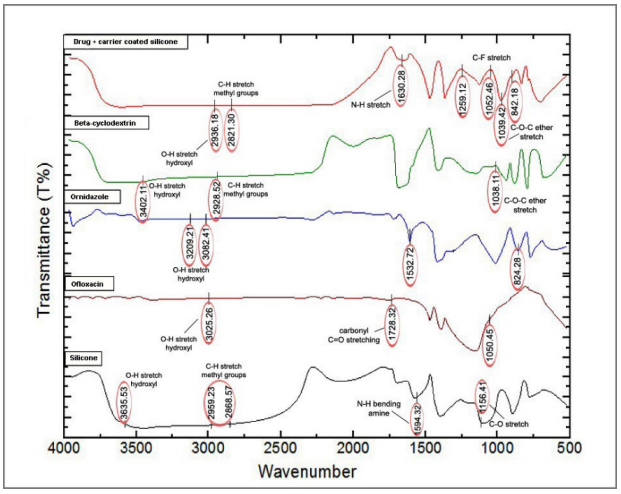


Figure 3: FTIR spectrum of coated and uncoated catheter samples (Ofloxacin+Ornidazole+Beta cyclodextrin).

Plain silicone catheter

FTIR spectrum of uncoated silicone showed most characteristic C-H stretch vibrations of methyl groups at a peak 2953.12 cm^{-1} and 2901.36 cm^{-1}. A strong methyl band, a weak methyl band and a methylene rocking vibration band are assigned for strong asymmetric stretch Si-CH_3 (1464.42 cm^{-1}), weak asymmetric stretch Si-CH_3 (1286.32 cm^{-1}) and Si-CH_3 rocking vibration (758.47 cm^{-1}) respectively.

Cefixime

Cefixime is a type of cephalosporin antibiotic. A prominent characteristic peak at 3025.26 cm^{-1}, represented the stretching vibration of O-H group and intramolecular hydrogen bonding. The peak obtained at 1728.32 cm^{-1} represented the acidic carbonyl C=O stretching of the compound.

Ciprofloxacin

Ciprofloxacin was a type of fluoroquinolone antimicrobial drug. FTIR spectrum of the ciprofloxacin showed peaks at 3209.21 cm^{-1} corresponded to hydroxyl group of O-H stretch. C-H stretching in the compound was observed at the peak, 3082.41 cm^{-1}.

Tocopherol acetate

Asymmetric and symmetric C-H stretching of methyl and methylene groups in beta cyclodextrin was observed as intense peaks at 3011.32 cm^{-1} and 2945.46 cm^{-1} respectively. Carbonyl groups were observed at 1756.12 cm^{-1} due to C=O stretch.

Ofloxacin

Ofloxacin is a type of fluoroquinolone antibiotic. The fluorine and quinolone functional groups of the antibiotic were observed at peak 1728.32 cm^{-1} and 1050.45 cm^{-1}. The former peak was assigned to C=O carbonyl stretching and the later peak were assigned to N-H bending vibration of quinolones.

Ornidazole

Ornidazole was a type of nitroimidazole antimicrobial drug. The peaks corresponding to the symmetric and asymmetric (NO_2) Nitro group of the nitroimidazole (Ornidazole) were obtained at peak 1532.72 cm^{-1} (aromatic nitro-compound). The corresponding nitrate ion of the drug Ornidazole was also evident at peak 824.28 cm^{-1}.

Beta cyclodextrin

FTIR spectrum of pure beta-cyclodextrin showing a strong absorption peak at 2936.18cm^{-1} corresponded to O-H stretch in primary alcohol. Asymmetric alkyl C-H stretch vibrations of methylene groups were observed at peak 2821.30 cm^{-1}. The alkyl substituted ether C-O-C stretch was observed at 1038.11 cm^{-1}

Drug-carrier coated silicone

FTIR spectrum of the *drug-carrier* (synergistic drugs and carrier) coated silicone was analysed for detecting the chemical interactions among them. From the spectrum it was observed that addition of drugs and carriers did not alter the functional group of silicone. C-H vibration for methyl groups of silicone, terminal C-H stretch of drugs and asymmetric methyl groups of carriers was evident from a common peak obtained at 2956.31 cm^{-1}. The obtained peak showed that the functional group of silicone was not altered.

All the peaks assigned for each functional group of the compounds was shown in Figure. In Figure 2 and Figure 3, the catheter coated with Cefixime + ciprofloxacin +

Table 1: Minimum Biofilm Eradication Concentration (MBEC) of the coated catheters.

Sl. No.	Test organisms	Minimum Biofilm Eradication Concentration (MBEC) (mg/mL)	
		Cefixime+Ciprofloxacin	Ofloxacin and Ornidazole
1	*Escherichia coli*	0.64±0.57	0.61±0.75
2	*Staphylococcus aureus*	0.68±0.75	0.64±0.57
3	*Pseudomonas aeruginosa*	0.69±0.57	0.65±0.75
4	*Staphylococcus epidermidis*	0.65±1.05	0.59±0.57
5	*Klebsiella pneumoniae*	0.63±0.57	0.58±1.05

Tocopherol acetate and Ofloxacin + Ornidazole + Beta cyclodextrin were presented respectively.

Anti-biofilm assay

In Table 1, The anti-biofilm results of two different synergistic-drug combinations against the test bacteria was presented in Table 1.

Cefixime+Ciprofloxacin coated catheters showed anti-biofilm activity (eradication) against all the biofilm producing test organisms during the analysis. The drug combinations showed 0.64±0.57 mg/mL, 0.68±0.75 mg/mL, 0.69±0.57 mg/mL, 0.65±1.05 mg/mL and 0.63±0.57 mg/mL of MBEC against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Klebsiella pneumoniae* respectively.

Ofloxacin and Ornidazole coated catheters showed anti-biofilm activity (eradication) against all the biofilm-producing test organisms during the analysis. The drug combinations showed 0.61±0.75 mg/mL, 0.64±0.57 mg/mL, 0.65±0.75 mg/mL, 0.59±0.57 mg/mL and 0.58±1.05 mg/mL of MBEC against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Klebsiella pneumoniae* respectively.

DISCUSSION

One of the most common nosocomial infections among the patients in healthcare sectors are Urinary tract infections. Most of the time it has been reported that the infections were transmitted through urinary catheters and ureteral stents; and hence it's commonly known as Catheter or stent-associated infections. Quesada and Light (1993) reported that these types of infections are difficult to be treated with antibiotics; changing the catheters in patients are considered as safe methods.[25] Stickler, *et al*., (2003) stated that catheters are made of silicone or latex; which favours harbouring of bacterial agents on its surface. The irregular surface may allow the organisms to adhere and survive both externally and internally. This leads to transmission of infection due to biofilm formation by different types of bacteria like *Escherichia coli, Proteus mirabilis* and *Citrobacter* sp.[26] Danese (2002) explained a simplest way for the prevention of biofilm formation on the catheter surface by coating with broad-spectrum antimicrobial agent. These agents degrade naturally and eluted into the tissues associated with catheter inserted and prevents the growth and metabolism of biofilm forming organisms.[27] However, Pittet *et al*., (1994) earlier reported that, these types of antimicrobial catheters may not able to deliver the drugs at the catheter-inserted sites for prolonged periods due to lesser concentrations coated on its surface.[28] Bharadwaj *et al*., (2003) stated that when any single type of antibacterial agent is used alone for coating the catheter, chance of acquiring antibiotic-resistance by the microbes are high.[29] And hence in the present study we used two different antibacterial drugs from two different groups and combined for coating the catheters.

The antimicrobial drugs (Cefixime and Ofloxacin; Ornidazole and Ciprofloxacin) used in the present study were chosen according to different types of criteria reported from literature survey. Most significant criteria reported as they showed good synergistic activity to inhibit the growth of test isolates at greater level compared when the drugs where used alone. And next being capable of drugs to attack the cell components of biofilm producing pathogens or organisms associated with urinary tract infections. All these drugs were reported to be specific in attacking the cell components of bacteria to provide maximum bactericidal activity. Cefixime attacks the membrane and cytoplasmic components of a bacteria; Ofloxacin and ciprofloxacin are quinolone groups of drugs that are capable of attacking the DNA replicative enzymes. Ornidazole are nitroimidazole drugs that potentially breaks the replication chain of bacteria by interacting with enzyme DNA gyrase.[30,31]

Based on these, in our present study the results were found promising in retarding the growth of test bacteria. Catheter samples coated with the synergistic drugs when

subjected to FESEM analysis evidenced homogenous coating of drug and carrier combination did not give surface area on sample for microbial adherence. The crystallized drug-particles adhered on sample surface reduces the area for bacteria to adhere.

With reference to our findings, Yassin *et al*., (2019) demonstrated facile coating of urinary catheter with bio-inspired antibacterial coatings using silver nanoparticles. Similar to our findings, the researcher also evidenced homogenous and smooth surface of the catheter after coating with antibacterial silver nanoparticles. The coated catheters also showed greater anti-fouling potential against Gram-Positive bacteria including *Staphylococcus aureus* and *Staphylococcus epidermidis*.[32]

FESEM analysis not only confirms the adhesion of synergistic drugs on to catheter surface; it's clear that surface modified catheters would also have the ability to prevent the adhesion of biofilm producing microbes. Adhesion assay carried out by Reid *et al*., (1994) proved that ciprofloxacin, ofloxacin and norfloxacin coated urinary catheters malformed the adherent organisms like *Pseudomonas aeruginosa* significantly.[33]

FTIR analysis of the synergistic drug and carrier coated catheters showed that no chemical intervention was observed for the *drug-carrier coated* catheters materials. The presence of drug and carrier thus provided a significant impact for the catheters, which included; the enhanced antibacterial properties, persistence of drugs and durability of the biomedical materials. During the analysis, most characteristic peaks representing the vibrations and symmetric/asymmetric stretches of significant functional groups influenced by the compounds in catheters, carriers and synergistic drugs were found evident.

Similar work carried out by Kowalczuk, (2020) used two different fluoroquinolone drugs (Sparfloxacin and ciprofloxacin) for coating urinary catheters. The researchers reported that application of FTIR method proved, sparfloxacin is a good choice to coat the catheters as it showed unique absorption bands of the amine group in the range of 4000-3000cm^{-1}.[34] In another study, Yassin *et al*., (2019) proved that the coated urinary catheters displayed new broad absorption band representing to stretching vibrations of significant functional groups that are attributing the presence of antibacterial agents coated firmly on the silicone surface.[32] These cited details were found supportive to our present findings in terms of FT-IR analysis based on the obtained functional groups which are considered highly significant in attributing to antibacterial activity and biofilm eradication.

Biofilm eradication concentrations of the synergistic drugs against the test bacteria was evaluated. Cefixime+Ciprofloxacin coated catheters revealed Biofilm eradication concentrations ranging from 0.63 mg/mL to 0.69 mg/mL. The other combination (Ofloxacin and Ornidazole) showed slightly lesser biofilm eradication concentration ranging from 0.58 mg/mL to 0.65 mg/mL against the test bacteria. However, the obtained values were found significant in eradicating the biofilm forming capability of test organisms. A number of authors had examined the effectiveness of the antibiotics which could support the contemporary research.

Reid *et al*., (1994) reported that ciprofloxacin, ofloxacin and norfloxacin coated urinary catheters exhibited good biofilm eradication concentration ranging from 50 to 100 ug/mL; with 99% reduction in the number of adherent bacteria.[33] Mohammad *et al.,* (2013) studied *in vitro* the antimicrobial activities of central venous catheters impregnated with Levofloxacin and N-acetylcysteine (NAC), an anti-biofilm agent, and a broad-spectrum antibiotic in contradiction of a range of significant clinical pathogens.[35] Elayarajah *et al*., (2011) studied coated the urinary catheters using similar types of synergistic drugs (ofloxocin and Ornidazole) to prevent the growth of biofilm producing *E. coli* and *S. epidermidis*. The researchers tested the efficacy of coated catheters against preventing bacterial adhesion in artificial urine and found the number on the synergistic drug coated samples to be significantly lower compared to the uncoated catheter samples.[36] These findings were found supportive to our results in terms of biofilm eradication assays and proved that the clinical role of fluoroquinolone drugs in preventing and treating urinary tract infections associated with biofilm producing bacterial species are highly significant.

The present findings are unique and significant in the aspect that, the urinary catheters coated with synergistic drugs and drug carriers (tocopherol acetate, beta cyclodextrin) inhibits the growth of urinary tract pathogens on the surface of the catheters and its surrounding tissues. As future perspective, these experiments shall also be done as standard *in vivo* studies using albino white rats. Expected positive results from *in vivo* studies could be of great interest for successful catheterization and preventing the catheter-associated infections among the patients in any health care centre and hospitals.

CONCLUSION

FESEM analysis showed that drug and carrier were large uniform and continuous layer of parallelogram shapes on the catheters. FTIR spectrum of the *drug-carrier* (synergistic drugs and carrier) coated silicone was analysed for detecting the chemical interactions among them. From the spectrum it was observed that addition of drugs and carriers did not alter the functional group of silicone. C-H vibration for methyl groups of silicone, terminal C-H stretch of drugs and asymmetric methyl groups of carriers was evident from a common peak obtained at 2956.31 cm^{-1}. The obtained peak showed that the functional group of silicone was not altered. These *in vitro* outcomes suggest that the antimicrobial combinations used in the present research (Cefixime+Ciprofloxacin; and Ofloxacin and Ornidazole) with specific carriers (tocopherol acetate and beta cyclodextrin) can possibly use to combat bacterial colonization and catheter-associated infections.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FESEM: Fourier emission scanning electron microscopy; **FTIR:** Fourier transform infrared spectroscopy; **MBEC:** Minimal biofilm eradication concentration.

SUMMARY

The strength of this study is emphasized that, by using different groups of antibacterial drugs, synergistic antibacterial activity is achieved. Advantage of the synergistic drug selected inhibits the bacteria gaining resistance against these antibiotic drugs. The coating of drugs onto the silicone catheter surface is unique and simple to retard the adherence of bacteria and its proteins. Hence the coated catheters are considered safe, hygiene and ready to insert in the patients' genitals. Characterization studies like FESEM and FTIR analysis revealed proper coatings of synergistic drugs, which in turn assist in providing excellent inhibitory zones during the antibacterial studies against the pathogenic strains. The concentration of the synergistic drugs coated onto the urinary catheters exhibited good biofilm eradication against the test organisms.

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