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Repurposing of fluoxetine for antibacterial activities in catheter-associated urinary tract biofilm infections: an *in vitro* analysis

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ABSTRACT

BACKGROUND

Urinary tract infections are often initiated by indwelling catheters and bring about serious consequences, especially when they are caused by multidrug-resistant bacterial pathogens. The biofilms of uropathogens such as *Enterococcus faecalis* and *Escherichia coli* pose serious challenges. Therefore the scientific world is trying to experiment with alternative drugs to replace conventional antibiotics as the latter are more prone to cause the development of antibacterial resistance. Here, we evaluate the repurposing of the antidepressant fluoxetine as an antibacterial agent against the mentioned pathogens.

METHODS

To repurpose fluoxetine for its antibacterial activity against *Enterococcus faecalis* and *Escherichia coli*, the agar diffusion method was used. The minimal inhibitory concentration was found by the microdilution method. The drug was also analyzed as a coating on catheters to evaluate its efficiency against biofilm formation by pathogens.

RESULTS

The drug fluoxetine showed potential antibacterial and anti-biofilm activities. Its minimum inhibitory concentration was found to be 18.75 µg/mL and 37.5 µg/mL against *Enterococcus faecalis* and *Escherichia coli* respectively. The antibiofilm activity on polystyrene surfaces was also remarkable as it reduced the formation of *Enterococcus faecalis* and *Escherichia coli* biofilms by 70% and 74%, after being treated with 1x MICs and 2x MICs respectively.

CONCLUSIONS

Fluoxetine - one of the drugs of choice in treating depression, when repurposed, has shown considerable antibacterial and antibiofilm effects against two of the major catheter-associated urinary tract infection-causing bacteria - *viz. Enterococcus faecalis* and *Escherichia coli*. Therefore, further studies are needed to understand its applicability as an antibacterial agent.

Keywords: Biofilms, catheter-associated urinary tract infections, fluoxetine, repurposing

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INTRODUCTION

In modern medical science, indwelling medical devices have been playing a revolutionary role in the management of various diseases, and urinary catheters which relieve the discomforts of patients with urinary bladder complications are the most significant among them. Unfortunately, in hospitalized patients these indwelling catheters significantly increase the risk for iatrogenic infections that are common when the patient is immunocompromised. In the modern age, medical device-associated infections contribute to a major part of nosocomial infections and among these, urinary tract infections (UTIs) are one of the most significant hospital-acquired infections associated with catheters.[1,2] Catheter-associated urinary tract infections (CAUTIs) occur when the presence of uropathogenic bacteria is manifested in the urine of hospitalized patients with catheter usage and are the most common healthcare-associated infections that affect millions of patients globally.[3-5] The use of catheters facilitates the entry of microbial pathogens to the urinary tract and their colonization leads to various complications such as sepsis and bacteriuria, resulting in long hospital stays and in extreme cases, higher mortality rates causing socio-economic burdens.[6,7]

Prolonged use of urinary catheters favours polymicrobial infections including those by bacteria and fungi.[8] Reports suggest that *Escherichia coli* and *Enterococcus faecalis* are the most prevalent bacterial pathogens that cause CAUTIs.[9,10] These prevalent bacteria form biofilms by adhering to the catheter surfaces and establishing communications among them making their eradication complicated.[11] The biofilm-forming ability enables the microorganisms to resist several antibiotics through various resistance mechanisms, such as the physical protective effects of the extracellular polymeric substances, efflux pumps, and the transfer of antibiotic resistance genes between bacterial cells.[12-14] As a result, in most cases, the treatment of CAUTIs has been practically difficult.[15,16] Hence, there is an immediate need for alternative therapies that enable the prevention of colonization and biofilm formation on urinary catheters by bacterial pathogens such as *Enterococcus faecalis* and *Escherichia coli* and their eradication. However, the search for new drugs from the available sources is a time-consuming and expensive process as these drugs under development have to undergo many clinical trials before entering the market.

Among the several ways of drug development, repurposing already existing drugs for a new and novel application is one approach that has gained much attention because of the unique nature of this drug development process. The quest of repurposing the existing drug is significant as the drug has already gone through many safety, pharmacological efficacy, and human trials which reduces the cost, time, and risks associated with antibiotic innovation.[17] Considering all these facts, in the present study, an effort was made to evaluate the antibacterial activity of the antidepressant drug fluoxetine against two of the major CAUTI biofilm-forming bacterial pathogens, viz, *Enterococcus faecalis* and *Escherichia coli*.

METHODS

Research design

An *in vitro* analysis was carried out to screen the anti-depression drug fluoxetine against two of the major catheter-associated urinary tract infection-causing bacterial pathogens *viz.*, *Enterococcus faecalis* and *Escherichia coli*. The experiments and the following analyses were conducted at the Basic Medical Science Laboratory, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia, and at Biotech Research Centre, Salem- 636010, Tamil Nadu, India, during the period between November 4th, 2022 and January 2nd, 2023.

Reagents and materials

For the study, Mueller Hinton Broth, ampicillin, and rifampicin were procured from Hi

Media (USA) and fluoxetine from Sigma Aldrich. Fluoxetine (1 mg/ mL) was prepared in sterile double distilled water. The strains *Enterococcus faecalis* (ATCC 29212) and *Escherichia* coli (ATCC 25922) were obtained from American Type Culture Collection (ATCC). The antibiotics rifampicin (5 µg) and ampicillin (5 µg) were used as controls.

Determination of antibacterial activity

The antibacterial activity of fluoxetine against *Enterococcus faecalis* and *Escherichia coli* was investigated using the well diffusion method as per standard protocols.[18] In brief, overnight cultures of *Enterococcus faecalis* and *Escherichia coli* were grown in Mueller-Hinton Broth (MHB) at densities of 0.5 MacFarland units and were used for determining the antibacterial activity of fluoxetine against *Enterococcus faecalis* and *Escherichia coli.* The mentioned cultures were swabbed over the surface of sterile Mueller-Hinton Agar (MHA) plates, in which the wells of 6 mm diameters were made to allow the different concentrations of fluoxetine, and incubated at standard conditions overnight. Then, the plates were observed for zones of inhibition, which were measured in millimetres (mm), to determine the antibacterial activity of fluoxetine. The experiments were done in duplicate. Ampicillin and rifampicin were used as positive controls for *Enterococcus faecalis* and *Escherichia coli* respectively.

Minimum inhibitory concentration determination

The microdilution method was adopted, as described by Meiyazhagan et al.,[19] to find out the minimum inhibitory concentrations (MICs) of fluoxetine against *Enterococcus faecalis* and *Escherichia coli.* Here, in 96 well plates, 300 µg/mL of fluoxetine was serially diluted using 200 µl of MHB to achieve a final concentration of 2.3 µg/mL. Later, the plates were incubated under standard conditions after adding the overnight cultures of the above-indicated organisms. After incubation, the plates were observed for turbidity, and the optical density was read at 600 nm using a spectrophotometer. The experiments were done in triplicate.

Effect of fluoxetine on bacterial colony formation

To find out the effect of fluoxetine on *Enterococcus faecalis* and *Escherichia coli* colonization, the drug was serially diluted in 96-well plates using MHB, then the overnight cultures were added and ncubated for 96 hours. Then, the phosphate-buffered saline (PBS) wash was done to each well for the removal of unattached cells. The adherent cells were fixed with methanol and stained with crystal violet for several minutes. The ethanol and acetone (1:9) mixture was added to the stained cells and the plate was read at 570 nm using a spectrophotometer.[19] The experiments were done in triplicate. Cells without treatment acted as a negative control.

Biofilm inhibition efficiency of fluoxetine

The biofilm formation assay was performed in polystyrene microlitre plates as illustrated by Gowri et al.,[20] to ascertain the effect of fluoxetine on *Enterococcus faecalis* and *Escherichia coli* biofilms. Briefly, the biofilm formation was achieved after incubating the *Enterococcus faecalis* and *Escherichia coli* cultures in a 12-well plate for 96 hours. After the incubation, 1x MIC and 2x MIC concentrations of fluoxetine were added and the plate again incubated for 24 hours. The non-adherent cells were then removed by PBS wash, followed by methanol fixation and crystal violet staining. Then, the excess stain was removed by washing followed by air drying. The ethanol and acetone (1:9) mixture was added to the stained cells and the plate was read at 570 nm using a spectrophotometer. Cells without treatment were considered negative controls. Ampicillin and rifampicin were the positive controls for *Enterococcus faecalis* and *Escherichia coli* respectively. The experiments were done in triplicate.

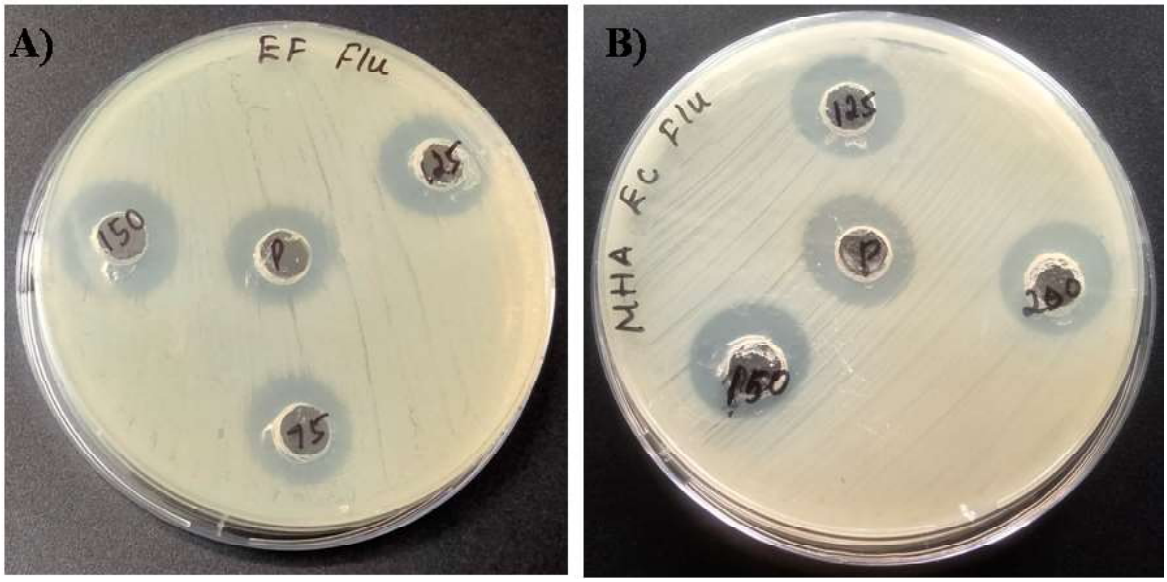

Figure 1. Fluoxetine exhibited zone of inhibition against A) *Enterococcus faecalis* B) *Escherichia coli*

Antibacterial activity of catheter coated with fluoxetine

To determine the antibacterial activity of a catheter coated with fluoxetine against *Enterococcus faecalis* and *Escherichia coli*, the *in vitro* catheter model was adopted as described by Goda et al.[21] Briefly, small pieces of sterile catheter tubes were dipped into 25 mg/mL of fluoxetine solution for 30 mins followed by air drying. For the assay, the air-dried fluoxetine-coated tubes were placed on the sterile MHA plates which were swabbed with *Enterococcus faecalis* and *Escherichia coli* followed by 24 hours of incubation. After incubation, the plates were observed for zones of inhibition. The experiments were done in duplicate.

Statistical analysis

Means and standard deviations were calculated for MIC determination, colony formation, and biofilm formation assays to analyze the statistical significance.

RESULTS

Determination of antibacterial activity of fluoxetine

The antibacterial activities of various concentrations of fluoxetine were investigated against *Enterococcus faecalis* and *Escherichia coli* and the zones of inhibition exhibited by different concentrations of fluoxetine against prevalent microbes involved in CAUTI are presented in Figure 1. As seen in the Figure, the antibacterial activity was achieved with 75 µg of fluoxetine against *Enterococcus faecalis* and 125 µg of fluoxetine against *Escherichia coli.* It is noted that, when the concentrations increased, the activity of fluoxetine was also higher against both of the microbes.

MIC determination

The MICs of fluoxetine were evaluated against *Enterococcus faecalis* and *Escherichia coli* involved in CAUTIs and the lowest concentration of fluoxetine which inhibited the growth of the respective bacteria was calculated, as presented in Figure 2. As shown, the calculated MICs of fluoxetine was 18.75 µg/mL against *Enterococcus faecalis* and 37.5 µg/mL against *Escherichia coli*.

Effect of fluoxetine on bacterial colonization

The activities of fluoxetine against colony formation by *Enterococcus faecalis* and *Escherichia coli* were studied and are represented in Figure 3. As shown, the presence of fluoxetine up to its MIC level in polystyrene

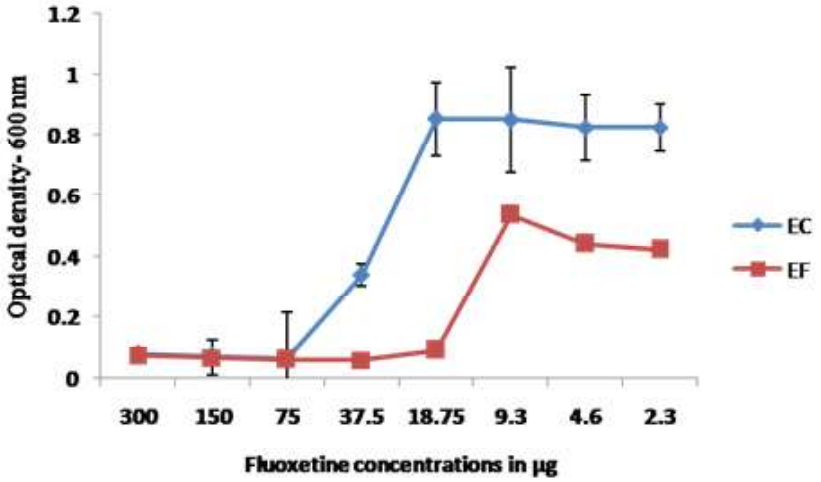

Figure 2. MICs of fluoxetine against *Enterococcus faecalis* and *Escherichia coli*
Values are for mean ± standard deviation (error bar)

plates did not allow any bacterial growth on the surface. Surprisingly, even traces of fluoxetine present in the wells were able to decrease the colony-forming ability of *Enterococcus faecalis* and *Escherichia coli* on the surface of the plate when compared with untreated wells which is reflected in the results.

Effect of fluoxetine on biofilm formation

The effects of fluoxetine on *Enterococcus faecalis* and *Escherichia coli* biofilm formation, after treatment with various concentrations of the drug, have been quantified and are shown in Figure 4. As seen in the Figure, the percentage of effects of fluoxetine on *Enterococcus faecalis* biofilm formation was calculated as 64.4 and 70 for 1x MICs and 2x MICs respectively. In contrast, *Escherichia coli* biofilm formation was reduced by 69% and 74% after being treated with 1x MICs and 2x MICs respectively.

Activity of fluoxetine coated catheters against the bacteria

Antibacterial activity was investigated for the fluoxetine-coated catheters against *Enterococcus faecalis* and *Escherichia coli* under suitable *in vitro* conditions as presented in Figure 5. As seen in the Figure, the drug-coated

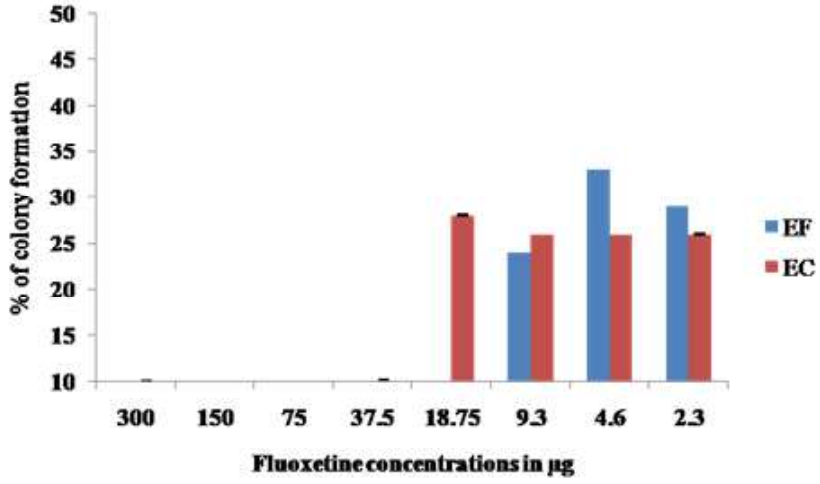

Figure 3. Graphical representation of fluoxetine effect on *Enterococcus faecalis* and *Escherichia coli* colony formation

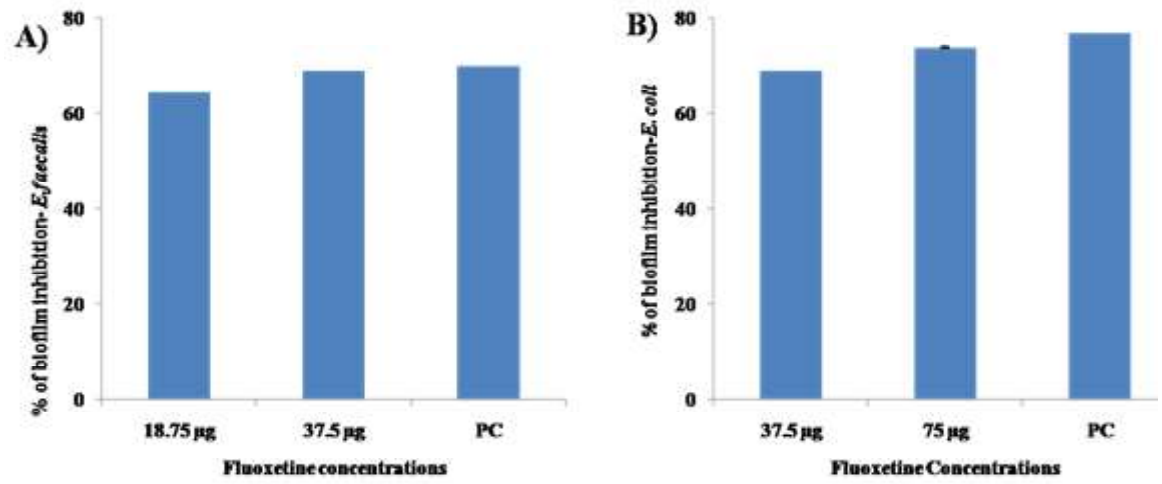

Figure 4. Percentage of biofilm inhibition of fluoxetine against A) *Enterococcus faecalis* B) *Escherichia coli*
Note: PC-Positive control

catheter tube exhibited a clear zone of inhibition around the silicone tube which represents the antibacterial activity of fluoxetine.

DISCUSSION

Catheter-associated urinary tract infection is one of the most significant hospital-associated infections causing serious clinical complications leading to high morbidity and mortality owing to their polymicrobial nature, which creates treatment challenges because of the antibiotic resistance and biofilm-forming ability of the prevalent organisms.[22,23] Considering these facts, there have been novel ways of searching for new antibacterial agents, and one of them is the repurposing of existing drugs for a different scope that has achieved much attention owing to their known pharmaceutical profiles. Here, the anti-depression drug fluoxetine was repurposed for its antibacterial activity against *E. faecalis* and *E. coli* which are prevalent in CAUTI. The fluoxetine antibacterial activity was explored and the lowest inhibitory concentration was determined. Recently, duloxetine had been evaluated against multi-drug resistant *E. coli* and

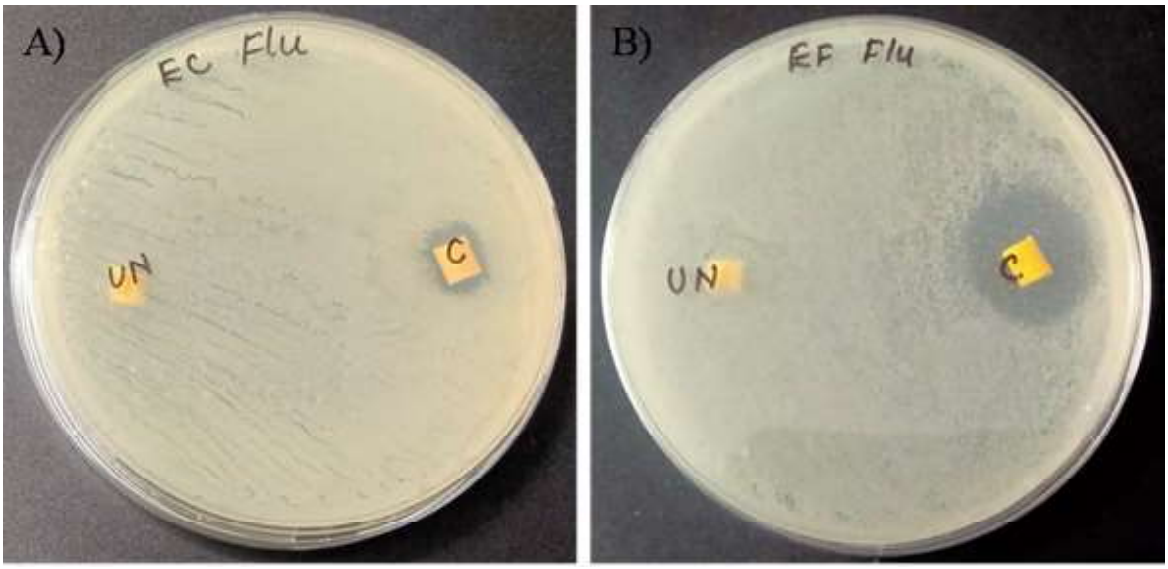

Figure 5. Antibacterial activity of fluoxetine coated catheter tube against A) *Escherichia coli*
B) *Enterococcus faecalis.* Note: UN- Uncoated, C- coated with drug fluoxetine

found to have excellent activity when combined with chloramphenicol.[24] Correspondingly, the antibacterial and anti-biofilm activity of the repurposed drug auranofin was investigated and the drug was recorded to possess potential activity against *Bacteroides fragilis*.[25] In the same way, the anti-depression drug sertraline was investigated for its antibacterial activity against *E. faecalis, Acinetobacter baumanii, Pseudomonas aeruginosa,* and *E. coli*.[26] Various repurposed drugs such as amodiaquine, curcumin, ibuprofen, ellagic acid, and quercetin were evaluated against two important *ESKAPE* pathogens and the evaluation revealed the antibacterial activity of curcumin and ellagic acid against *Staphylococcus aureus* and *Pseudomonas aeruginsa*.[27] The antiamoebic drug diiodohydroxyquinoline was repurposed for its antibacterial activity against *Clostridioides difficile*.[28] Likewise, mitomycin-c was repurposed for the antibacterial activity against *Klebsiella pneumoniae* and found to have enhanced antibacterial activity when combined with imipenem.[29]

In the present study, fluoxetine was studied for its effect on *E. faecalis* and *E. coli* colony formation which is the most important stage in biofilm formation. The insertion of a catheter may provide an entry for bacteria to initiate infection in the urinary tract by adhering to the catheter surface.[30] Once the bacteria are attached to the surface area, they can form a complex structure that protects the bacteria from external sources, such as host defence mechanisms resulting in the development of antibiotic resistance, making the management of CAUTI critical.[31] Therefore, the quest is to concentrate on each stage of biofilm formation, from adhesion to mature biofilm, while studying biofilm prevention.[32,33] Therefore, the effect of fluoxetine was studied on *E. faecalis* and *E. coli* colony formation and it was found that the drug was able to prevent colony formation of *E. faecalis* and *E. coli* on surfaces, and thereby inhibiting biofilm formation. Similarly, etoposide-A was repurposed for its anti-biofilm activity against *S. aureus* and has shown excellent activity in removing the biofilm which formed on hydroxyapatite.[34] Also, the drug penfluridol was repurposed for its antibacterial and anti-biofilm activities against *E. faecalis,*[35]with promising results.

Besides the antibacterial and antibiofilm activity, fluoxetine was studied for the effect of a coating on the silicone catheter tube which was evaluated against *E. faecalis and E. coli*. The coating of the catheter tube is an excellent approach to prevent the microbial colonization of uropathogens in the inner and outer surfaces of the tube. Hence, our study showed the efficacy of fluoxetine-coated silicone catheter tubes against *E. faecalis* and *E. coli* in the *in-vitro* bladder model which imitates the general process. The drug fluoxetine was able to suppress the growth of *E. faecalis and E. coli* which was represented by the clear zone of inhibition. Similarly, the antibacterial activity of silver nanocomposite-coated catheter tubes was studied against *E. coli* and *S. aureus* representing biofilm inhibition.[36] In another report by Abbott et al.[37] on repurposing fosfomycin, it was said that the drug showed excellent activity against *E. faecalis* in bladder infection *in-vitro* model. In short, fluoxetine can be used as an alternative for CAUTI when the mechanisms of action will be explored and *in vivo* efficacy of the drug is analyzed.

CONCLUSION

This study demonstrated that the antidepressant drug fluoxetine can make an excellent antibacterial drug and an anti-biofilm coating component. Further, *in vivo* analysis of fluoxetine is needed to determine the efficacy against CAUTI.

CONFLICT OF INTEREST

The authors declare that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization MMP; methodology and experiments MMP, MAA; result analysis and interpretation MMP, RS, MAA; writing of original draft RS, MAA; review and editing MMP. All authors have read and agreed to the published version of the manuscript.

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None.

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