Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 1, 2024, pp. 483-497.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

EFFECT OF CHITOSAN OR N-ACETYL CYSTEINE COMBINATIONS WITH SOME ANTIBIOTICS ON BIOFILM FORMATION ON INTRAUTERINE DEVICES

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An intrauterine device (IUD) is one of the most often used contraceptive tools. However, it represents a stable surface for microbial attachment and a perfect environment for the biofilm to grow and thrive. Thus, it might act as an infection reservoir of the reproductive system. This study aimed to isolate and identify microbes forming biofilms on IUDs and to provide some remedial measures to counteract biofilm formation. A total of 110 IUD samples were collected from women attending family planning clinics. They were treated to isolate and identify microorganisms forming biofilms by conventional methods. Biofilm assay was done using the tissue culture plate method to assess the isolates' degrees of biofilm formation and test the effect of selected antibiotics and antibiofilm agents on biofilm formation and disruption of preformed biofilm. A scanning electron microscope was also used to assess the effect of the tested agents on biofilm formation and disruption of preformed biofilms on IUD segments. 177 isolates were recovered from 110 IUDs including Candida spp. (51, 28.8%), Staphylococcus aureus (49, 27.7%), coagulase-negative Staphylococci (28, 15.8%), Pseudomonas spp. (21, 11.9%), E. coli (16, 9%) and Klebsiella spp. (12, 6.8%). Klebsiella spp. was the most biofilm producer, different isolates showed variable degrees of biofilm formation. The tested antibiotics exhibited remarkable inhibitory effects of biofilm formation either alone or in combination with Chitosan and N-acetyl cysteine as antibiofilm agents

Keywords: *Biofilm; Intra uterine device; Antibiofilm, Chitosan, N- Acetyl cysteine*

INTRODUCTION

The intrauterine devices (IUDs) are long-term, extremely effective contraceptive means with the advantage of reversibility. IUDs are commonly used in underdeveloped nations[1]. Similar to other medical implants, IUDs pose a risk for upper genital tract infection because they include foreign components that could serve as a breeding ground for biofilm[2]. Implant associated infections (IAIs) represent a big problem that may lead to increased mortality and is mostly brought on by biofilm formation on implant sites[3]. Many microorganisms are frequently involved in implant associated infections (IAIs) such as *Staphylococcus* spp.[4], *Pseudomonas aeruginosa*[5], *Enterococcus* spp.[6], *Escherichia coli*[7], and *Candida* spp.[8]

Implant associated infections are now treated by administering high-dose antibiotics in accordance with the infection's severity; if symptoms don't improve, implant removal will be required. As a result of biofilms and the rising incidence of bacteria that are resistant to conventional antimicrobial agents, conventional antimicrobial treatments are frequently unsuccessful at treating these

Received : 25/9/2023 & Accepted : 31/10/2023

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diseases[9]. Therefore, it is crucial to create novel methods to manage device colonization and biofilm growth.

Previous investigations have shown that bacteria within biofilms are more antibiotic-resistant than planktonic ones. Combination therapy is therefore preferred over the use of a single antibiotic[10]. Utilizing potent anti-biofilm compounds or biofilm-dissolving chemicals is a promising tactic[11]. Disturbance of biofilms by antibiofouling agents increase the antimicrobial sensitivity of bacteria associated with biofilms[12], Therefore, it is possible that combination of antimicrobial agents with antibiofilm agent would work together effectively[13].

Chitosan (CS) and its derivatives stood out among the newly evaluated substances for their broad-spectrum antibacterial action and efficiency against planktonic and biofilm cells[14].They were found to be effective against yeasts, filamentous fungi, gram-positive and gram-negative bacteria[15]. Due to its exceptional biological characteristics, including biodegradability, toxicity-freeness, allergy-free conduct, and antibacterial activity, CS as a biopolymer has gained significant interest for biomedical applications[16].

The ability of N-Acetyl cysteine (NAC), a well-known disulfide bond disrupter and antioxidant glutathione (GSH) prodrug, to stop the biofilm from adhering to the substrate makes it easier to administer antibiotics[17]. When used in conjunction with antibiotics to treat lower respiratory tract infections, N-acetylcysteine (NAC) which is a mucolytic drug, has been shown to have antibiofilm and antimicrobial activity[18,19].

In this work, biofilm forming microorganisms on IUDs were investigated, their antimicrobial profiles were assessed, and the effect of antibiotics either alone or in combination with CS or NAC on the inhibition of biofilm formation and disruption of preformed (mature) biofilms were evaluated in order to prevent and treat biofilms and their associated infections.

MATERIAL AND METHODS

Sample collection and processing

One hundred and ten cupper IUD samples were removed and collected from women tended to remove their IUDs at family planning clinic of Minia university hospital for obstetrics and gynecology, Minia, Egypt. They were submitted straight away to the microbiology lab, where each sample was put in 10 ml of pH-balanced brain heart infusion (BHI) broth and vortexed for 30s. After being gently mixed, suspensions were diluted in reduced BHI broth before being promptly plated as 100 µl of each dilution onto nutrient agar plates and incubated aerobically for 24-48 hours at 37°C. HiMedia, India, provided all of the media.

Identification of isolates

Isolates were recovered and identified using conventional methods[20].

Antibiotic sensitivity testing of the isolates

The antibiotic sensitivity testing was performed according to the guidelines of the CLSI[21] by the disk diffusion method using commercial disks including cefoperazone, cefipime, clindamycin, amoxicillin/clavulanic acid, amikacin, sulfamethoxazole/ trimethoprim, ciprofloxacin, Levofloxacin, and erythromycin. All discs were purchased from Oxoid, UK.

Detection of Biofilm formation by isolates using microtitre plate method

By using a crystal violet assay, isolates' capacity for and degree of biofilm development were found in vitro. Briefly, bacterial isolates were seeded into BHI supplemented with 0.25% glucose (BHIg) and placed on 96-well microtitre plates. The plates were then incubated for 18 to 24 hours at 35°C. To the wells containing CS or NAC solution that had been serially diluted twice with BHIg to obtain a final volume of 200 µL per well, a cell suspension calibrated to a 0.5 McFarland standard and a volume of 100 µL were added. Following incubation, the wells were evacuated and gently washed with sterile phosphate buffered saline, allowed to air dry, then stained for 30 min. with 200 µL of 0.01% crystal violet. Each well received three further washes with 200 µL[22].

Interpretations of biofilm production

All strains were categorized into the following categories based on Stepanovic et

al.'s[22] criteria: weak, moderate, and strong biofilm producers. All strains were divided into the following categories: weak biofilm producers; moderate biofilm producers; strong biofilm producers.

Detection of MIC50

Cefoperazone (Sigma, Egypt), Levofloxacin (Amoun, Egypt), both alone and in combination with CS (Sigma, Germany) and NAC (Sedico, Egypt), were investigated for their capacity to suppress the growth of biofilms. The Clinical and Laboratory Standards Institute (CLSI)'s microdilution method[21] was used to calculate the minimal inhibitory concentration (MIC50) for each drug. Standardized cell suspensions ($1x10^6$cells/ml in SGB) were planted in certain microtitre plate wells with cefoperazone, levofloxacin, chitosan, or N-acetyl cysteine. The optical density was determined at 600 nm after a 24-hour incubation period at 37°C. The negative and blank controls were medium alone and phosphate buffered saline (PBS; pH 7.2). The MIC was considered to be the lowest concentration that caused growth to be reduced by 50%.

Determination of the effect of antibiotics alone and if combined with antibiofilm agents on the biofilm formation (adherence)

Effect of combination of antibiotics and antibiofilm agents on biofilm formation was assessed using microtiter plate test[22]. 200 µL of test solutions (cefoperazone or levofloxacin alone or in combination with chitosan or N-Acetyl Cystiene) were added to wells in a 96-well microplate at MIC50 concentrations and inoculated at 1% (v/v). The proper media supplemented with 5% sucrose was added, each experiment was carried out in triplicate and incubation was done for 24 hours at 37 °C. By using crystal violet assay, the formation of the biofilm was measured. As negative controls, optical density data from wells with liquid media, medication solutions, and no inoculums were used, whereas positive controls included OD from wells with deionized water, inoculums and liquid media. Results were attained by using the formula below:

% biofilm formation inhibition
= 100 – (OD assay/OD control) × 100

Determination of the effect of antibiotics alone and if combined with antibiofilm agents on disruption of preformed (mature) biofilms

Effect of antibiotics and antibiofilm agents on preformed (mature) biofilms was assessed using the protocol of Stepanovic *et al*.[23]. A 96-well microplate was used, each well received 200 µL of media that was inoculated at 1% (v/v) and incubated for 48 hours at 37 °C. With great care, the medium was aspirated after the 48 hours, and the wells were then cleaned with phosphate buffer. Then, 200 µL of media containing the medication combination was added, and incubated at 37 °C for 24 hours. The crystal violet assay was used to measure the formation of the biofilm. As a positive control, media containing sterile deionized water was employed, whereas medium was considered as a negative control. The following formula was used to get the desired outcomes:

Mature biofilm reduction percentage
= 100 – (ODassay/ODcontrol) × 100

Scanning Electron Microscopy (SEM)

Testing the effect of combination of antibiotics and antibiofilm agents on adherence and mature biofilms using scanning electron microscope was done as follows: IUD segments were employed as a surface to investigate how the drugs affected bacterial biofilm formation. IUD segments (1 cm in length) were placed in 5 ml of trypticase soy broth containing $5x10^6$ cfu/ml of *pseudomonas aeruginosa* for 90 minutes in order to test the medicines' capacity to prevent the growth of biofilm. Each tube was then supplemented with one of the following medications (cefoperazone alone, CEP/NAC, or CEP/chitosan) at MIC50 concentration, and incubated at 25°C for 24 hours[24]. IUD segments were cultured with bacterial cultures at 25°C for 48 hours to determine how the tested drugs affected the mature biofilms that had already formed. Pieces were inserted in new test tubes containing TSB medium supplemented with (cefoperazone alone, CEP/NAC, and CEP/chitosan) at MIC50 concentration after incubation, and they were then twice-washed with normal saline without disrupting the biofilms. Control tubes received normal saline addition and were incubated for 24 hours.

Scanning Electron microscope Examination

IUD segments were fixed for 1.5 hours in 2.5% (vol/vol) glutaraldehyde in Dulbecco PBS (PH 7.2), washed with PBS, and then dehydrated using a succession of ethanol solutions. Samples were coated in gold-palladium after being dried. On a JSM-840 SEM (JEOL Ltd., Tokyo, Japan), SEM investigations were conducted[24].

Statistical analysis

One way ANOVA test was used to evaluate significant differences between the percentages of inhibition of biofilm formation and destruction of preformed (mature) biofilms by antibiotics alone and after combination with chitosan and NAC. The test was done using SPSS, 20 software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Results

A total of 177 isolates were recovered from 110 IUDs. 101 (91.8 %) IUDs showed positive cultures, while 9 (8.2 %) was negative in culture. Out of 101 IUDs, 56 (55.4%) samples showed mixed infections while 45 (44.6 %) showed single isolate. *Candida spp.* (51, 28.8%) was the most prevalent isolated microorganism followed by *Staphylococcus aureus* (49, 27.7%), *coagulase negative Staphylococci* (28, 15.8%), *Pseudomonas* spp. (21, 11.9%), *E. coli* (16, 9%) and *Klebsiella* spp. (12, 6.8%).

Antibiotic sensitivity testing

S. aureus had highest resistance percentage to amoxicillin/clavulanic acid (42.8%) while coagulase negative *Staphylococci* showed the highest resistance to erythromycin (50%) as shown in **Fig. (1).** *E. coli* was highly resistant to Amoxicillin/clavulanic acid (62.5%), *Klebsiella* spp. showed the highest resistance to cefepime (58.3%) and *Pseudomonas* spp. was mostly resistant to ciprofloxacin (19%) as shown in **Fig. (2).**

Detection of Biofilm formation by isolates using microtitre plate method:

Results revealed that 151 of 177 (88.7%) isolates exhibited the capacity to produce biofilms. Isolates showed different degrees of biofilm formation. 106 (59.9%) were high biofilm formers, 45 (25.4%) were moderate biofilm formers and 26 (14.7%) were weak (non biofilm) formers. The majority of bacteria that produced biofilms were *Klebsiella* spp. (100%), followed by *Pseudomonas* spp. (95.2%), *Candida* spp. (88.2%), *S. aureus* (87.7%), coagulase negative *staph* (71.4%), and *E. coli* (68.7%). **Fig. (3)** displayed the distribution of different degrees of biofilm formation among the tested isolates.

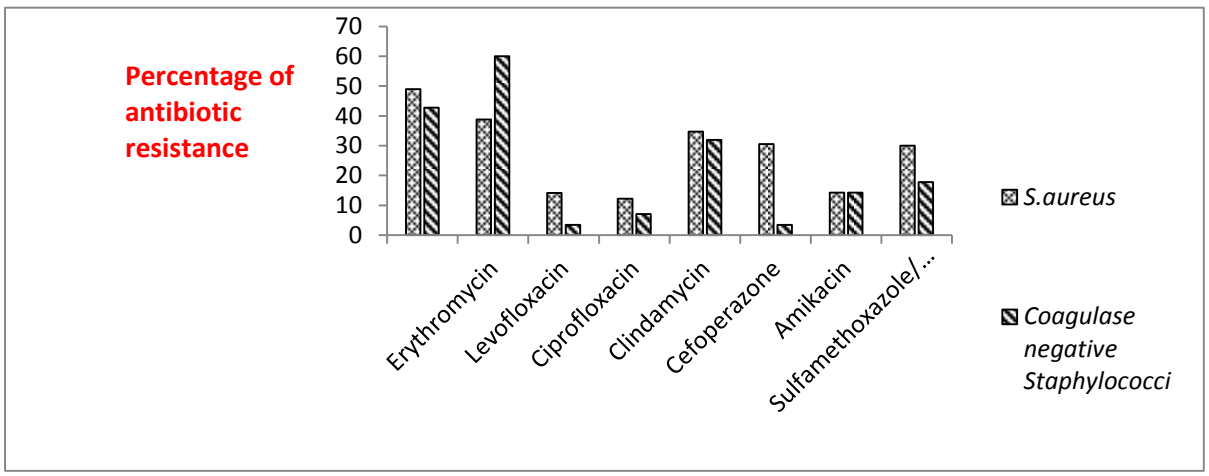


Fig.1: Percentage of antibiotic resistance among isolated gram positive bacteria.

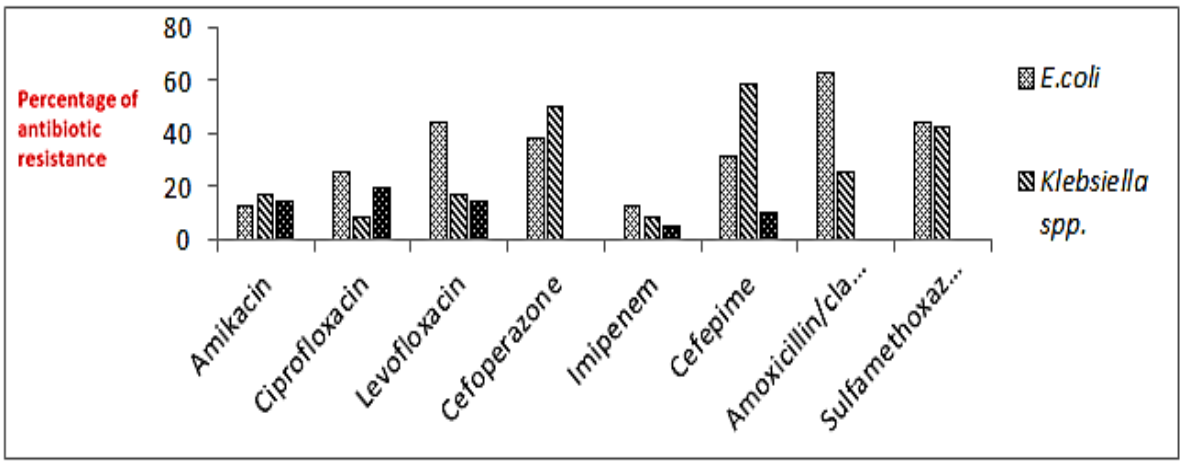


Fig.2: Percentage of antibiotic resistance among gram negative isolated bacteria.

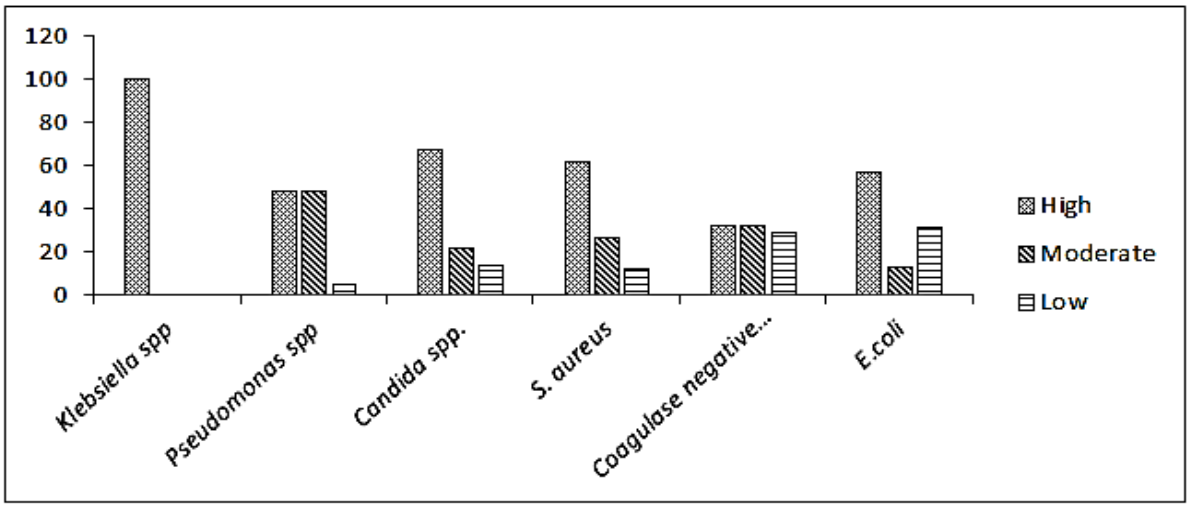


Fig.3: Distribution of biofilm formation degrees among the tested isolates.

degrees among the tested isolates

Determination of MIC50

MIC50 values for antibiotics (levofloxacin and cefoperazone) and antibiofilm agents (chitosan and N-acetyl cystiene) are listed in **Table (1)**.

Determination of the effect of antibiotics alone and if combined with antibiofilm agents on the biofilm formation (adherence) and on disruption of mature biofilms

It was observed that the tested antibiotics alone reduced the biofilm adherence of the tested isolates and their reduction ability was increased by combination with the tested antibiofilm agents as shown in **Table (2). Table (3)** showed the reduction effect of the antibiotics alone and in combination with antibiofilm agents on the preformed or mature biofilms. **Table (4)** showed comparison of the effects of different antibiotics and combinations.

Scanning Electron Microscopy (SEM)

SEM photos demonstrated how the tested drugs affected the morphology of the cells and the biofilm mass texture. Cefoperazone alone gave a good effect on the adherence and the preformed (mature) biofilm. Combination of CEP with NAC and CS increased the inhibition of biofilm formation on IUD surface **Fig. (4).**

Table 1 : MIC50% of used drugs for tested isolates.

Drug	MIC50% (µg/ml)
Levofloxacin	512
Cefoperazone	1024
N-Acetyl Cystiene	20
Chitosan	7

Table 2: Effect of antibiofilm agents and antibiotics on the biofilm adherence of isolates.

Microorganism	Drug at MIC50	% of Reduction
S. aureus	CEP	63
	CEP/NAC	87
	CEP/ CS	80
	Levo	59
	Levo/NAC	86
	Levo/CS	62
Coagulase –ve *staph.*	CEP	68
	CEP/NAC	100
	CEP/ CS	100
	Levo	53
	Levo/NAC	66
	Levo/CS	62
***Pseudomonas* spp.**	CEP	60
	CEP/NAC	78
	CEP/CS	84
	Levo	53
	Levo/NAC	87
	Levo/ CS	60
E. coli	CEP	56
	CEP/NAC	83
	CEP/ CS	93
	Levo	57
	Levo/NAC	75
	Levo/CS	65
***Klebsiella* spp.**	CEP	68
	CEP/NAC	100
	CEP/ CS	85
	Levo	53
	Levo/NAC	96
	Levo/CS	61

CS: Chitosan, NAC: N-acetyl cysteine, Levo: Levofloxacin, CEP: cefoperazone.

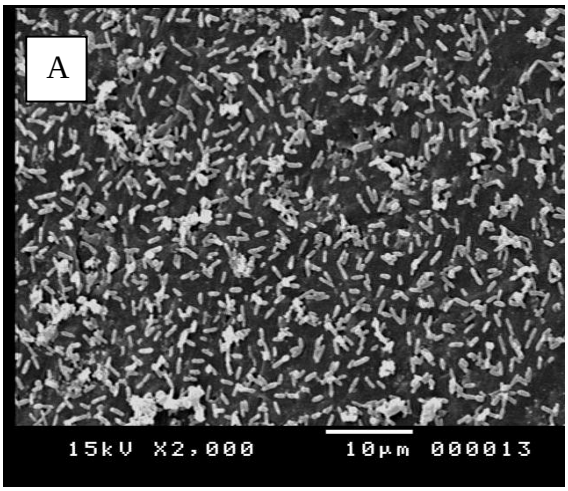
Table 3: Effect of antibiotics and antibiofilm agents on preformed (mature) biofilm**.**

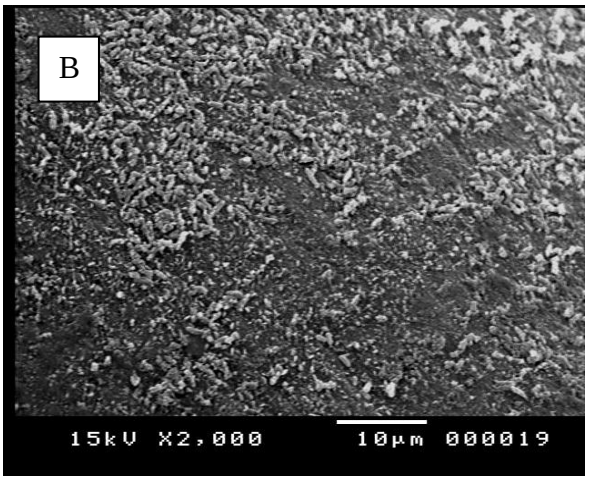
Microorganism	Drug at MIC50	% of Reduction
S.aureus	CEP alone	53
	CEP/NAC	76
	CEP/ CS	60
	Levo alone	66
	Levo/NAC	81
	Levo/CS	72
Coagulase–ve *staph.*	CEP alone	53
	CEP/NAC	78
	CEP/ CS	62
	Levo alone	52
	Levo/NAC	75
	Levo/CS	65
Pseudomonas spp.	CEP alone	55
	CEP/NAC	76
	CEP/ CS	60
	Levo alone	54
	Levo/NAC	89
	Levo/CS	60
E.coli	CEP alone	56
	CEP/NAC	79
	CEP/ CS	63
	Levo alone	59
	Levo/NAC	80
	Levo/CS	63
Klebsiella spp.	CEP alone	65
	CEP/NAC	77
	CEP/ CS	91
	Levo alone	58
	Levo/NAC	98
	Levo/CS	64

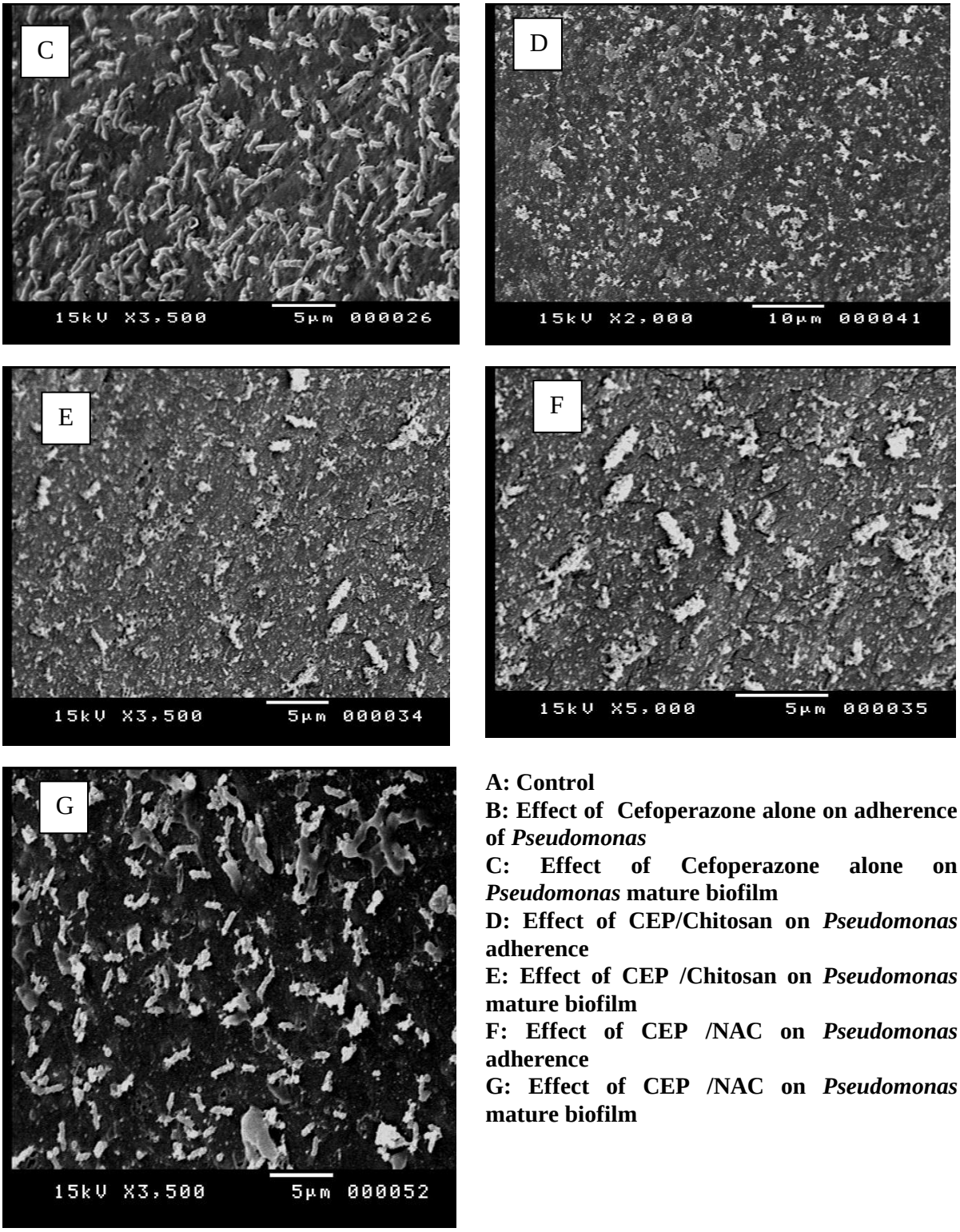
Table 4: Comparison between effects of antibiotics and their combinations with CS and NAC.

Inhibition of adherence		**Sig**	**Disruption of mature biofilm**		**Sig**
CEP	CEP/NAC	.000	CEP	CEP/NAC	.002
	CEP/ CS	.000		CEP/ CS	.239
	Levo alone	.555		Levo alone	1.000
	Levo/NAC	.006		Levo/NAC	.000
	Levo/CS	1.000		Levo/CS	.498
CEP/NAC	CEP	.000	CEP/NAC	CEP	.002
	CEP/ CS	1.000		CEP/ CS	.313
	Levo	.000		Levo	.005
	Levo/NAC	.607		Levo/NAC	.628
	Levo/CS	.000		Levo/CS	.130
CEP/ CS	CEP	.000	CEP/ CS	CEP	.239
	CEP/NAC	1.000		CEP/NAC	.313
	Levo	.000		Levo	.378
	Levo/NAC	.757		Levo/NAC	.013
	Levo/CS	.000		Levo/CS	.995
Levo	CEP	.555	Levo	CEP	1.000
	CEP/NAC	.000		CEP/NAC	.005
	CEP/ CS	.000		CEP/ CS	.378
	Levo/NAC	.000		Levo/NAC	.000
	Levo/CS	.684		Levo/CS	.679
Levo/NAC	CEP	.006	Levo/NAC	CEP	.000
	CEP/NAC	.607		CEP/NAC	.628
	CEP/ CS	.757		CEP/ CS	.013
	Levo	.000		Levo	.000
	Levo/CS	.004		Levo/CS	.004
Levo/CS	CEP	1.000	Levo/CS	CEP	.498
	CEP/NAC	.000		CEP/NAC	.130
	CEP/ CS	.000		CEP/ CS	.995
	Levo	.684		Levo	.679
	Levo/NAC	.004		Levo/NAC	.004

Difference is considered significant at the level of 0.05 or less.







A: Control
B: Effect of Cefoperazone alone on adherence of *Pseudomonas*
C: Effect of Cefoperazone alone on *Pseudomonas* mature biofilm
D: Effect of CEP/Chitosan on *Pseudomonas* adherence
E: Effect of CEP /Chitosan on *Pseudomonas* mature biofilm
F: Effect of CEP /NAC on *Pseudomonas* adherence
G: Effect of CEP /NAC on *Pseudomonas* mature biofilm

Fig. 4: Effect of antibiotics either alone or in combination on *Pseudomonas* biofilm on IUD surface.

Discussion

The most popular way of preventing conception is using intrauterine devices (IUDs)[25]. Chronic wounds linked to diabetes, cardiovascular disease, and IUD infections are now included in the list of illnesses caused by bacterial biofilms[26].

In this study, when IUD samples were screened for biofilm forming microorganisms, *Candida spp.* (28.8%) was the most prevalent microorganism followed by *Staphylococcus aureus* (27.7%), *coagulase negative Staphylococci* (15.8%), *Pseudomonas* spp. (11.9%), *E. coli* (9%) and *Klebsiella* spp.

(6.8%). Different results were obtained by where IUDs were predominantly composed of *Escherichia coli* (27%), *Candida albicans* (20%), *Staphylococcus epidermidis* (18%), *Staphylococcus aureus* (16%), *Candida dubliniesis* (12%), *Pseudomonas aeruginosa* (5%), and *Neisseria gonorrhoeae* (2%)[27]. Al-Kattan *et al.*,[28], found that *E. coli* was found to be most isolated bacteria (61.5%) followed by *staphylococcus aureus* 43.6%, *Pseudomonas* spp. 15.3%, *candida albicans* 10.3%, and *Neisseria gonorrhoea* 5.1%. Another study detected different prevalence of microorganisms as, *E. coli* (24.1%), *Enterococcus faecalis* (23.2%), *Candida* spp.(18.9%), *Staph aureus* (16.4*%),* Coagulase negative *staph* (9.5%), *Klebsiella pneumonia* (5.2%) *and Proteus* (2.6%)[29]**.**

In the current study, it was found that 100% of *Klebsiella* isolates were biofilm formers while 95.2% *Pseudomonas spp.*, 87.7% *S. aureus*, 71.4% of coagulase negative *staph*., and 68.7% *E. coli* had biofilm formation ability. Study of Mishra *et al.,*[30] using TCP method found that 30% *Klebsiella,* 27.3%, *S. aureus,* 18.2% *S. epidermidis,* 15% of *E. coli,* and10% *Pseudomonas* had biofilm formation ability. Also, Hassan *et al.,*[31], found that the biofilm forming ability of isolates was 52.9% for *Staphylococcus epidermidis,* 46.6% *Escherichia coli,* 35.2% *Staphylococcus aureus,* and 30% *Klebsiella pneumonia.*

The majority of implantable medical devices are vulnerable to microbial adhesion and the development of biofilms, which is the main factor in implant-associated infections[32]. A promising method to prevent planktonic cells from adhering to implant surfaces initially is to coat implants with antibacterial and antibiofilm chemicals. A promising method to prevent planktonic cells from adhering to implant surfaces initially is to coat implants with antibacterial and antibiofilm chemicals. There are several methods for creating antibiofilm coatings with both natural and manmade materials[33].

In this study, biofilm assay using TCP method revealed that levofloxacin reduced biofilm adherence at 512 µg/ml by 57% for *E. coli* and by 53% for *Klebsiella* and *Pseudomonas* while study of[34], reported that levofloxacin inhibited biofilm adherence by 65% for *E. coli* at concentration of 32 µg/ml, 65% for *Klebsiella* at 2 µg/ml and 75% for *Pseudomonas* at 8µg/ml. Also in the current study, levofloxacin had reducing effect on mature biofilm at 512 µg/ml by 59%, 58% and 54% for *E. coli*, *Klebsiella* and *Pseudomonas* respectively. El-Gebaly *et al.,*[35], demonstrated that levofloxacin reduced mature biofilms up to 70% for *E. coli* at 256 µg/ml, 65% for *Klebsiella* at 8 µg/ml and 63% for *Pseudomonas* at16 µg/ml.

Many natural substances have been used in recent years in an effort to stop infections linked to biofilms. Among the many such substances were lectins[35], and chitosan (CS)[36]. In example, investigations including Ex vivo and in vivo interactions with CS in diverse forms have examined CS as an antibacterial agent against a wide range of species, such as bacteria, yeasts, and fungus[37]. According to selected studies, CS antimicrobial coatings were developed for use on a variety of implantable medical devices, including central venous catheters (17.5%), orthopaedic implants (15.0%), and urinary catheters (12.5%)[38], But no studies were done on IUDs.

The current work confirmed that chitosan acheived a remarkable reduction of the adherence and eradication of mature biofilms of all bacterial isolates, when combined with levofloxacin and cefoperazone. This result is on accordance with some previous studies. For example, Tin *et al.,*[39] confirmed the synergistic effect of chitosan with sulfamethoxazole for improving antibiotic activity against *Pseudomonas aeruginosa* biofilms. Zhang *et al.,*[40], tested streptomycin combination with chitosan, this combination could effectively destroy established or preformed biofilms and inhibit biofilm formation by Gram-positive bacteria. Mu et al.,[41] noticed that *Listeria monocytogenes* biofilms were dispersed after short or long-term treatment with a chitosan-gentamicin mixture. Mu *et al.,*[42] revealed that the ability of chitosan to inhibit or disrupt *L. monocytogenes* biofilms was increased when paired with an aminoglycoside antibiotic such amikacin, but not with clindamycin, vancomycin, or erythromycin.

Study of Asli *et al.,*[12] revealed that chitosan had ability to prevent the development of *S. aureus* biofilm at a concentration of up to 16 mg/ml, also it exhibited synergy with erythromycin as well as with ciprofloxacin.

Breser *et al.*,[43] stated that antibiotic effectiveness against several coagulase-negative Staphylococcus lifestyles was enhanced by the combination of chitosan and cloxacillin. The combination approach not only increased preformed biofilm eradication and increased bacterial biofilm inhibition, but it also decreased intracellular bacterial viability. Lu *et al.*,[44] compared levofloxacin alone, with combination of levofloxacin with chitosan, they reported that such combination had a greater ability to disrupt *Salmonella* biofilms and reduce bacterial burden in organs.

Antibiotics are currently used in treating of infections associated with biofilms, but the biofilm matrix is not specifically targeted[45]. So in this study we tried using N-acetyl-L-cysteine (NAC) as a matrix-disruptive agent to ease and potentiate the effect of antibiotics. We noticed that combination of NAC with both levofloxacin and cefoperazone markedly increased the inhibition of initial biofilm formation and mature biofilms of all tested isolates. This agree with previous reports which stated that NAC has been demonstrated to have beneficial modulatory effects when used with a number of widely used antibiotics[46].

Marchese *et al.*,[47] reported that early and preformed biofilms were reduced by 66.80% and 60.73%, respectively, when fosfomycin and NAC were used in combination. Combinations had a greater impact than either element acting alone. El-Feky *et al.*,[48] tested the effect of ciprofloxacin combined with NAC in combination on both biofilm formation and pre-formed biofilms on ureteral stents by various Gram-negative and Gram-positive bacteria, this combination achieved much higher inhibitory effect than that acheived by individual drugs.

Moon *et al.*,[49] observed that rather than eliminating the already-existing bacterial biofilm, NAC may be utilized to prevent *Prevotella intermedia* from forming one. Eroshenko *et al.*,[50] found that NAC and rifampicin have a synergistic impact against staphylococcal biofilms. In a study done by Feng *et al.*,[51], Combining NAC with tigecycline had a partial synergistic effect on planktonic cells and a synergistic effect on *A. baumannii* embedded in biofilms. In a study done by Pijls *et al.*,[52], a synergistic effect resulted from combination of NAC with vancomycin and vibramycin with heating, resulted in the complete eradication of the *staphylococcus aureus* biofilm.

Manoharan *et al.*,[53] study revealed that combination treatments with NAC and oxacillin, teicoplanin, or amoxicillin/clavulanate, caused biofilms to be disrupted by over 90% in both MRSA and MSSA strains. NAC showed synergistic effect with the tested antibiotics. Additionally, combining antibiotics can lower the effective dose. Aiyer et al.,[54] tried combination of colistin, ciprofloxacin, and tobramycin with NAC against *Stenotrophomonas*, Burkholderia, and *Achromobacter* spp. both Planktonic cells and mature biofilms. They observed significant reduction in both planktonic and biofilm forms.

Pinto *et al.*,[45], observed the effect of combination of moxifloxacin and NAC nanosystems, they observed that such combination increased antibiofilm effect towards biofilms of *S. aureus.* Valzano *et al.*,[55] reported that NAC/ Colistin combination showed relevant antibiofilm synergistic activity on *P. aeruginosa* biofilms.

Although the exact mechanism of action is unknown, NAC's impact on biofilms can be broken down into three categories: (i) antibacterial characteristics; (ii) biofilm detachment; and (iii) inhibition of bacterial adhesion and extracellular polysaccharide (EPS) formation. Numerous studies conducted in the past attested to the effectiveness of NAC as an and an anti-biofilm and antibacterial against various species, particularly *Pseudomonas aeruginosa* biofilms[56]. This study's Scanning electron microscope (SEM) results revealed the effectiveness of combination of antibiotics with NAC to combat *Pseudomonas aeruginosa* biofilms on IUD surfaces.

Conclusion

Many bacterial species are included within biofilms formed on IUDS. NAC and Chitosan showed a great synergistic activity with antibiotics against biofilm formation and preformed biofilms for both Gram-negative and Gram-positive bacteria. NAC had stronger effect than chitosan in increasing antibiotic effect on both initial adherence and preformed

biofilms. It will be recommended to use these agents as adjuvants with antibiotics to treat implant associated infections (IAIs) as they help to disrupt biofilms, potentiate the antibiotic action and decrease the dose and side effects of antibiotics.

List of abbreviations

Abbreviation	Complete text
CS	Chitosan
GSH	Glutathione
IAIs	Implant associated infections
IUD	Intrauterine device
NAC	N-Acetyl-cysteine
SEM	Scanning Electron Microscope

Authors' contributions

The study's inception and design involved input from all authors. Heba Ahmed Mohamed and Gehad Mostafa Elheiny handled the preparation of the materials, the gathering and analysis of the data. Gehad Mostafa Elheiny and Heba Ahmed Mohamed wrote the first draft of the manuscript. The final manuscript was read and approved by all writers.

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Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 1, 2024, pp. 483-497.

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تأثير اضافة الشيتوزان أو أن اسيتيل السيستين مع المضادات الحيوية على تكوين الأغشية الحيوية على الأجهزة داخل الرحم

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يعد الجهاز الرحمي (اللولب) أحد أدوات منع الحمل الأكثر استخدامًا. ومع ذلك، فهو يمثل سطحًا مستقرًا للالتصاق الميكروبي وبيئة مثالية لنمو الأغشية الحيوية وازدهارها. وبالتالي، قد يكون بمثابة خزان للعدوى في الجهاز التناسلي. هدفت هذه الدراسة إلى عزل وتحديد الميكروبات التي تشكل الأغشية الحيوية على اللوالب الرحمية وتقديم بعض التدابير العلاجية لمواجهة تكوين الأغشية الحيوية. تم جمع ما مجموعه ١١٠ عينة من اللوالب من النساء المترددات على عيادات تنظيم الأسرة. وتم علاجهم لعزل وتحديد الكائنات الحية الدقيقة التي تشكل الأغشية الحيوية بالطرق التقليدية. تم إجراء مقايسة الأغشية الحيوية باستخدام طريقة لوحة زراعة الأنسجة لتقييم درجات تكوين الأغشية الحيوية المعزولة واختبار تأثير المضادات الحيوية المختارة وعوامل المضادات الحيوية على تكوين الأغشية الحيوية وتعطيل الأغشية الحيوية المشكلة. تم أيضًا استخدام المجهر الإلكتروني الماسح لتقييم تأثير العوامل التي تم اختبارها على تكوين الأغشية الحيوية وتعطيل الأغشية الحيوية المشكلة مسبقًا على شرائح اللولب. تم استخراج ١٧٧ بكتيريا من ١١٠ لولب بما في ذلك(٢٨.٨% ، ٥١.) كانديدا، (٤٩، ٢٧.٧%)، المكورات العنقودية الذهبيةو (٢٨، ١٥.٨%)، المكورات العنقودية سلبية التخثر (٢١، ١١.٩%)، سودوموناس ايروجينوسا و(١٦، ٩%) الإشريكية القولونية و (٦.٨% ، ١٢). كانت الكليبسيلا الأكثر إنتاجًا للأغشية الحيوية، وأظهرت العزلات المختلفة درجات متفاوتة من تكوين الأغشية الحيوية. أظهرت المضادات الحيوية التي تم اختبارها تأثيرات مثبطة ملحوظة لتكوين الأغشية الحيوية إما بمفردها أو بالاشتراك مع الشيتوزان والان-أسيتيل سيستين كعوامل مضادة للأغشية الحيوية.