

Effect of Levofloxacin and Vitamin C on Bacterial Adherence and Preformed Biofilm on Urethral Catheter Surfaces

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Abstract

The effect of levofloxacin (LEV) and vitamin-C (VIT-C) individually or in combinations on initial bacterial adherence and pre-formed (mature) biofilms on the surface of urethral catheters was studied. The isolated and studied uropathogens in the present study showed considerable diversity, where the major pathogens were identified as *E.coli*, *Klebsiella sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Proteus sp.* and *Pseudomonas sp.* Using the static adherence assay, addition of LEV and VIT-C each alone at sub MIC concentrations (0.25 MIC and 0.5 MIC) and (80 and 100 mg ml⁻¹) respectively, reduced the initial adherence ability of bacteria to the catheter by 35-94%. Besides, the inhibitory effect on the mature biofilm was estimated to be 40-90% in the presence of (MIC and 2MIC) LEV or (80 and 100 mg/ml) VIT-C. The highest inhibitory effects on both the initial adherence and mature biofilm were recorded when 0.5 MIC levofloxacin was added. Combination of LEV and VIT-C significantly increased the inhibitory effect of both initial biofilm formation and the mature biofilm to 80-100%. In addition, scanning electron microscope (SEM) was used to verify the effect of the tested drugs on biofilm production. The obtained results confirmed the significant role of this combination in inhibition of urethral catheter biofilm formation.

Keywords: Ascorbic acid (vitamin-C); Biofilm; Fluoroquinolones; Levofloxacin; Urinary tract

Introduction

Catheter associated urinary tract infection (CAUTI) is one of the most common types of hospital acquired infection [1,2]. Several studies have confirmed its significant contribution in increasing the morbidity, mortality, hospital stay and costs [3]. The normal function of the urinary tract is usually altered by the presence of a catheter and this makes it easier for bacteria to become established in the bladder and cause serious infection [1]. A wide range of persistent catheter related infections may be related to the ability of bacteria to form biofilms [4,5]. In this regards, the treatment of device related infections with conventional antimicrobial agents frequently fails because microorganisms growing in biofilms are more tolerant or phenotypically resistant to antimicrobial agents than planktonic (free) cells [4]. A mature biofilm can even tolerate antibiotics at concentrations of 10-1000 times more than required to kill planktonic bacteria [6]. In this regard, fluoroquinolones (The most common clinically used members of the quinolones antibiotics) have been reported as an effective treatment in case of both young and mature biofilms because of their good penetrative qualities [7]. Norrby et al. [8] have reported that among the recently developed fluoroquinolones, levofloxacin is widely used in clinical practice and it is less likely to select resistant strains compared with other group members. Therefore, levofloxacin represent a promising candidate to treat tedious infections such as those of biofilm forming bacteria [9].

However, the use of levofloxacin alone may jeopardous its high activity to fast development of resistance. Fortunately, several reports have stated that the antibacterial effect of levofloxacin was enhanced by ascorbic acid (VIT-C) [10]. Interestingly, Habash et al. [11] have reported that the use of ascorbic acid (VIT-C) provided a degree of protection against adhesion by some uropathogens and colonization of biomaterials utilized within the urinary tract. Therefore, it is conceivable that an antibiofilm/antimicrobial agent combination would be synergistic [12].

In this prospective, this study investigated the adherence capacity of common isolated uropathogens on urinary catheters. The effect of levofloxacin and vitamin-C either individually or in combinations on the adherence ability as well as the mature formed biofilms by uropathogens on urinary catheters was evaluated.

Materials and Methods

Unless otherwise specified, all tests were conducted under aseptic conditions and in triplicates and the presented data is mean \pm standard error.

Uropathogens

Previously, uropathogenic bacterial isolates were collected from catheters and stents from 115 inpatients at Beni Seuf University hospital. All isolated uropathogens in the present study were subjected to systematic identification based on morphological characteristics and biochemical reactions using API Test Kits (20E and 20NE API, bioMerieux, France; data not shown). The antibiogram and minimum inhibitory concentration (MIC) for all isolates were determined (data not shown). Among the isolated and preliminary identified bacterial uropathogens, 2 isolates of each common species (*E.coli*, *Klebsiella sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Proteus sp.* and *Pseudomonas sp.*) were selected to be used in this study. Selection was based on the pre-

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determined MIC in order to have one with the highest MIC and the other with lowest MIC (Table 1). All isolates were stored on slants of corresponding culture media at 4°C according to [13].

Drugs

Stock solutions of levofloxacin (5000 µg/ml) and Vitamin-C (1000 mg/ml) were prepared in sterile distilled water. Both drugs were purchased from Sedico, Egypt and stored according to manufacturer instructions.

Determination of minimum inhibitory concentrations (MIC) of levofloxacin

Minimum inhibitory concentrations of levofloxacin were determined using the microbroth dilution method according to Clinical Laboratory Standard Institute (CLSI 2009) [14].

Effect of levofloxacin and vitamin-C (alone and in combination) on initial biofilm formation and on the preformed biofilm

Bacterial cultures in 5 ml tryptic soy broth (TSB) of the tested uropathogens were diluted 100 times with fresh TSB and distributed as portions of 5 ml into test tubes. One of the following solutions: LEV (0.25 MIC and 0.5 MIC), VIT-C (80 and 100 mg/ml) and LEV/VIT-C (0.25 MIC/80 mg/ml and 0.5 MIC/100 mg/ml) were added to each tube as triplicates. One set of seven pieces of urethral catheters segments (each of 1 cm length) was added to each tube and all tubes were statically incubated at 37°C for 24 h. After incubation, catheter segments were rinsed 3 times with phosphate buffer saline (PBS, pH 7.2), placed in 10 ml fresh saline and sonicated for 30 seconds to dislodge the adherent cells. The sonicated saline was serially diluted and cultured on tryptic soy agar plates and the number of adherent bacteria that indicates degree of adherence was determined by the viable count technique [15]. Control was conducted by addition of normal saline and treated in the same regimen, with the addition of saline in lieu of TSB. The effect of the tested agents on the pre-formed (mature) biofilm was tested by incubating seven pieces of the 1cm urethral catheter segments in test tubes containing the selected bacterial suspension and 5 ml of TSB to allow biofilm formation. After incubation at 37°C for 24 h., segments were removed and rinsed 3 times with (PBS, pH 7.2) to remove any non-adherent bacteria. Catheter segments were then suspended for 24 h at 37°C in one of the following treatment solutions: saline (control), levofloxacin (MIC and 2 MIC), vitamin-C (80 and 100 mg/ml) and LEV/VIT-C (MIC/80 and 2 MIC/100 mg/ml). After incubation, the number of viable adherent cells was determined as previously described.

Scanning Electron Microscopy (SEM)

Catheter segments were fixed in 2.5% (v/v) glutaraldehyde in PBS (pH 7.2) for 1.5 h, rinsed with PBS and then dehydrated through an ethanol series. Samples were dried and gold-palladium coated. SEM examinations were made on a JSM-840 SEM (JEOL Ltd., Tokyo, Japan) [13].

Statistical analysis

One-way ANOVA was employed to evaluate any significant difference between the values obtained without the drug (controls) and the values obtained in the presence of different drug concentrations. Differences were done using INSTAT statistical software (GraphPad Instant V 2.03, GraphPad Software Inc., USA).

Species	Isolate No.	MIC of levofloxacin (µg ml ⁻¹)
<i>E. coli</i>	27	128
	98	4
<i>Klebsiella sp.</i>	56	4
	58	2
<i>Citrobacter sp.</i>	22	2
	77	64
<i>Enterobacter sp.</i>	29	64
	94	2
<i>Proteus sp.</i>	102	16
	131	128
<i>Pseudomonas sp.</i>	86	2
	133	16

Table 1: MIC's of levofloxacin of the tested uropathogens.

Results

Effect of levofloxacin and/or vitamin-C on biofilm production

Levofloxacin at 0.25 MIC inhibited the initial adherence of all tested bacterial uropathogens. The recorded inhibition was widely ranged from 40-77% (Table 2). This inhibitory effect was increased with different extents when the concentration increased to 0.5 MIC. Similarly, VIT-C at 80 mg/ml showed also an inhibitory effect ranging from 60-80% on all tested bacterial uropathogens. Again this inhibitory effect was slightly increased by increasing VIT-C concentration to 100 mg/ml. Combination between 0.25 MIC of (LEV) and 80 mg/ml of (VIT-C) inhibited the bacterial adherence of all tested isolates with almost the same range as VIT-C alone. However, when the concentration increased to 0.5 MIC and 100 mg/ml, the recorded inhibitory effect was always above 90 %. In most cases, upon combination of LEV and VIT-C (0.25 MIC/80 mg/ml), the inhibitory effect on the biofilm formation ability was increased (Table 2). The highest inhibitory effect was observed on *Proteus sp.*131 while the lowest inhibitory effect (60.7%) was observed on *Enterobacter sp.* 94. When the concentration of LEV/VIT-C combination was increased to 0.5 MIC/100 mg/ml, the inhibition was always above 90% and in 2 cases complete inhibition was recorded. Interestingly, the same pattern of inhibitory effect was always consistent regardless the sensitivity or resistance of the tested uropathogens. For instance, the highest inhibitory effect on *E. coli* 27 (most resistant isolate) and *Pseudomonas sp.* 86 (most sensitive isolate) was recorded when a combination of 0.5 MIC/100 mg/ml VIT-C was used (Figure 1). Again, the lowest inhibitory effect against both bacteria was recorded when 0.25 MIC LEV was used alone.

Effect of levofloxacin and vitamin-C on the pre-formed (mature) biofilm

No significant inhibitory effect was recorded on all tested uropathogens when 0.25 and 0.5 MIC concentrations were tested (data not shown); therefore, levofloxacin was tested at higher concentrations (MIC and 2 MIC). Application of LEV at MIC inhibited the mature biofilm by a range of 20-65% (Table 3). Increasing this concentration to 2 MIC increased the inhibitory effect to different extents (up to 91%). Similarly, VIT-C at 80 mg/ml showed a wide range of inhibition (27-79%). The increase in VIT-C concentration to 100 mg/ml was accompanied with an increase in the inhibitory effect (Table 3). Combination between LEV and VIT-C at MIC/80 mg/ml showed almost similar inhibitory effect to that recorded for VIT-C alone (33-82%). The highest inhibitory effects (83-99.6%) were recorded when a combination of 2 MIC/100 was used (Table 3).

Uropathogen	Levofloxacin			Vitamin-C			Levofloxacin/Vitamin-C		
	Conc. µg/ml	V.C cfu×10 ³ ± SEM	Reduction %	Conc. mg/ml	V.C cfu×10 ³ ± SEM	Reduction%	Conc. (µg/ml)/ (mg/ml)	VC cfu×10 ³ ± SEM	Reduction %
<i>E. coli</i> (27)	CTR	160 ± 5			160 ± 5			160 ± 5	
	32 ^a	56 ± 0.6*	65	80	48 ± 1.4*	70	32/80 ^c	40 ± 0.68*	75
	64 ^b	24 ± 0.64*	85	100	20 ± 0.76*	87.5	64/100 ^d	0.5 ± 0.07*	99.7
(98)	CTR	40 ± 0.86			40 ± 0.86			40 ± 0.86	
	1 ^a	12 ± 0.56*	70	80	8 ± 0.6*	80	1/80 ^c	10 ± 0.8*	75
	2 ^b	1.4 ± 0.17*	96.5	100	3.2 ± 0.4*	92	2/100 ^d	0.3 ± 0.05*	97.25
<i>Klebsiella sp.</i> (56)	CTR	56 ± 0.64			56 ± 0.64			56 ± 0.64	
	1 ^a	34 ± 0.6*	40	80	14 ± 0.97*	60	1/80 ^c	21 ± 0.97*	75
	2 ^b	19 ± 1*	65	100	8.4 ± 0.75*	85	2/100 ^d	0.1 ± 0.02*	99.8
(58)	CTR	25 ± 1			25 ± 1			25 ± 1	
	0.5 ^a	13 ± 1.1*	50	80	6.2 ± 0.7*	75	0.5/80 ^c	5.7 ± 0.4*	77
	1 ^b	7.5 ± 0.56*	70	100	3.8 ± 0.4*	85	1/100 ^d	0.45 ± 0.02*	90
<i>Citrobacter sp.</i> (22)	CTR	170 ± 10.4			170 ± 10.4			170 ± 10.4	
	0.5 ^a	58.8 ± 1.6*	65	80	68 ± 1.3*	60	0.5/80 ^c	51 ± 1.6*	70
	1 ^b	8.8 ± 0.7*	94.8	100	42 ± 1.6*	75	1/100 ^d	1.7 ± 0.25*	99
(77)	CTR	120 ± 0.57			120 ± 0.57			120 ± 0.57	
	16 ^a	54 ± 0.76*	55	80	36 ± 1.3*	70	16/80 ^c	30 ± 1.3*	75
	32 ^b	18 ± 1*	85	100	13 ± 1.1*	89	32/100 ^d	9 ± 0.8*	92
<i>Enterobacter sp.</i> (29)	CTR	140 ± 5.7			140 ± 5.7			140 ± 5.7	
	16 ^a	70 ± 5*	50	80	35 ± 1*	75	16/80 ^c	32 ± 1.1*	77
	32 ^b	15 ± 0.76*	89	100	14 ± 1*	90	32/100 ^d	0	100
(94)	CTR	56 ± 0.76			56 ± 0.76			56 ± 0.76	
	0.5 ^a	32 ± 1*	43	80	12 ± 0.49*	78.6	0.5/80 ^c	22 ± 0.68*	60.7
	1 ^b	4 ± 0.4*	93	100	8.4 ± 0.3*	85	1/100 ^d	0.5 ± 0.05*	99
<i>Proteus sp.</i> (102)	CTR	140 ± 5.7			140 ± 5.7			140 ± 5.7	
	4 ^a	42 ± 1.5*	70	80	49 ± 1.3*	65	4/80 ^c	32.2 ± 1.1*	77
	8 ^b	21 ± 1*	85	100	21 ± 0.76*	85	8/100 ^d	2.8 ± 0.3*	98
(131)	CTR	60 ± 1			60 ± 1			60 ± 1	
	32 ^a	14 ± 0.4*	77	80	13 ± 0.55*	78	32/80 ^c	9 ± 0.5*	85
	64 ^b	11 ± 0.5*	82	100	5.4 ± 0.3*	91	64/100 ^d	0	100
<i>Pseudomonas sp.</i> (86)	CTR	65 ± 1.4			65 ± 1.4			65 ± 1.4	
	0.5 ^a	32.5 ± 1.2*	45	80	29 ± 1.5*	70	0.5/80 ^c	19.5 ± 1.2*	80
	1 ^b	19.5 ± 1.8*	85	100	1.9 ± 0.3*	83	1/100 ^d	5.8 ± 0.44*	99.9
(133)	CTR	68 ± 1.5			68 ± 1.5			68 ± 1.5	
	4 ^a	41 ± 1.1*	40	80	16 ± 0.5*	77	4/80 ^c	14 ± 1*	80
	8 ^b	6 ± 0.52*	75	100	7.5 ± 0.3*	89	8/100 ^d	4 ± 0.7*	94

a) The used concentration was equal to 0.25 MIC of levofloxacin alone.
b) The used concentration was equal to 0.5 MIC of levofloxacin alone.
c) The used concentration was equal to 0.25 MIC of levofloxacin and 80 mg vitamin C/ml.
d) The used concentration was equal to 0.5 MIC of levofloxacin and 100 mg vitamin C/ml.
(V.C) means the viable cell counts on the surface of urethral catheters.
(CTR) means the control without drug.
*P<0.05: Significant value, compared to controls.

Table 2: Effects of levofloxacin, vitamin-C alone and in combination on initial adherence of the tested uropathogens on the catheter surfaces.

The highest inhibitory effect of LEV at MIC was observed on *Proteus sp* 102 (65%) while the lowest inhibitory effect (20%) was recorded on *Klebsiella sp.* 58. When LEV was used at 2 MIC, the highest inhibitory effect (91%) was recorded against *Pseudomonas sp.* 133. Again *Klebsiella sp.* 58 was the least inhibited (47%) by 2 MIC of LEV (Table 3). VIT-C at 80 mg/ml showed the highest inhibitory effect (79%) on *Pseudomonas sp.* 133 and the lowest effect (27%) on

Klebsiella sp. 58. Similarly pattern was observed when VIT-C was used at 100 mg/ml (Table 3). Again, similar pattern of inhibitory effect on the mature biofilm pre-formed was observed regardless the degree of sensitivity or resistance of the tested uropathogens. A combination of LEV/VIT-C (2 MIC/100 mg/ml) had the highest inhibitory effect on both *E. coli* 27 (resistant isolate) and *Pseudomonas* 86 (sensitive isolate). Still both uropathogens were least inhibited when LEV was

Uropathogen	Levofloxacin			Vitamin-C			Levofloxacin/Vitamin-C		
	Conc µg/ml	V.C cfu×10 ³	Reduction %	Conc. mg/ml	V.C cfu×10 ³	Reduction %	Conc. (µg/ml)/ (mg/ml)	V.C cfu×10 ³	Reduction %
<i>E. coli</i> (27)	CTR	130 ± 10.4			130 ± 10.4			130 ± 10.4	
	128 ^a	80 ± 1.1*	39	80	64 ± 1*	51	128/80 ^c	25 ± 1.5*	81
	256 ^b	39 ± 4.5*	70	100	24 ± 1.3*	82	256/100 ^d	5 ± 0.57*	96
(98)	CTR	60 ± 5			60 ± 5			60 ± 5	
	4 ^a	33 ± 1.2*	45	80	50 ± 1.1*	60	4/80 ^c	18 ± 0.64*	70
	8 ^b	16 ± 0.68*	74	100	10 ± 0.9*	85	8/100 ^d	2.4 ± 0.3*	96
<i>Klebsiella sp.</i> (56)	CTR	52 ± 1.1			52 ± 1.1			52 ± 1.1	
	4 ^a	16 ± 0.64*	40	80	18 ± 0.79*	65	4/80 ^c	13 ± 0.9*	75
	8 ^b	4.2 ± 0.45*	65	100	16 ± 1*	70	8/100 ^d	1 ± 0.25*	95
(58)	CTR	60 ± 1			60 ± 1			60 ± 1	
	2 ^a	48 ± 1.1*	20	80	44 ± 0.7*	27	2/80 ^c	40 ± 1*	33
	4 ^b	32 ± 0.57*	47	100	39 ± 0.57*	35	4/100 ^d	10 ± 0.57*	83
<i>Citrobacter sp.</i> (22)	CTR	180 ± 5			180 ± 5			180 ± 5	
	2 ^a	67 ± 1.5*	63	80	72 ± 0.76*	60	2/80 ^c	45 ± 1*	75
	4 ^b	43 ± 0.57*	76	100	27 ± 0.57*	85	4/100 ^d	7.2 ± 0.4*	96
(77)	CTR	150 ± 5			150 ± 5			150 ± 5	
	64 ^a	75 ± 1*	50	80	72 ± 1.1*	52	64/80 ^c	63 ± 1.2*	58
	128 ^b	45 ± 1.5*	70	100	53 ± 1.5*	65	128/100 ^d	15 ± 1*	90
<i>Enterobacter sp.</i> (29)	CTR	140 ± 1.1			140 ± 1.1			140 ± 1.1	
	64 ^a	90 ± 5*	36	80	80 ± 5*	43	64/80 ^c	55 ± 1*	61
	128 ^b	16 ± 0.76*	89	100	42 ± 1.1*	70	128/100 ^d	0.5 ± 0.15*	99.6
(94)	CTR	48 ± 1			48 ± 1			48 ± 1	
	2 ^a	20 ± 1*	59	80	16 ± 0.76*	66.7	2/80 ^c	14 ± 0.76*	70
	4 ^b	10 ± 0.76*	79	100	2 ± 0.28*	82	4/100 ^d	7.6 ± 0.36*	96.5
<i>Proteus sp.</i> (102)	CTR	140 ± 5			140 ± 5			140 ± 5	
	16 ^a	62 ± 1*	65	80	63 ± 1.5*	55	16/80 ^c	42 ± 1*	70
	32 ^b	84 ± 1.3*	72	100	53 ± 1.5*	62	32/100 ^d	21 ± 1*	85
(131)	CTR	50 ± 1			50 ± 1			50 ± 1	
	128 ^a	20 ± 1.1*	60	80	28 ± 1*	45	128/80 ^c	18 ± 0.28*	65
	256 ^b	15 ± 0.76*	70	100	18 ± 1*	65	256/100 ^d	6.5 ± 0.5*	87
<i>Pseudomonas sp.</i> (86)	CTR	60 ± 1.2			60 ± 1.2			60 ± 1.2	
	2 ^a	37.5 ± 0.8*	37.5	80	28.6 ± 0.9*	55	2/80 ^c	21.6 ± 0.87*	64
	4 ^b	12 ± 0.76*	80	100	12.8 ± 0.7*	79	4/100 ^d	3.9 ± 0.45*	93.5
(133)	CTR	76 ± 0.76			76 ± 0.76			76 ± 0.76	
	16 ^a	28 ± 1*	63	80	16 ± 0.76*	79	16/80 ^c	14 ± 0.76*	82
	32 ^b	6.9 ± 0.49*	91	100	8.4 ± 0.3*	89	32/100 ^d	1.5 ± 0.28*	98

- a) The used concentration was equal to MIC of levofloxacin alone.
 b) The used concentration was equal to 2MIC of levofloxacin alone.
 c) The used concentration was equal to MIC of levofloxacin and 80 mg vitamin C/ml.
 d) The used concentration was equal to 2MIC of levofloxacin and 100 mg vitamin C/ml.
 (V.C) means the viable cell counts on the surface of urethral catheters.
 (CTR) means the control without drug.
 *P<0.05: Significant value, compared to controls.

Table 3: Effects of levofloxacin, vitamin-C alone and in combination on the preformed biofilm on the catheter surfaces.

used alone at MIC (Figure 2). The effect of levofloxacin and vitamin-C either individually used or in combination was ascertained by SEM where at 0.5 MIC or VIT-C (100 mg/ml) a decrease in the number of adherent cells of all tested uropathogens was observed (Figure 3). This was always accompanied with morphological changes where the cells were elongated with disorganized membrane especially when vitamin-C was used (Figure 3B and 3D). A distinct decrease in number

accompanied with scattering, swallowing and malformation of the adherent cells were observed upon combination of both levofloxacin and vitamin-C (Figure 3F).

Discussion

Static adherence assay demonstrated that the effect of levofloxacin and vitamin-C on biofilm inhibition was concentration dependent.

Levofloxacin at sub-MIC concentrations (0.25 MIC and 0.5 MIC) reduced biofilm synthesis by 40-96%. The inhibitory effect of sub-MIC of levofloxacin against biofilm formation was also similarly reported by Drago et al. [16] who have recorded a significant inhibition of bacterial adherence to uroepithelial cells when both ciprofloxacin and levofloxacin were used at sub-inhibitory concentrations. Similarly, it was reported that the presence of sub-inhibitory concentrations (0.5, 0.25, or 0.125 MIC) of all the tested antimicrobial agents significantly reduced the biofilm formation and adherence of *P. aeruginosa* and *S. aureus* to plastic surfaces [17].

Both levofloxacin and vitamin-C had similar inhibitory effect on the preformed biofilm. However, higher concentrations of levofloxacin were needed for reduction of pre-formed biofilm than those required in case of initial microbial adherence. Levofloxacin at concentrations of MIC and 2MIC reduced the viable cell counts of the mature biofilm by 36-95% of the controls. Similarly, it was demonstrated that a high concentration of ciprofloxacin (another member of quinolones antibiotics) was necessary for significant reduction of a pre-formed biofilm, although the drug could reduce the adhesion and survival of the *Pseudomonas aeruginosa* at subinhibitory concentrations [15,18]. Vitamin-C was tested for its ability to inhibit biofilm formation and there was up to 92% reduction in the number of viable adherent bacteria on catheters treated with 80 and 100 mg vitamin-C ml⁻¹. Also a reduction of viable counts of the mature biofilm by up to 89% was

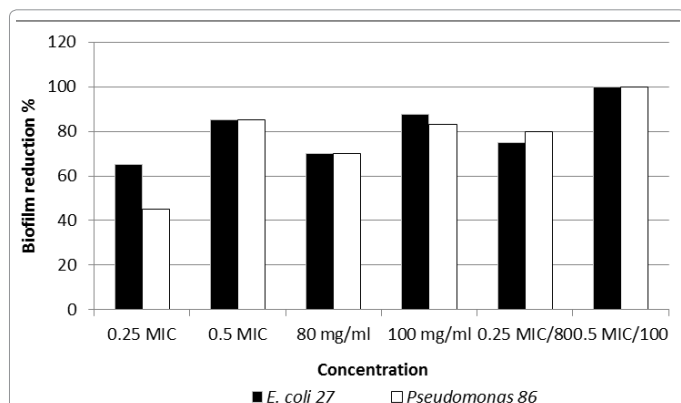


Figure 1: Effect of levofloxacin and vitamin-C at different concentrations on biofilm production on urethral catheter surfaces by *E. coli* 27 and *Pseudomonas* 86.

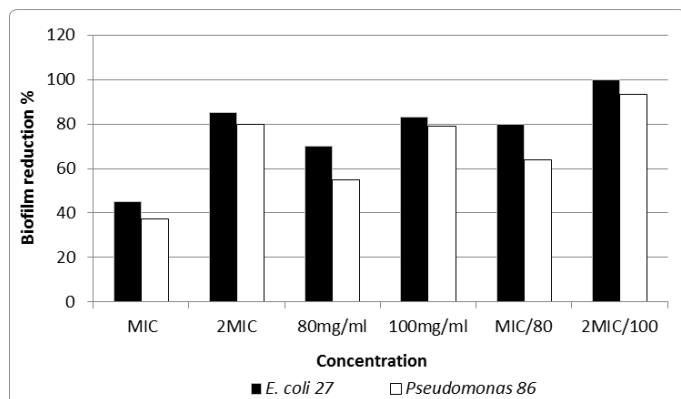


Figure 2: Effect of levofloxacin and vitamin-C at different concentrations on the preformed biofilm on urethral catheter surfaces by *E. coli* 27 and *Pseudomonas* 86.

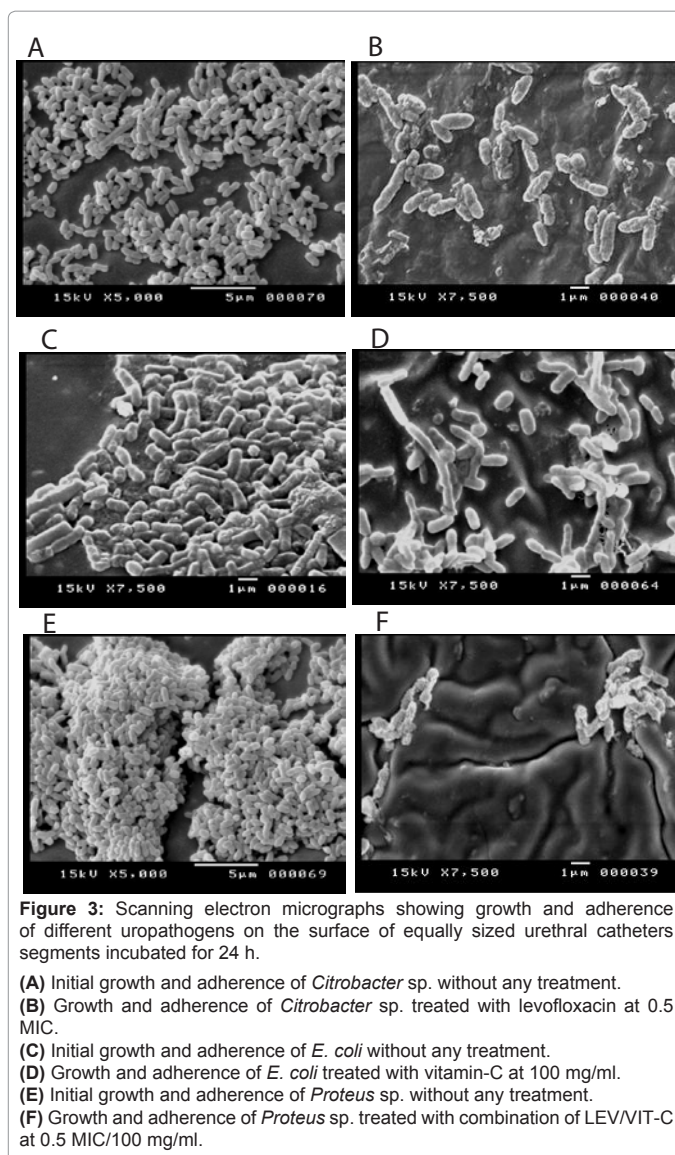


Figure 3: Scanning electron micrographs showing growth and adherence of different uropathogens on the surface of equally sized urethral catheters segments incubated for 24 h.

- (A) Initial growth and adherence of *Citrobacter* sp. without any treatment.
- (B) Growth and adherence of *Citrobacter* sp. treated with levofloxacin at 0.5 MIC.
- (C) Initial growth and adherence of *E. coli* without any treatment.
- (D) Growth and adherence of *E. coli* treated with vitamin-C at 100 mg/ml.
- (E) Initial growth and adherence of *Proteus* sp. without any treatment.
- (F) Growth and adherence of *Proteus* sp. treated with combination of LEV/VIT-C at 0.5 MIC/100 mg/ml.

observed. Ascorbic acid (vitamin-C) is a naturally occurring furanone [19], and could be exploited to control quorum sensing in bacteria [20]. Therefore, the inhibitory effect of vitamin-C might be due to its anti-quorum sensing activity where ascorbic acid was reported to have competitive inhibition with autoinducer-2 (AI-2) [20]. Previously, Habash et al. [11] demonstrated that vitamin-C supplementation can provide a degree of protection against adhesion by some uropathogens and colonization of biomaterials utilized within the urinary tract and this agrees with the results reported in the present study. Again, it has been reported that there was a steady decrease in viable cell counts with increasing vitamin-C concentrations that might have impacted growth [20]. Combination of levofloxacin with vitamin-C (LEV/VIT-C) increased the inhibitory effects of the initial microbial adherence to the catheter surface up to 100% with almost complete eradication of the pre-formed biofilm. The enhancement of the inhibitory effect of LEV/VIT-C combinations could be explained by the fact that reducing pH of the medium by vitamin-C addition could increase the activity of levofloxacin according to what have been reported by [17].

A similar pattern was reported by El-Feky et al. [21]; where

ciprofloxacin and N-acetylcysteine combinations had better inhibitory effect on both biofilm formation and pre-formed biofilm than that recorded for individually used drugs. However, levofloxacin is more advantageous than ciprofloxacin as lower concentrations of it (sub-MIC) showed almost the same inhibitory effect to ciprofloxacin at higher concentrations. Levofloxacin was also reported to have almost double the renal excretion rate of ciprofloxacin and this makes it an ideal agent for urinary tract infections (UTIs) [22]. Similarly, vitamin-C is more preferable than N-acetylcysteine due to both terms; safety and cost. In conclusion, these results showed the tremendous increase in inhibitory effect of the biofilm formation upon combination of both levofloxacin and vitamin-C and this represents a promising approach for prevention and treatment of biofilm associated infections.

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