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Effect of Ciprofloxacin and N-acetylcysteine on Bacterial Adherence and Biofilm Formation on Ureteral Stent Surfaces

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Abstract

The aim of this study was to evaluate the effect of ciprofloxacin (CIP), N-acetylcysteine (NAC) alone and in combination on biofilm production and pre-formed mature biofilms on ureteral stent surfaces. Two strains each of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebseilla pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris,* recently isolated from patients undergoing ureteral stent removal and shown to be capable of biofilm production, were used in this study. The inhibitory effects of ciprofloxacin, N-acetylcysteine and ciprofloxacin/N-acetylcysteine combination were determined by static adherence assay. Ciprofloxacin (MIC and 2 MIC) and N-acetylcysteine (2 and 4 mg/ml) inhibited biofilm production by ≥60% in all tested microorganisms. Disruption of pre-formed biofilms of all tested microorganisms was found to be ≥78% in the presence of ciprofloxacin (MIC and 2 MIC) and ≥62% in the presence of N-acetylcysteine (2 and 4 mg/ml), compared to controls. Ciprofloxacin/N-acetylcysteine showed the highest inhibitory effect on biofilm production (94–100%) and the highest disruptive effect on the pre-formed biofilms (86–100%) in comparison to controls. N-acetylcysteine was found to increase the therapeutic efficacy of ciprofloxacin by degrading the extracellular polysaccharide matrix of biofilms. These data are statistically significant. The inhibitory effects of ciprofloxacin and N-acetylcysteine on biofilm production were also verified by scanning electron microscope (SEM). In conclusion, Ciprofloxacin/N-acetylcysteine combinations have the highest inhibitory effect on biofilm production and the highest ability to eradicate pre-formed mature biofilms.

Key words: Biofilm, ciprofloxacin, mature biofilm, N-acetylcysteine

Introduction

Ureteral stents are commonly used in urologic practice (Mohan-pillai *et al.*, 1999). Although urinary catheterization is valuable, it may lead to stent obstruction, stent migration, stent encrustation, stone formation and biofilm formation (Lojanapiwat, 2005). Biofilm is a population of cells growing on a surface and enclosed in an exopolysaccharide matrix which may lead to complete blocking to the lumen of the catheter (Desgrandchamps *et al.*, 1997). Biofilm development initiates when bacteria transfer from a planktonic (free) existance to a lifestyle in which microorganisms are firmly attached to abiotic or biotic surfaces. After attachment, exopolysaccharide glycocalyx polymers are produced, a matrix inside which microcolonies increase and coalease to form biofilms (Costerton *et al.*, 1987). The longer the stents remain in place, the greater the tendency of these microorganisms to develop biofilms (Stickler, 1996).

Several approaches have been studied to prevent the formation of biofilms. Some of these depend on coating catheters with silver, antiseptics or by producing radio-opacity by silicone material and some depend on the use of antimicrobial agents or non antimicrobial agents (Flowers *et al.*, 1989). Ciprofloxacin is active against a wide range of urinary tract pathogens and able to inhibit bacterial colonization of urinary catheters *in vitro* (Reid *et al.*, 1994). N-acetylcysteine (NAC) is a non-antibiotic drug that has antibacterial properties. It is a mucolytic agent that disrupts disulphide bonds in mucus and reduces the viscosity of secretions. NAC is widely used in medical practice *via* inhalation, oral and intravenous routs and has an excellent safety profile

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(Kao *et al.*, 2003). NAC was found to decrease biofilm formation by a variety of bacteria and reduces the production of extracellular polysaccharide matrix while promoting the disruption of mature biofilms (Pézer-Giraldo *et al.*, 1997). Since the antimicrobial susceptibility of biofilm-associated bacteria is enhanced in disrupted biofilms (El-Azizi *et al.*, 2005), it is conceivable that an antibiofilm/antimicrobial agent combination would be synergistic (Olofsson *et al.*, 2003). In this work, we study the effect of ciprofloxacin, N-acetylcysteine each alone and in combination on biofilm formation on ureteral stents surfaces to select a suitable treatment for biofilm-associated infections to decrease hospitalization time and cost.

Experimental

Materials and Methods

Strains. Two strains each of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebseilla pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* recently isolated from urine samples and stent segments collected from patients undergoing ureteral removal, identified according to standard procedures (Benson, 2002 and Sheretz *et al.*, 1990) and shown to be capable of biofilm production (Christensen *et al*., 1985), were employed in this study.

Drugs. Preparation of stock solutions of ciprofloxacin (Bayer, Milan, Italy) was performed in accordance with the manufacturer's instructions. Two concentrations (2 and 4 mg/ml) of NAC (Sedico, Egypt) were evaluated.

Determination of minimum inhibitory concentrations (MIC) of ciprofloxacin. Minimum inhibitory concentrations of ciprofloxacin were determined by agar dilution method, according to Clinical Laboratory Standards Institute (CLSI) (2007).

Effect of ciprofloxacin, N-acetylcysteine (alone and in combination) on the pre-formed biofilms on stent surfaces and on biofilm production by the tested microorganisms. Seven pieces of JJ uretral stent segments (1 cm length) were incubated in bacterial suspensions that contained $5\times10^5 - 1\times10^6$ cfu/ml of bacteria in 5 ml of Trypticase soy broth (TSB, BBL, USA) to allow biofilm formation. After incubation at 37°C for 24 h, segments were removed and rinsed three times with phosphate buffer saline (PBS, 7.2) to remove non-adherent bacteria.

To test the effect of the tested agents on the preformed mature biofilms, catheter segments were suspended for 24 h at 37°C in one of the following treatment solutions: saline (control), ciprofloxacin (MIC and 2 MIC), N-acetylcysteine (2 and 4 mg/ml) and CIP/NAC (MIC/2 mg/ml and 2 MIC/4 mg/ml). After incubation, catheter segments were rinsed, placed in 10 ml fresh sterile saline and sonicated for 30 seconds to dislodge the sessile adherent cells. Serial dilutions of the sonicated saline were cultured. The number of sessile bacteria that indicates degree of adherence was determined by the viable count technique (Reid *et al.*, 1994).

To test the effect of the tested agents on bacterial adherence to stent surfaces, bacterial cultures in 5 ml TSB were washed, diluted with fresh TSB, standardized to contain $5\times10^5 - 1\times10^6$ cfu/ml and distributed into test tubes. One of the following solutions: Ciprofloxacin (MIC and 2 MIC), N-acetylcysteine (2 and 4 mg/ml) and CIP/NAC (MIC/2 mg/ml and 2 MIC/4 mg/ml) were added to each tube. Normal saline was added to control tubes. At the same time, one segment of ureteral stents was added to each tube. After 24 hours incubation, the number of viable adherent cells were determined as described before. Each assay was repeated at least three times. Data were expressed as mean cfu ± S.E.M.

Scanning Electron Microscopy (SEM). Catheter segments were fixed in 2.5% (vol/vol) glutaraldehyde in Dulbecco 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) (Soboh *et al.*, 1995).

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 SPSS, 11 statistical software (SPSS Inc., Chicago, IL).

Results

A total of 12 biofilm-producing strains (2 strains of each microorganism) were isolated and identified from urine samples and stents segments collected from patients undergoing ureteral stent removal.

Minimum inhibitory concentrations of ciprofloxacin. The MICs of ciprofloxacin were 1 and 2 µg/ml for *S. aureus*, *S. epidermidis* and *P. vulgaris*, 2 and 32 µg/ml for *E. coli*, 1 and 32 µg/ml for *P. aeruginosa* and 0.5 and 64 µg/ml for *K. pneumoniae*.

Inhibition of biofilm production. The inhibitory effects of ciprofloxacin and N-acetylcysteine were found to be concentration dependent. Ciprofloxacin at MIC inhibited biofilm synthesis by ≥75.6% in *S. aureus*, *S. epidermidis* and *P. vulgaris*, ≥67% in *P. aeruginosa* and *E. coli*, 60–95.8% in *K. pneumoniae* ($p<0.05$). At 2 MIC, reduction of biofilm synthesis was ≥89.3% in *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa* and *P. vulgaris* and 80–95.8% in

K. pneumoniae (P<0.05). N-acetylcysteine showed a significant inhibitory effect (p<0.05) on biofilm production. At a concentration of 2 mg/ml, reduction of biofilm synthesis was ≥60% while at 4 mg/ml, biofilm synthesis was reduced by ≥76.7% in all tested strains. Ciprofloxacin/N-acetylcysteine combinations were found to have the highest inhibitory effect on biofilm production (p<0.01). As CIP/NAC reduced the number of viable cell counts by 94–100% in comparison to controls in all tested microorganisms (Table I).

Disruption of pre-formed mature biofilms. Ciprofloxacin and N-acetylcysteine were found to have significant effects (p<0.05) on pre-formd mature biofilms. Ciprofloxacin (MIC and 2 MIC) reduced viable cell counts by ≥78% in the pre-formed mature biofilms of all tested strains. N-acetylcysteine (2 and 4 mg/ml) resulted in a breakdown of pre-formed biofilms and a reduction in the number of viable cell counts by ≥62%. On the other hand, Ciprofloxacin/N-acetylcysteine showed the highest ability to disrupt

Table I
Effects of ciprofloxacin, N-acetylcysteine alone and in combination on biofilm synthesis by the tested microorganisms

Microorganisms		Ciprofloxacin			N-acetylcysteine			Ciprofloxacin/N-acetylcysteine		
		Conc. (µg/ml)	viable cell counts Mean cfu ×10³ ± S.E.M	% reduction	Conc. mg/ml	viable cell counts Mean cfu ×10³ ± S.E.M	% reduction	Conc.	viable cell counts Mean cfu ×10³ ± S.E.M	% reduction
S. aureus	(64)	*CTR*	9 ± 2.08	–			–			
		1 [a]	2.2 ± 0.17*	75.6	2	3.5 ± 0.25*	61.1	1/2[c]	0.02 ± 0.01**	99.8
		2 [b]	0.4 ± 0.05*	95.6	4	2.1 ± 0.32*	76.7	2/4[d]	0 ± 0**	100
	(4)	CTR	10 ± 2.3							
		2 [a]	1.4 ± 0.1*	86	2	1.5 ± 0.11*	85	2/2 [c]	0.6 ± 0.11**	94
		4 [b]	0.8 ± 0.1*	92	4	0.8 ± 0.05*	92	4/4 [d]	0.01 ± 0**	99.9
S. epidermidis	(42)	*CTR*	100 ± 11.5	–						
		1 [a]	23 ± 1.5*	77	2	34 ± 1.5*	66	1/2 [c]	2.3 ± 0.1**	97.7
		2 [b]	9 ± 1.15*	91	4	13 ± 1.5*	87	2/4 [d]	0.1 ± 0**	99.9
	(82)	CTR	10 ± 1.7	–						
		2 [a]	1.8 ± 0.15*	82	2	4 ± 1*	60	2/2 [c]	0.5 ± 0.05**	95
		4 [b]	0.2 ± 0.1*	98	4	1 ± 0.26*	90	4/4[d]	0.01 ± 2.08**	99.9
E. coli	(14)	*CTR*	43 ± 2.5	–						
		32 [a]	20 ± 2.5*	72	2	13 ± 1.2*	69.8	32/2 [c]	0.16 ± 0.02**	99.9
		64 [b]	4.3 ± 0.6*	90	4	4 ± 1.1*	90.7	64/4 [d]	0 ± 0**	100
	(43)	CTR	100 ± 4	–						
		2 [a]	33 ± 3.2*	67	2	34 ± 2.8*	66	2/2 [c]	0.3 ± 0.15**	99.7
		4 [b]	9 ± 0.57*	91	4	12 ± 1.7*	88	4/4 [d]	0.06 ± 0.01**	99.94
K. pneumoniae	(69)	*CTR*	60 ± 4.7	–						
		64[a]	24 ± 2.3*	60	2	21 ± 1.5*	65	64/2 [c]	3.1 ± 0.057**	94.8
		128[b]	2.5 ± 0.16*	95.8	4	4.4 ± 0.46*	92.7	128/4	0.16 ± 0.023**	99.7
	(30)	CTR	10 ± 1.15	–						
		0.5 [a]	4 ± 0.8*	60	2	1.4 ± 0.2*	86	0.5/2 [c]	0.06 ± 0**	99.4
		1 [b]	2 ± 0.5*	80	4	0.5 ± 0.1*	95	1/4[d]	0 ± 0**	100
P. aeruginosa	(63)	*CTR*	44 ± 2.8	–						
		32 [a]	14 ± 3.6*	68.2	2	12 ± 1.1*	72.7	32/2 [c]	1.3 ± 0.23**	97
		64 [b]	3.3 ± 0.1*	92.5	4	7 ± 1.5*	84	64/4 [d]	0.08 ± 0.01**	99.8
	(59)	CTR	50 ± 3.4	–						
		1 [a]	13 ± 1.7*	74	2	18 ± 2*	64	1/2 [c]	0.8 ± 0.1**	98.4
		2 [b]	5 ± 1*	90	4	3 ± 0.5*	94	4/4 [d]	0.01 ± 0.001**	99.98
P. vulgaris	(16)	*CTR*	14 ± 2.3	–						
		2 [a]	2.8 ± 0.2*	80	2	1.4 ± 0.17*	90	2/2 [c]	0.06 ± 0.017**	95.7
		4 [b]	1.5 ± 0.23*	89.3	4	0.7 ± 0.26*	95	4/4 [d]	0.02 ± 0.01**	99.9
	(18)	CTR	70 ± 2.8	–						
		1 [a]	14 ± 1.7*	80	2	25 ± 3.4*	64.3	1/2 [c]	3.6 ± 0.23**	94.9
		2 [b]	7 ± 2.6*	90	4	15 ± 1.7*	78.6	2/4 [d]	0.05 ± 0.003**	99.9

CTR: without drug (control).
[a]: At MIC. [b]: At 2 MIC. [c]: MIC/2 mg/ml; [d]: 2 MIC/4 mg/ml; * P<0.05: Significant value, compared to controls.
** P<0.01: Significant value, compared to controls, ciprofloxacin group and NAC group.

pre-formed biofilms ($p<0.01$). As CIP/NAC combinations at MIC/2mg/ml and 2 MIC/4mg/ml reduced the number of viable cell counts by 86–100%, in comparison to controls in all tested microorganisms (Table II). Our results revealed that Ciprofloxacin/N-acetylcysteine combination showed the highest ability to inhibit biofilm synthesis and disrupt pre-formed mature biofilms. The inhibitory effects of the tested agents were also verified by (SEM) and the results are shown on figure 1.

Discussion

Microbial biofilms may pose a public health problem for patients requiring catheterization. The microorganisms in biofilms are protected from antimicrobial agents and host defense mechanisms due to the presence of large quantities of exopolysaccharides, slow-growing condition of bacteria, expression of possible biofilm specific resistance genes (Yasuada, 1996). In addition, biofilms facilitate the spread of antibiotic

Table II
Effects of ciprofloxacin, N-acetylcysteine alone and in combination on pre-formed mature biofilms on stent surfaces

Microorganisms		Ciprofloxacin			N-acetylcysteine			Ciprofloxacin/N-acetylcysteine		
		Conc. (µg/ml)	viable cell counts Mean cfu $\times 10^3$ ± S.E.M	% reduction	Conc. mg/ml	viable cell counts Mean cfu $\times 10^3$ ± S.E.M	% reduction	Conc.	viable cell counts Mean cfu $\times 10^3$ ± S.E.M	% reduction
S. aureus	(64)	*CTR*	9 ± 2.08	–			–			
		1 [a]	1.4 ± 0.11*	84.4	2	2.5 ± 0.23*	72.2	1/2 [c]	0.3 ± 0.05**	96.7
		2 [b]	0.8 ± 0.05*	91.1	4	1.2 ± 0.4*	86.7	2/4 [d]	0.02 ± 0**	99.8
	(4)	CTR	10 ± 2.3	–						
		2 [a]	1.6 ± 0.17*	84	2	3 ± 1.5*	70	2/2 [c]	0.4 ± 0.05**	96
		4 [b]	1 ± 0.3*	90	4	0.8 ± 0.23*	92	4/4 [d]	0.06 ± 0.005**	99.4
S. epidermidis	(42)	*CTR*	100 ± 11.5							
		1 [a]	22 ± 1.7*	78	2	35 ± 2.5*	65	1/2 [c]	3.2 ± 0.25**	96.8
		2 [b]	14 ± 1.7*	86	4	11 ± 1.7*	89	2/4 [d]	0.4 ± 0.05**	99.6
	(82)	CTR	10 ± 1.7							
		2 [a]	1.8 ± 0.11*	82	2	3.2 ± 0.02*	68	2/2 [c]	0.08 ± 0.11**	99.2
		4 [b]	0.9 ± 0.28*	91	4	1.7 ± 0.11*	83	4/4 [d]	0 ± 0**	100
E. coli	(14)	CTR	43 ± 2.5							
		32 [a]	9 ± 0.5*	79	2	14 ± 1.1*	67.4	32/2 [c]	0.5 ± 0.2**	98.8
		64 [b]	4.3 ± 0.23*	90.2	4	5 ± 1*	88.4	64/4 [d]	0 ± 0**	100
	(43)	CTR	100 ± 4							
		2 [a]	20 ± 0.23*	80	2	18 ± 0.23*	82	2/2 [c]	1.2 ± 0.05**	98.8
		4 [b]	12 ± 0.28*	88	4	9 ± 0.23*	91	4/4 [d]	0.05 ± 0.01**	99.95
K. pneumoniae	(69)	*CTR*	60 ± 4.7							
		64 [a]	9 ± 2*	85	2	16 ± 1.7*	73.3	64/2 [c]	0.66 ± 0.04**	98.9
		128 [b]	5.3 ± 0.34*	91.2	4	4.8 ± 0.4*	92	128/4 [d]	0.2 ± 0.05**	99.7
	(30)	CTR	10 ± 1.1							
		0.5 [a]	2.2 ± 0.23*	78	2	3.8 ± 0.23*	62	0.5/2 [c]	1.4 ± 0.05**	86
		1 [b]	1.3 ± 0.28*	87	4	2.2 ± 0.23*	78	1/4 [d]	0.08 ± 0.01**	99.2
P. aeruginosa	(63)	CTR	44 ± 2.8							
		32 [a]	8.2 ± 0.28*	81.4	2	14 ± 2.3*	68.2	32/2 [c]	1.6 ± 0.17**	96
		64 [b]	4.2 ± 0.23*	90.5	4	7 ± 0.3*	84	64/4 [d]	0.09 ± 0.02**	99.8
	(59)	CTR	50 ± 3.4							
		1 [a]	6.3 ± 0.34*	87.4	2	16 ± 4*	68	1/2 [c]	1.4 ± 0.05**	97.2
		2 [b]	3 ± 0.5*	94	4	5 ± 2*	90	2/4 [d]	0.06 ± 0.02**	99.9
P. vulgaris	(16)	CTR	14 ± 2.3							
		2 [a]	3 ± 1*	78.6	2	4.7 ± 0.23*	66.4	2/2 [c]	1.2 ± 0.28**	95.7
		4 [b]	0.8 ± 0.17*	94.3	4	2 ± 0*	85.7	4/4 [d]	0.04 ± 0.01**	99.7
	(18)	CTR	70 ± 2.8							
		1 [a]	12 ± 2.8*	82.9	2	13 ± 1.1*	67.1	1/2 [c]	0.3 ± 0.11**	99.6
		2 [b]	6.3 ± 0.4*	91	4	8 ± 1*	88.6	2/4 [d]	0.03 ± 0**	99.95

CTR: without drug (control).
[a]: At MIC. [b]: At 2 MIC. [c]: MIC/2 mg/ml; [d]: 2 MIC/4 mg/ml; * $P<0.05$: Significant value, compared to controls.
** $P<0.01$: Significant value, compared to controls, ciprofloxacin group and NAC group.

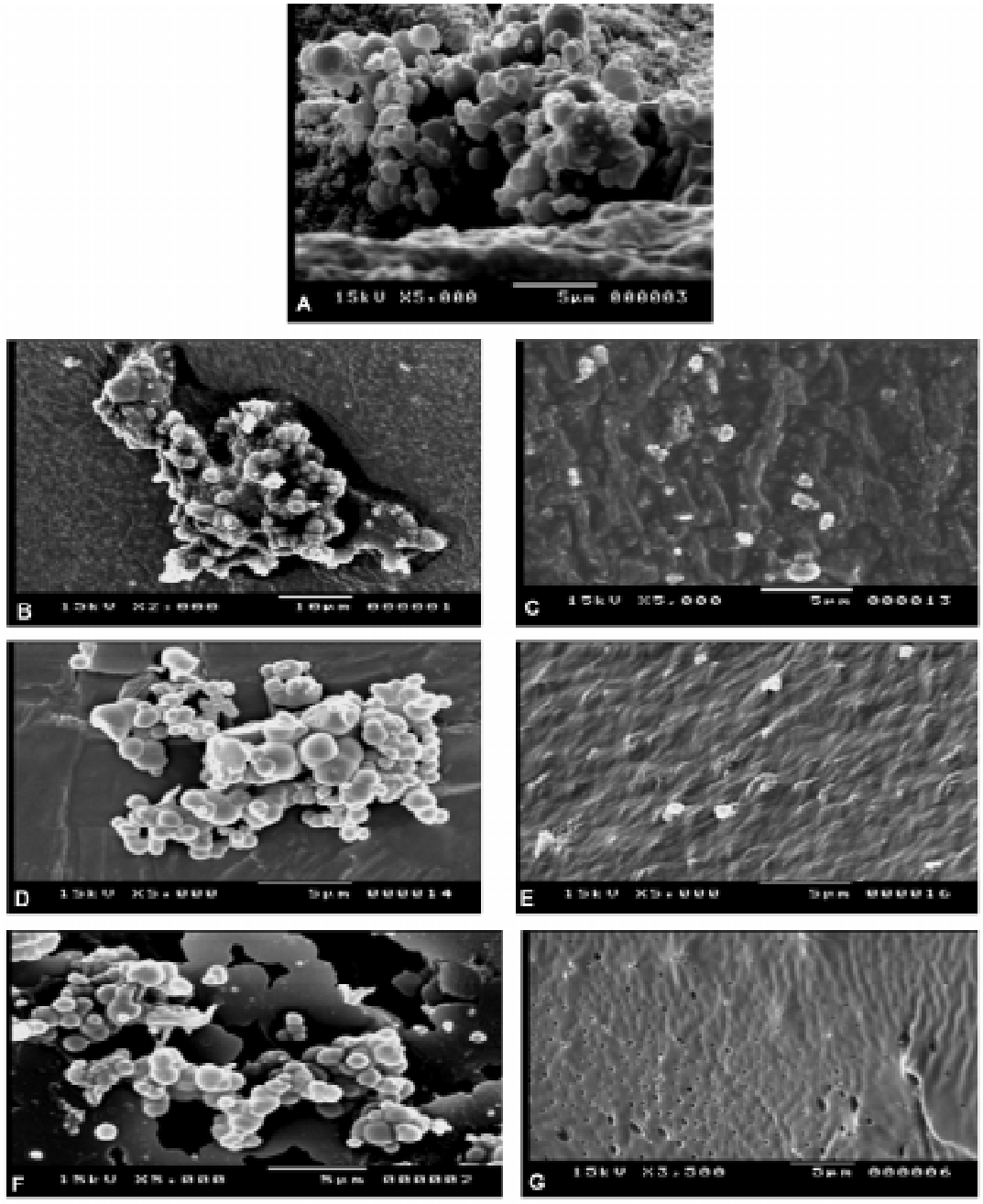

Fig. 1. Scanning electron micrographs showing the effect of Ciprofloxacin, N-acetylcysteine each alone and in combination on a performed *S. aureus* biofilm developed *in vitro* on stent surface.

A. *S. aureus* biofilm on the surface of a uretral stent incubated with *S. aureus* suspension for 24 h (× 5000) (control).
B. The morphological response of *S. aureus* biofilm exposed to MIC concentration. There was a decrease in the amount of biofilm mass adhered to stent surface.
C. The effect of ciprofloxacin at 2 MIC on *S. aureus* biofilm. Cells appeared swollen, scattered and with irregular cell wall. No biofilm mass observed.
D. **T**he effect of N-acetylcysteine (2 mg/ml) on *S. aureus* biofilm. Cotton like mass disappeared and cells appeared swollen with disrupted cell wall (× 5000).
E. **T**he effect of N-acetylcysteine (4 mg/ml) on *S. aureus* biofilm. Cells appeared scattered with no biofilm mass
F. **T**he effect of ciprofloxacin-N-acetylcysteine combination (MIC/2 mg/ml) on *S. aureus* biofilm. Cell appeared swollen, disrupted and scattered (× 5000).
G. The effect of CIP/NAC combination (2 MIC/4 mg/ml) on *S. aureus* biofilm. No bacterial cells observed.

resistance by promoting horizontal gene transfer (Fux *et al*., 2005) and provide foci of infection for other parts of the body *via* bacterial detachment and biofilm sloughing (Marshall, 1992). Bacteria within a biofilm can be 1000 times more resistant to antibiotics than their planktonic counterparts. This obviously presents particular difficulties in the treatment of infection-associated with indwelling urinary catheters. Therefore,

prevention of bacterial adherence to either indwelling catheters or host cells may be helpful to reduce the risk of biofilm-associated infections (Elves and Feneley, 1997). Also degrading polysaccharide matrix increases the rate of penetration of antimicrobial agents through biofilms and enhancing their efficacy against biofilm-associated infections (Di Bonaventura *et al*., 2004). Some antimicrobial agents such as clarithromycin were found to alter bacterial surface structure and reduce adherence by affecting expression of microbial adhesins (Baskin *et al.*, 2002). Reid *et al*. (2001) reported that oral administration of ciprofloxacin and ofloxacin in 40 patients with ureteral stents, led to drug levels on all the device surfaces that were higher than the minimum inhibitory concentration of *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*. In addition, no bacteria were isolated from patients' urine and no biofilm were detected. They found also that adsorption of ciprofloxacin and ofloxacin to the film surrounding stents was higher than that adsorbed to stent devices themselves. On the other hand, N-acetylcysteine, Bandazac lysine, protamine sulfate and sodium salicylate were found to have the ability to reduce or inhibit bacterial adherence and to disrupt mature biofilms (Pézer-Giraldo *et al.*, 1997).

Although Sub-MICs and MICs may not kill all bacteria, they are capable of modifying physicochemical characteristics, architecture of outermost surface and interfering with some important bacterial functions, such as adherence, surface hydrophopicity, fimbriation or motility and alginate production. Therefore, the final result is the reduction of their pathogenicity (Oviedo *et al*., 2000).

Static adherence assay showed that ciprofloxacin (MIC and 2 MIC) reduced biofilm synthesis by ≥60% and reduced viable cell counts of pre-formed mature biofilms by ≥78%. N-acetylcysteine (2 and 4 mg/ml) reduced biofilm synthesis by ≥60% and resulted in a reduction in the number of viable cell counts of preformed mature biofilms by ≥62%. A similar decrease in viable cells dislodged from catheters had been reported by Wojnicz *et al.* (2007), Vidya *et al*. (2005) and Reid *et al*. (1994) who studied the effect of ciprofloxacin at sub-MIC, MIC and above MIC on microbial adherence after incubation for 24 h. Pézer-Giraldo *et al.* (1997) reported the significant effect of N-acetylcysteine on the inhibition of biofilm formation and reported also that its effect is concentration dependent which is in agreement with our results.

Majtán and Hoštacká (1996) studied the effect of suprainhibitory concentrations of ciprofloxacin, pefloxacin and aminoglycosides on alginate production, an important virulence factor produced by *P. aeruginosa*, which plays an important role in biofilm formation. They found that suprainhibitory concentrations (2 MIC and 4 MIC) of the tested antibiotics have a significant inhibitory effect on alginate production by *P. aeruginosa*.

In this study, ciprofloxacin and N-acetylcysteine combinations (CIP/NAC) had the highest inhibitory effect on microbial adherence to stent surfaces (94–100%, compared to controls) and the highest ability to eradicate the pre-formed mature biofilms (86–100%, compared to controls). The inhibitory effects of the tested combinations were significantly ($P<0.01$) higher than the effects of ciprofloxacin and N-acetylcysteine each alone. These effects were also verified by scanning electron microscope. A similar reduction in the number of viable cell counts dislodged from catheters was described after incubation for 24 h with other lock solutions, such as minocycline-rifampicin (Sherertz *et al*., 2006), taurolidine-citrate (Shah *et al*., 2002) and in some cases, minocycline-EDTA (Raad *et al*., 2003).

Soboh *et al.* (1995) found that the combination of ciprofloxacin at MIC and protamine sulfate 50 µg/ml reduced the number of bacterial cell counts adherent to catheter surfaces by ≥99.9%. SEM showed that the majority of biofilm population exhibited extensive elongation, membrane disorganization and apparent protrusions in the cytoplasmic membrane which agree with our results. Similar results were obtained by Marchese *et al*. (2003) who reported that when fosfomycin at 2000 mg/l and NAC at 2 mg/ml used in combination, initial and mature biofilms were reduced by 66–80 and 60–73%, respectively. The effect of combinations were more than that of each alone. As NAC was found to increase the therapeutic effect of fosfomycin. Aslam *et al.* (2007) found that combination of NAC and tigecycline was synergistic. As no bacteria was detected in cases of methicillin resistant *S. aureus* (MRSA), methicillin resistant *S. epidermidis* (MRSE), methicillin sensitive *S. aureus* (MSSA) and *K. pneumonaie*.

In conclusion, our results showed the great effect of the tested combinations on bacterial adherence inhibition and their ability to disrupt pre-formed mature biofilms. N-acetylcysteine increases the therapeutic efficacy of ciprofloxacin when used in combination with it. Ciprofloxacin and N-acetylcysteine combination can be used as a catheter lock solution for the treatment and eradication of biofilms formed on ureteral stent surfaces.

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