Zagazig J. Pharm. Sci., June 1995,
Vol. 4, No.1, pp. 59 - 70 ISSN 1110-5089

MODULATION OF THE BIOFILM OF *PSEUDOMONAS AERUGINOSA* BY QUINOLONES IN AN *IN-VITRO* MODEL OF VASCULAR CATHETER COLONIZATON

Mahmoud Yassien, Ali Ahmedy, Salwa Rasmy, Mohamed Toama, and Nancy Khardori *

** School of Medicine, Southern Illinois University, Springfield, Illinois, USA and Microbiology Department, Faculty of Pharmacy, Cairo University, Egypt*

ABSTRACT

The effect of quinolones ciprofloxacin (CPX), norfloxacin (NOR), pefloxacin (PFX), and ofloxacin (OFX) on the adherence and on the preformed biofilms of *P. aeruginosa* to vascular catheters (VC) was studied in an in-vitro model using modified Robbins device. The presence of adherent viable bacteria was determined by culture on blood agar plates and quantitation of CFUs after dislodging the adherent cells into normal saline by sonication. The glycocalyx formation and adherence were also studied by scanning electron microscopy (SEM). In the presence of subinhibitory concentration of quinolones (0.5-, 0.25-, & 0.125-MIC) no or slight growth was observed around the segments and the number of the adherent cells was reduced to 21-23%, 40-46%, and 55-70% of the controls, respectively. Significant reduction in the glycocalyx formation and adherent bacteria was observed by SEM. In a separate set of experiments, the catheter segments were precolonized with *P. aeruginosa* for 24h and exposed to the quinolones in concentration 50 µg/ml (5 MIC) and 100 µg/ml for 2hrs. Slight or no growth was observed around the treated segments, while a heavy growth was reduced to less than 5% of the controls in VC treated with 50 µg/ml of the quinolones and complete removal of the adherent cells was achieved with 100 µg/ml. By SEM a very small amount of glycocalyx and a few scattered microcolonies were seen after treatment with 50 µg/ml, no organisms were seen in the presence of 100 µg/ml. These data show that the subinhibitory concentrations of CPX, NOR, PFX, and OFX sgnificantly inhibit the adherence of *P. aeruginosa* to vascular catheters. Clinically achievable concentration of quinolones were able to eradicate the preformed biofilm on the VC.

INTRODUCTION

The use of various medical devices including indwelling vascular catheters, cardiac pacemakers, prosthetic heart valves, chronic ambulatory peritoneal dialysis catherters and prosthetic joints has greatly facilitated the management of serious medical and surgical illnesses. Bacterial infections following colonization and biofilm formation on these devices are a major cause of morbidity in patients receiving prosthetic devices[1]. Biofilms have also been demonstrated on tissues in chronic bacterial diseases that are characterized by resistance to antimicrobial chemotherapy. Extracellular slime or glycocalyx production is an important factor in the adherence of bacteria and their protection from host defence mechanisms. Extensive glycocalyx production has been demonstrated on cultures of *Staphylococcus epidermidis* , *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, and *Bacteroides* sp. [2].

Different approaches (including the use of various antimicrobial agents) to eradicate biofilm bacteria have been studied. The combination of tobramycin and piperacillin[3], the combination of tosufloxacin and erythromycn[4], clindamycin[2] and clarithromycin[5,6] have been reported to be effective in eradication of biofilm associated bacteria. However, the clinical usefulness of these experimentally effective strategies remains to be established[5].

In this study, we investigated the effect of subinhibitory concentrations of quniolones on glycocalyx production and adherence of *Pseudomonas aeruginosa* to the plastic surfaces and to the vascular catheters in an in-vitro model of vascular catheter colonization. The interaction between preformed biofilms of *P. aeruginosa* and the quniolones was also studied.

EXPERIMENTAL

Organisms:

A total of 50 *P. aeruginosa* isolated recentlly from clinical specimens (mainly from blood), submitted to Clinical Pathology Department, Kasr El-Aini Hospital, Cairo University were used.

Isolation, purification and maintenance of the isolates was carried out on brain heart infusion agar or Mueller-Hinton agar media. The organisms were stored in skim milk at -70°C. Before use, the organisms were subcultured in Mueller-Hinton broth for 18-24 h at 37°C. The inoculum was standardized to contain 10^5-10^6 CFU/ml by McFarland standards and verified by viable counts.

Antimicrobial agents:

Antimicrobial agents were kindly provided by the following manufacturer: Miles Laboratories Inc., West Haven CT (ciprofloxacin); Merck Sharp and Dohme, Rahway NJ (norfloxacin); Roger Bellon Laboratories, Neuilly Surseine, France (pefloxacin); Ortho pharmaceuticals, Raritan, NJ (ofloxacin). The quniolones were stored and diluted according to manufacturer's instructions. All four quninolones were used in all the experiments except where pefloxacin was used for scanning electron microscopy where pefloxacin was used.

Glycocalyx (slime) production:

The isolates were examined for slime production as described by Christensen et al.[7]. In brief, two to three colonies of *P. aeruginosa* were inoculated into 5 ml of Trypticase Soy Broth (TSB) in plastic conical tubes (Falcon, Oxnard, CA) in triplicate. Glucose free basal medium that lacks the substrate for polysaccharides (glycocalyx) was used as a control. The cultures were incubated at 37°C for 18-20 hrs. The contents were aspirated; one tube was examined unstained and one each stained with trypan blue and safranin. The isolate was judged to be slime positive if there was a visible unstained or stained film lining the wall of the tube. Formation of a ring at the liquid-air interface was not considered a positive test. The presence of adherent microcolonies was also detected by phase contrast microscopy.

Quantitative measurement of glycocalyx production was determined by the method of Christensen et al.[8]. Briefly, stationary 18 hrs cultures of *P. aeruginosa* in 5 ml of Trypticase Soy Broth (TSB) were washed, diluted with fresh TSB and standardized to contain 5×10^5 - 10^6 CFU/ml. Aliquots (0.2 ml) of the diluted cutlures were added to the wells of sterile polystrene 96 flat bottom tissue culture plates. The plates used were Microtest III (Falcon no. 3072, Becton Dickinson). The manufacturer sterilized these plates with gamma irradiation, and electrically charges the surfaces to diminish the hydrophobicity of the polystyrene. Following an 18 hrs incubation at 37°C, the contents of the tissue culture plates were gently aspirated, using a pasteur pipette connected to low vaccum. The plates were then washed four times with sterile PBS (pH 7.2). Slime and adherent organisms were fixed overnight with Bouin fixative. The fixative was removed by washing the wells several times with 50% ethanol. The wells were stained by Huckr crystal violet. Placing the plates under running distilled water, the excess stain was removed, and the plates were then air dried. The optical density of the stained adherent films was read with a Micro ELISA Auto Reader (Titer TEk, Flow Labs, McLean, VA) at a wavelength of 550 nm. Measurements were performed in triplicate and repeated three times. The values for each isolate were then averaged. The bacteria were classified into three categories. If the average OD was less than 0.12, the isolate was considered nonadherent. If the OD was greater than 0.12, but less than 0.24, the isolate was considered weakly adherent. If the OD was greater than 0.24, the isolate was considered strongly adherent.

Effect of subinhibitory concentration of quinolones on the glycocalyx production and adherence of *P. aeruginosa*:

Minimum inhibitory concentrations of the quinolones (ciprofloxacin, norfloxacn, pefloxacin, ofloxacin were determined by broth microdilution technique[9]. The concentrations of the drugs in the medium ranged from 0.125 µg/ml to 128 µg/ml. The MIC was defined as the lowest concentration of the drug which inhibited visible growth at 37°C after overnight incubation.

The quinolones in 1/2 MIC, 1/4 MIC, and 1/8 MIC were used to study their effect on glycocalyx production and adherence as described above. Various concentrations of antimicrobial agents (0.2 ml) were added to the wells in triplicate. Drug free medium was used in control experiments.

Interaction between preformed biofilms of *P. aeruginosa* and quinolones:

The biofilm formation of *P. aeruginosa* was carried out by the same procedure used for measurement of glycocalyx production in the 96-well flat bottom tissue culture plates. After 48 hrs of incubation at 37°C, the contents of each well were aspirated and washed twice with normal saline. Then, 0.2 ml of various quinolone concentrations (12.5 µg/ml to 400 µg/ml) were added to the wells. Drug free saline solution was used as control. The plates were incubated at 37°C for 48 hrs.

After incubation, the contents of each well were aspirated and the wells washed twice with normal saline. The adherent cells were fixed in place with Bouin's fixative solution. The wells were stained by Hucker crystal violet and the excess stain removed by washing under running distilled water. After air drying, the optical density of the stained film was read by the micro ELISA Autoreader at a wavelength of 550 nm.

Zagazig J. Pharm. Sci., June 1995,
Vol. 4, No.1, pp. 59 - 70

In-vitro model of vascular catheter colonization:

a) Preparation of inoculum:

The 18 hrs old culture, grown in TSB with 0.3% yeast extract, was harvested by centrifugation and washed twice in normal saline. The suspension was standardized using McFarland standards. The inoculum size was confirmed by viable count on trypticase soy agar plates. A 500 ml bag of sterile normal saline containing 5% dextrose (D_5NS) was used as the reservoir for colonizing the catheter segments. Inocula sizes ranging from 10^5 to 10^9 colony forming units (CFU) were added to the infusate.

b) Colonization of catheters:

A modified Robbins device was used to study the formation of biofilm and colonization of catheters by *P. aeruginosa*[10]. The device is constructed of an acrylic block, 42 cm long, with a lumen of 2 x 10 mm. Twenty-five evenly spaced sampling ports are designed in a way so that the catheter material (0.5 cm^2) attached to sampling plugs lies flush with the inner surface without disturbing flow characteristics. The design prevents the occurrence of artifacts of cell sedimentation on the biomaterial and allows salient fluid dynamic features to be defined. The device was modified to fit 10 mm segments of vascular catheters across the lips of the sampling ports. We have demonstrated colonization of catheter segments in this model both on the external and the luminal surfaces. After placing the catheter segments in the sampling inserts, the entire apparatus was sterilized with ethylene oxide.

c) Biofilm formation:

To study the formation of biofilms of *P. aeruginosa* on vascular catheters, the bags of D_5NS containing the organism were infused through the modified Robbins device. An intravenous infusion pump (Gemini PC2; IMED, San Diego, CA) and an infusion set (Travenol Lab., Deerfield, IL) was used to infuse 30 ml of solution per h (Fig. 1 and 2).

After infusing 500 ml D_5NS solution, six vascular catheter segments were removed with sterile forceps and two of these were cultured on agar plates by embedding them slightly in agar. Four segments were sonicated in precooled saline for 30 seconds at 30% cycle and 3.5 output to dislodge the sessile (adherent) cells. Two of these segments were cultured on agar plates, while the others were washed in normal saline three times by vortexing them for 10 sec. before culturing them on agar plates.

The rest of catheter segments, remaining in the device, were flushed by running 250 ml of normal saline through the device over 1 hr to remove planktonic (free-floating) bacteria. After flushing, the segments were divided into six groups. Group one (3 segments each) was cultured on agar plates without any further processing. Groups two and three (3 segments each) were sonicated for 30 seconds at 30% cycle and 3.5 output. Segments from group 2 were cultured on agar without further processing. Group 3 segments were washed with normal saline once by vortexing for 10 seconds. Group four segments were sonicated and washed twice. Group 5 (5 segments) was sonicated for 2 minutes, vortexed for 1 minute and the number of cells released from the segments into normal saline was determined by viable counts. Segments from groups one and three were also used for scanning electron microscopy.

d) Catheter cultures:

The presence of viable cells on the catheter segments was evaluated by embedding the catheter segments into Mueller Hinton agar plates. The plates were incubated at 37°C and examined for growth around the segments after 24 and 48 hrs.

e) Scanning electron microscopy:

The presence of organisms (dispersed or in microcolonies) and a semiquantitative analysis of biofilm including glycocalyx formation was ascertained by scanning electron microscopy[11]. Catheter segments were prepared for scanning microscopy by fixation for 3 hrs at 4°C in 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 0.15% ruthenium red. The segments were rinsed in fresh 0.1 M cacodylate buffer for 10 min. (repeated 3 times). This was followed by refixation for 60 min at 4°C in 1.5 osmium in the same buffer. The catheters were washed in 0.1 M cacodylate buffer for 10 min. (repeated 3 times). The segments were dehydrated in a series of aqueous ethanol solution (30 to 100%) and dried by critical point dryer (Tousimis, Critical Point Dryer, Autosamdri 814) with carbon dioxide. The specimens were mounted on aluminum stubs with silver paste, allowed to dry for 3 h and then coated with

gold/palladium by using Polaron cool-sputter coater E5100 II. The segments were examined in scanning electron microscope (S-500; Hitachi, Mountain view, CA) at 20 KCV.

f) Effect of subinhibitory concentrations of quinolones on glycocalyx production and adherence of *P. aeruginosa*:

For studying the effect of subinhibitory concentration of the quinolones on glycocalyx production and bacterial adherence in this in-vitro model of vascular catheter colonization, the quinolones at the 1/2, 1/4, and 1/5 MIC were added to the inoculated infusate bags (500 ml D_5NS) during colonization. Drug free infusate was used as control. For scanning lectron microscopy experiments pefloxacin was the quinolone used.

g) Interaction between preformed biofilms of *P. aeruginosa* and quinolones:

For studying the effect of quinolones on the preformed biofilms of *P. aeruginosa*, the 500 ml bags of D_5NS containing the organism were infused through the device (30 ml per hr). Then 250 ml bags of saline solution containing the quinolones (50 and 100 µg/ml) were infused through the device (125 ml per hr) for 2 hrs. Drug free saline bags were used as control.

RESULTS

Glycocalyx (slime) production:

The results of qualitative tests showed that 20 of the 50 *P. aeruginosa* isolates were positive for glycocalyx production. Quantitatively, 7 isolates were judged to be strongly adherent, while, the others (13 isolates) were classified as moderately adherent.

Effect of subinhibitory concentrations of quinolones on glycocalyx production and adherence of *P. aeruginosa*:

The MICs of the quinolones for the 20 adherent isolates ranged from 1.0 µg/ml to 80 µg/ml. The MICs for the two isolated used for in-vitro colonization of vascular catheters ranged from 2 µg/ml and 12 µg/ml. Figure 1 shows that all four quinolones at the subinhibitory concentrations significantly reduced the optical density of the biofilm of *P. aeruginosa*. The optical density of the biofilms was reduced to 30-33% of the control in the presence of 1/2 MIC of the quniolones and to 61-67% in the presence of 1/8 MIC. Ciprofloxacin was slightly more effective than NOR and PFX in reducing the biofilm formation and OFX had the least activity.

Interaction between the preformed biofilms of *P. aeruginosa* and quinolones:

Treatment of the preformed biofilms of *P. aeruginosa* with the quinolone concentrations ranging from 12.5 µg/ml to 400 µg/ml caused significant reduction in the optical density of the preformed biofilms. Treatment with 400 µg/ml (approximately 5-100 times MIC) of the tested drugs caused reduction in the optical density to 39-60% of the controls and to 69-77% at 12.5 µg/ml. The agents in order of decreasing activity were Pefloxacin, Norfloxacin, Ofloxacin and Ciprofloxacin (Fig. 2).

In-vitro model of vascular catheter colonization:

Two isolates of glycocalyx producing *P. aeruginosa* were used in these experiments. The preliminary studies showed that the optimal inoculum size for colonization of the catheter segments was 10^6-10^8 CFU. The organisms were grown from all segments cultured without any further processing. When the catheters were ultrasonicated without prior flushing and subsequent washing, growth was also detected around all the segments. The organisms grown under these conditions represent both planktonic (free-floating) and sessile (adherent) bacteria. Segments that were flushed to remove planktonic bacteria but not ultrasonicated also showed growth of organism indicating the presence of adherent bacteria. Catheters that were flushed before ultrasonication but not washed afterwards showed bacterial growth, representing dislodged adherent organisms. No growth was detected in the segments that were flushed and ultrasonicated, followed by washing. This sequence led to the loss of planktonic bacteria by flushing and dislodgement of sessile bacteria by ultrasonication that were subsequently washed off (Table I and Fig. 4).

Effect of subinhibitory concentrations of quinolones on glycocalyx production and adherence of *P. aeruginosa* in the in-vitro model of vascular catheter colonization:

Catheters colonized in the presence of sub-MIC (1/2 to 1/8 MIC) of quinolones showed growth of the organism with and without sonication if they were not flushed before sonication, indicating the presence of planktonic bacteria. No growth was seen in the unflushed, sonicated segments, if washed

after sonication. A few or no organisms were grown from catheter segments with or without sonication after flushing. These included the catheter segments without subsequent washing, indicating the reduction or absence of adherent bacteria (Fig. 4 [a,b,c]). The results (Fig. 3) show that the presence of sub-MICs of quinolones significantly inhibits the adherence of *P. aeruginosa* to vascular catheter. There was a correlation between this inhibitory effect and the concentration of the quinolones (Fig. 3 and Table 1).

Scanning electron microscopy showed a large amount of glycocalyx and adherent microcolonies on the untreated, flushed, unsonicated and unwashed segments, (Fig. 5). While, complete removal of the adherent bacteria and traces of glycocalyx was observed on the untreated, flushed, sonicated and washed segments (Fig. 6). In the presence of sub-MICs of pefloxacin, a small amount of glycocalyx was present and a significant reduction in the number of the adherent bacteria was observed. This effect increased as the concentration of PFX increased i.e. the lowest number of adherent cells was observed in the presence of 1/2 MIC (Fig. 7 [a,b,c]).

Interaction between preformed biofilms of *P. aeruginosa* and quinolones in the in-vitro model of vascular catheter colonization:

The flushed, unsonicated and unwashed catheter segments did not show any growth when treated with 100 µg/ml of PFX. With 50 µg/ml, a few organisms were cultured on some of the segments. As shown in Figure (8 [a,b]). Scanning electron microscopy revealed significant eradication of the biofilms on segments treated with PFX (50 µg/ml) for 2 hrs. Complete eradication of the biofilms was achieved by treatment with 100 µg/ml of Pefloxacin.

DISCUSSION

Adherence of micro-organisms to specific substrates is considered a crucial step in the initiation of colonization and subsequent infection[22]. Extracellular slime or glycocalyx, produced by bacteria plays an important role in the development and persistence of biomaterial-associated infections. The matrix of bacteria and endogenous factors (collectively termed as a biofilm layer) which envelops the surface of a colonized implant anchors the bacteria firmly to the surface of the device[10]. The adherent bacteria are protected

Table 1. The effect of sub-MIC of Pefloxacin on the adherence of *Pseudomonas aeruginosa* in an in-vitro model of vascular catheter colonization.

Catheter Treatment	Organism Expected	Untreated (control)	Treated		
			1/2-MIC	1/4-MIC	1/8-MIC
-UNE-UNS -UNW	FR & AD	+++	+	++	++
-FL-UNS -UNW	AD	++	+/-	+	+
-FL-SN -UNW	DG	+	+/-	+/-	+/-
-FL-SN -WS	none	-	-	-	-

UNF = unflushed
UNS = unsonicated
UNW = unwashed
FL = flushed
SN = sonicated

FR = free
AD = adherent
DG = dislodged
WS = washed

+++ = heavy growth
++ = moderate growth
+ = slight growth
- = no growth
+/- = very few or now growth

from host defense mechanisms and the activity of antimicrobial agents[12]. Biofilm associated bacteria are a major concern in the treatment of device related infections because of their resistance to a wide range of antimicrobial agents[19, 24,25].

The effect of subinhibitory concentrations of some of the antibiotics on glycocalyx formation and adherence of bacteria has been studied by various investigators. Clindamycin and trospectomycin have been used in a previous study. Both antibiotics in concentrations less than 2 µg/ml, significantly reduced the glycocalyx formation[13]. As reported by Lamb et al.[14], the subinhibitory concentrations of clindamycin inhibit the adherence of *Bacteroides* sp. and *E. coli* to bone surfaces in-vitro. On the other hand, the data reported by Michael Dunne[16] have shown that sub-MICs of cefamandole and vancomycn have a variable effect on the formation of bacterial biofimls of selected strain of *S. epidermidis*.

Our results show that the subinhibitory concentrations (1/2 to 1/8 MIC) of the quinolones significantly inhibit the glycocalyx production and adherence of *P. aeruginosa* to inert surfaces of the microtiter plates. Such inhibitory effect was dependent on the concentration; the maximum inhibitory effect was achieved by 1/2 MIC of the quinolones (optical density was 30-33% that of the controls). At 1/8 MIC, the optical density was 61-67% of the controls. Among the quinolones used, there was no significant difference in their inhibitory effect on the glycocalyx production and adherence of bacteria. Among the isolates tested, there was a difference in the inhibitory effect of the quinolones and such differences decreased as the concentration of the quinolones increased. Carsent-Etesse et al. [4] reported similar effect of subinhibitory concentrations of vancomycin and teicoplanin on the adherence of *S. epidermidis* to tissue culture plates.

It is now documented that many chronic infections involve colonization by bacteria growing as adherent biofilms within an extended polysaccharide glycocalyx[27]. Despite some efforts, eradication of biofilm associated bacteria has been found to be difficult[1,2,8,21]. The slow growth rate and the presence of glycocalyx matrix in the biofilm structure are considered important mechanisms for resistance of biofilm associated bacteria to antimicrobial agents. Therefore, the reduction in glycocalyx formation can potentially render these bacteria more susceptible to antimicrobial agents[5,9,26]. Yasuda et al.[27] reported that treatment of the biofilms of *P. aeruginosa* with clarithromycin (for 5 days) caused decrease in the quantity of hexose and alginate and also the quantity of polysaccharides. Clarithromycin eradicated glycocalyx produced by *P. aeruginosa* by unknown mechanism[27].

In our studies, the treatment of two-day old biofilms of *P. aeruginosa* in the wells of microtiter plates with high concentrations of the quinolones (12.5 µg/ml to 400 µg/ml) resulted in reduction in the optical density of the adherent films, as a result of eradication of the membranous structure that covered the bacterial colonization. Also, the results revealed that Pefloxacin was the most effective quinolones in eradicating the biofilm produced by *P. aeruginosa,* although it was not the most effective antimicrobial agent as determined by in-vitro susceptibility testing. The activity to eradicate the biofilm may be independent of the antimicrobial activity. Clarithromycin efficiently eradicated the biofilms formed by *P. aeruginosa*, although it is highly resistant to clarithromycin. Our results indicate that the tested quinolones may be useful for treatment of chronic infections that involve colonization of bacteria growing as adherent biofilms within extended glycocalyx.

The two most common mechanisms of colonization of vascular catheters are contamination of insertion skin site and contamination of catheter hub. Gram-negative *bacilli* may also be involved in infections due to contaminated infusate[17]. The modified Robbins Device and the delivery system are analogous to that used for intravenous therapy in humans. The same model was used previously by Khardori et al [13] for studying the effect of subinhibitory concentrations of clindamycin and trospectomycin on the adherence of *S. epidermidis* to the vascular catheters. The results suggested that trospectomycin and clindamycin in subinhibitory concentrations may have a role in the prevention of the adherence of *S. epidermidis* to vascular catheters.

When the catheters were flushed, ultrasonicated and washed, no bacterial growth was seen in the presence or absence of antimicrobial agents. These three procedures remove adherent, as well as, planktonic bacteria. When catheters were colonized in the absence of antimicrobial agents, bacterial growth was observed in segments flushed but not sonicated. In the presence of antimicrobial agents (1/2 MIC, 1/4 MIC and 1/8 MIC) the catheters

Zagazig J. Pharm. Sci., June 1995,
Vol. 4, No.1, pp. 59 - 70

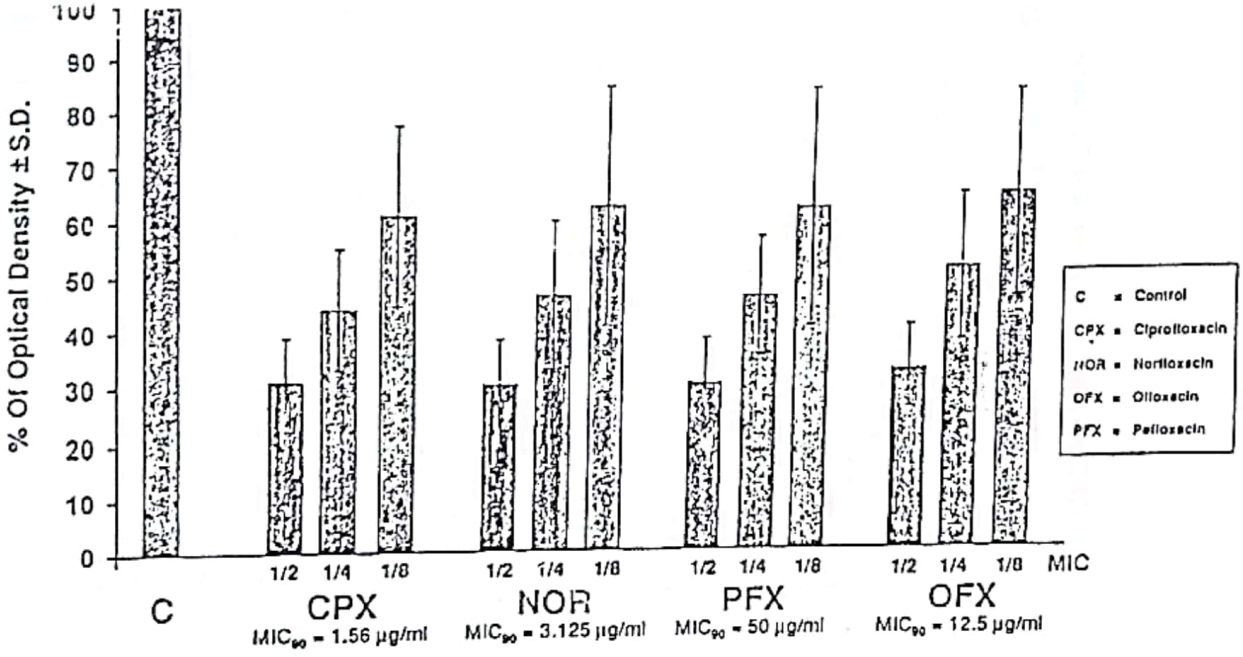


Figure 1: Effect of Subinhibitory Concentrations (1/2 MIC, 1/4 MIC, 1/8 MIC) off Quinolones on Glycocalyx Production and Adherence of 20 Clinical Isolates of *P. aeruginosa.*

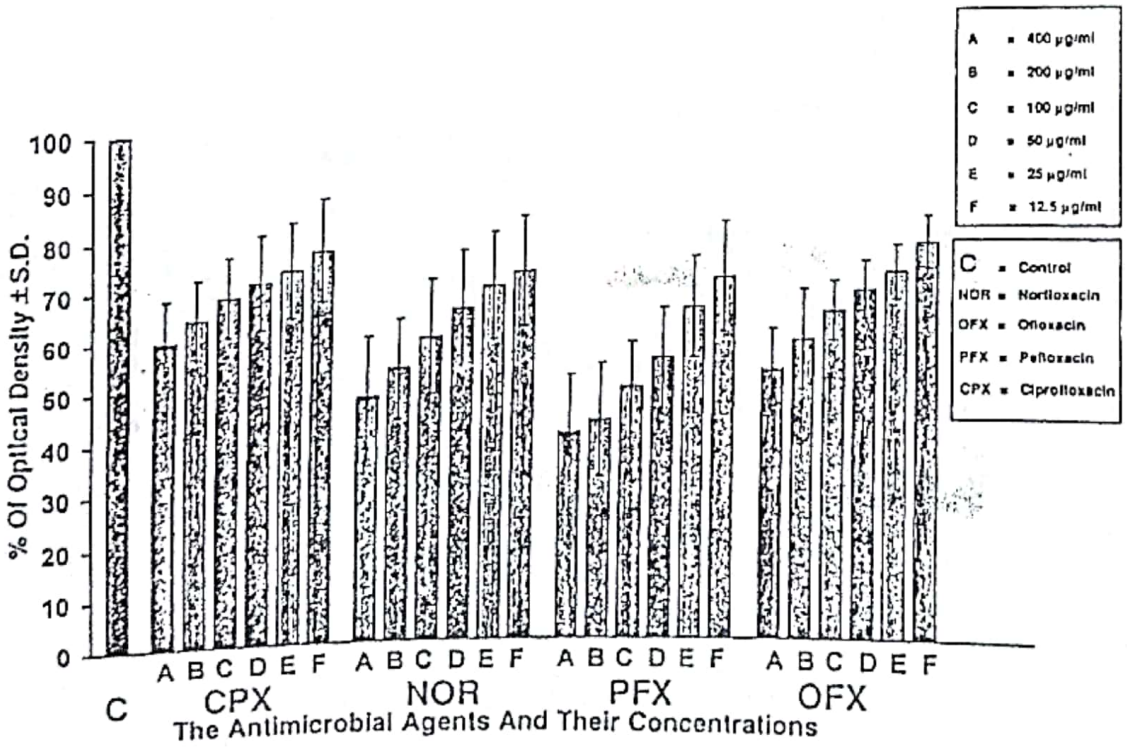


Figure 2: Interaction Between Quinolones and Biofilms Formed by 20 Clinical Isolates of *P. aeruginosa.*

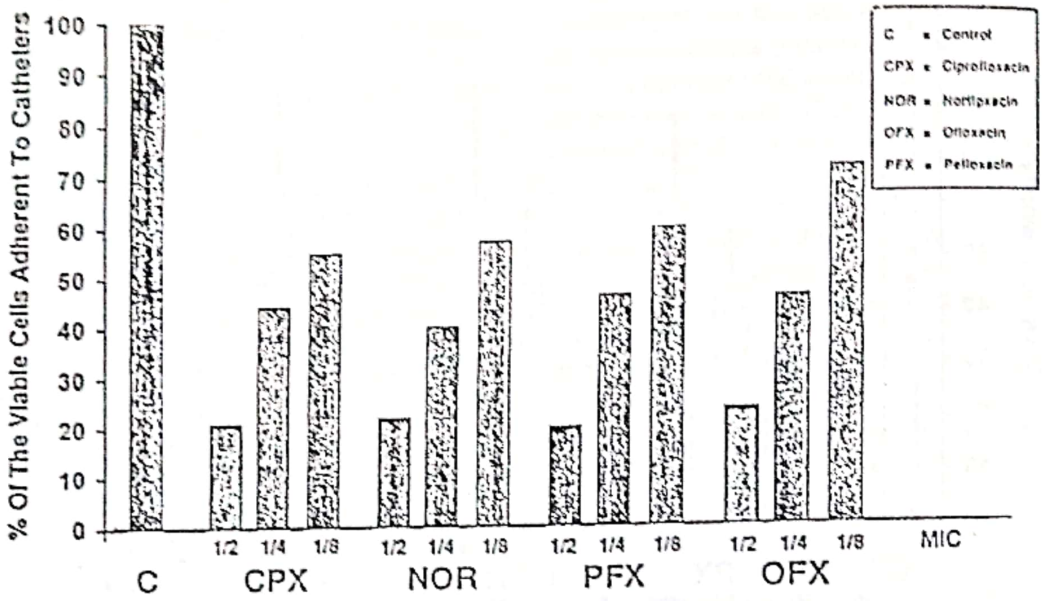
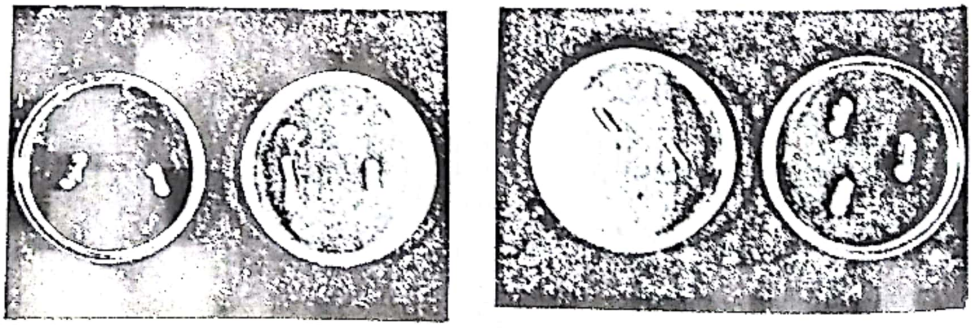


Figure 3: Effect of Subinhibitory Concentrations (1/2 MIC, 1/4 MIC, 1/8 MIC) of Pefloxacin on the Adherence of *P. aeruginosa* in an In-Vitro Model of Vascular Catheter Colonization.



a_1: Sonication without washing a_2: Sonication with washing b_1: Flushing with sonication b_2: Flushing without sonication

Figure 4: Cultured Catheters Showing: Reduction of growth around segments depending on subsequent treatments.

Zagazig J. Pharm. Sci., June 1995.
Vol. 4, No.1, pp. 59 - 70

Figure 5: Scanning electron micrographs showing a large amount of microcolonies of *P. aeruginosa* covered with glycocalyx in absence of antimicrobial agents.

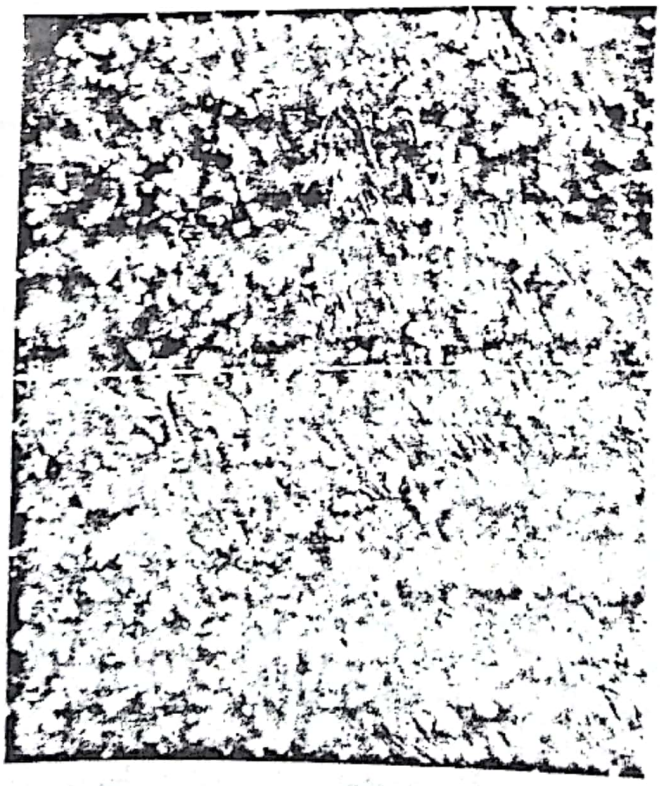
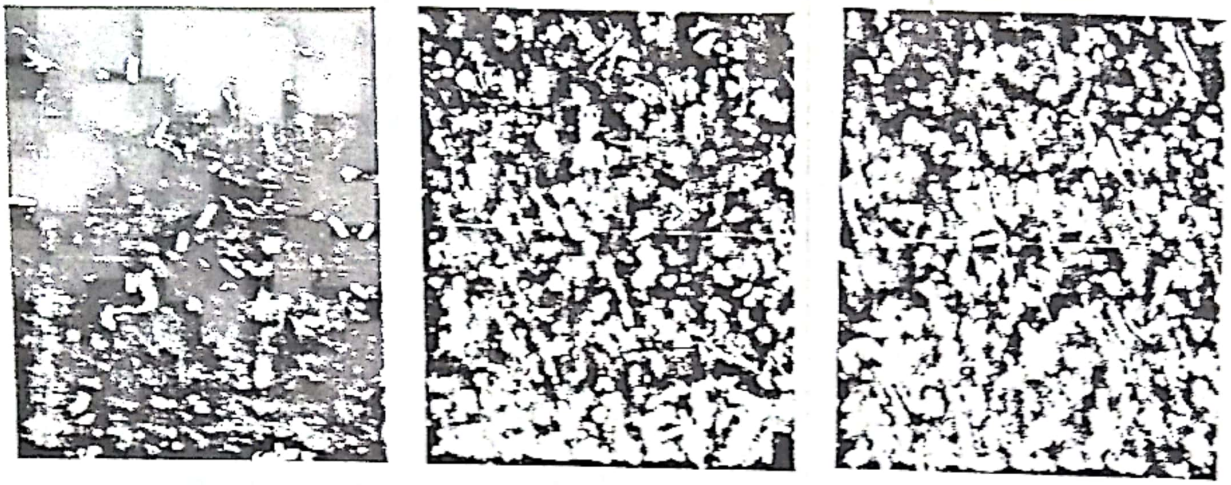
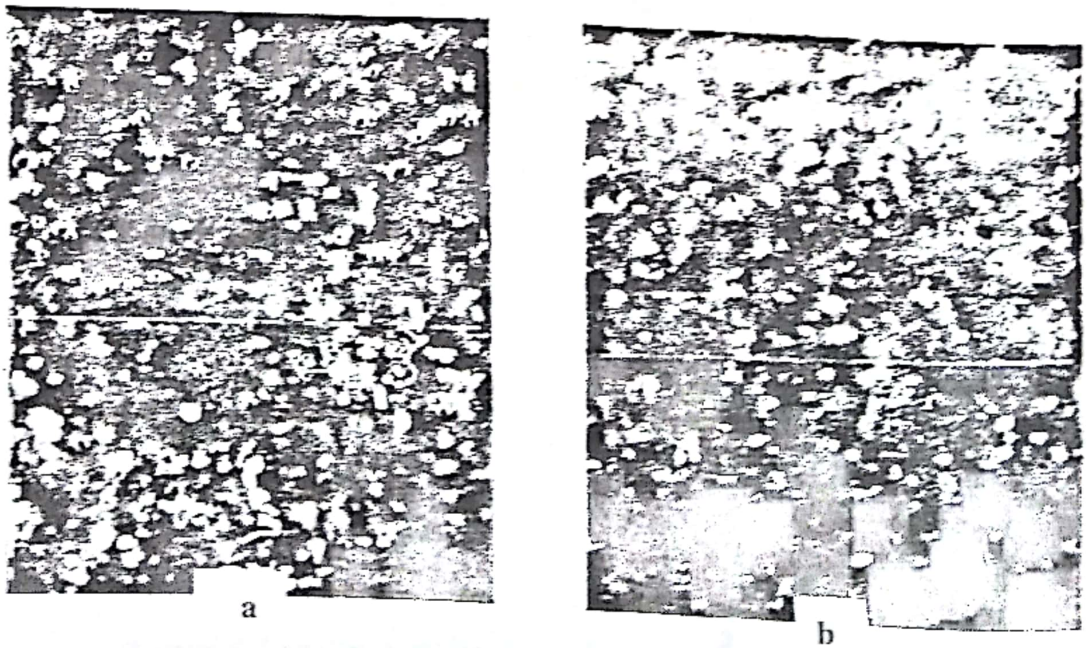


Figure 6: Scanning electron-micrographs showing traces of glycocalyx and no microcolonies of bacteria after ultrasonication and washing of the segments.



a b c

Figure 7: Scanning electron micrographs showing reduction in glycocalyx and microcolonies of bacteria in presence of Pefloxacine at a concentration of
a- 1/8 MIC
b- 1/4 MIC
c- 1/2 MIC



a b

Figure 8: Scanning electron micrographs showing: **a**-significant reduction in the number of microcolonies and glycocalyx when treated with 50 µg/ml of pefloxacine for 2 hours. **b**-traces of glycocalyx and no microcolonies after treatment with 100 ug/ml of pefloxacine for 2 hours.

showed no or slight growth after flushing and in the absence of sonication indicating a significant inhibition in the adherence of the tested organisms to the vascular catheters. These findings were further evaluated by observation of the glycocalyx formation and adherent bacteria on the surface of the catheter segments by scanning electron microscopy. Significant reduction in the adherent bacteria in the presence of 1/2 MIC, 1/4 MIC and 1/8 MIC of pefloxacin was observed. Maximum reducing effect was detected in the presence of 1/2 MIC. Reid et al.[23], reported that pretreatment of urinary tract silicone latex catheters in-vitro with subinhibitory concentration (0.1 µg/ml) of Ciprofloxacin for 1, 24, and 48 hrs significantly reduced the adherence of *P. aeruginosa* AK1. Our data show that the subinhibitory concentrations of the used quinolones significantly inhibit the glycocalyx production and the adherence of *P. aeruginosa* to the vascular catheters. Low doses of the quinolones may be useful as prophylactic agents to prevent biofilm associated infections.

The activity of the quinolones in eradicating the preformed biofilm and removal of the adherent bacteria in the in-vitro model of vascular catheter colonization was confirmed by scanning electron microscopy. The colonized catheters that were treated with 50 µg/ml of pefloxacin had most of the adherent cells removed and a very small amount of glycocalyx remained. No adherent cells were observed on the segments that were treated with 100 µg/ml for 2 hrs. The activity of cprofloxacin in eradicating the preformed biofilms of *P. aeruginosa* was also reported by Reid et al.[23]. The adherent biofilms of *P. aeruginosa* were eradicated by 24 hrs of interaction with 50 and 100 µg/ml of Ciprofloxacin. On the other hand, Nickel et al.[19], showed that the presence of 8 hrs old biofilms produced by *P. aeruginosa* on the urinary tract catheters, significantly increases the bacterial resistance to tobramycin. The activity of tobramycin against the sessile bacteria of *P. aeruginosa* was enhanced in combination with pipracillin[3].

The results indicate that the four quinolones, ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin can be in low doses to prevent biofilm associated infections. Treatment of preformed biofilms (sessile bacteria) of *P. aeruginosa* with clinically achievable concentrations of quinolones resulted in eradication of the membranous structures of the biofilms and consequently may increase the penetration of quinolones and other antimicrobial agent into the bacterial biofilms. Therefore, the quinolones may be effective in the treatment of biofilm associated infections as single agents and/or enhance the activity of other antimicrobial agents.

REFERENCES

1. Anwar H. and J. W. Costerton, **Antimicro. Agents Chemother.**, 34: 166-1671, (1990).
2. Anwar, H.; M. Dasgupta; K. Lam and J.W. Costerton, **Antimicrob. Agents Chemother.**, 24: 647-655, (1989).
3. Anwar, H.; J. L. Strap and J. W. Costerton, **Antimicrob. Agents Chemother.**, 36: 890-893, (1991).
4. Carsenti-Etesse, H.; J. Durant; J. Entenza; V. Mondain; C. Pradier; E. Bernard and P. Dellamonica, **Antimicrob. Agents Chemother.**, 37: 921-923, (1993).
5. Chaurd, C.; J. E. Lucet; P. Rohner; M. Herrmann and R. Auckenthaler, **J. Infectious Dis.**, 163: 1369-1373, (1991).
6. Christensen, G.D.; W.A. Simpson; A. Bisno and E. Beachy, **Infect. Immun.**, 37: 318-326, (1982).
7. Christensen, G.D.; W.A. Simpson; J.J. Younger; L.M. Baddour; F.F. Barret; D.M. Melton and E. Beachey, **J. Clinical Microbiol.**, 22: 996-1006, (1985).
8. Dall, L.; W. G. Barnes; J. W. Lane and J. Mills, **J. Infect. Dis.**, 156: 736-740, (1987).
9. Evans, D.J.; M.R.W. Brown; D.G. Allison and P. Gilbert, **Antimicrob. Agents Chemother.**, 25: 585-591, (1990).
10. Franson, T.R.; N.K. Sheth; H.D. Rose; P.G. Sohule, **J. Clin. Microbiol.**, 20: 5000-505, (1984).
11. Fujimaki, K.; M. Ideda; M. Takahata and T. Yasuda, **Chemotherapy** (Tokyo), 40: 886-893, (1992).
12. Johson, G. M.; D.A. Lee; W.E. Regelman; E.D. Gray; G. Peter; P.G. Quie, **Infect. Immun.**, 253-264, (1982).
13. Khardori, N.; E. Wong; H. Nguyen; C. Jeffery-Wiseman; . Wallin; R.P. Tewari and G.P. Bodey, **J. Infect. Disease**, 164: 108-13, (1990).
14. Lamb, D. W.; K.J. Mayberry-Carson; B. Toper-Meyer; J.W. Costerton; K.P. Ferguson, **Zentralbe Microbiol.**, 16 (suppl.): 278-86, (1987).
15. Marrie, J.J. and J. W. Costerton, **J. Clin. Microbiol.**, 19: 687-693, (1984).
16. Michael, D. W., 34: 390-393, (1984).
17. Nafziger, D. and R. Wanzel, **J. Crit. III**, 5: 857-63, (1990).
18. National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, M7-A. National Committee for Clinical

Laboratory Standards, Vilanova, PA.
19. Nickel, J.C.; A.C. Cristina and J.W. Costerton, **Can. J. Surg.**, 28: 50-54, (1985).
20. Nickel, J.C.; J. Heaton; A. Morales and J.W. Costerton, **J. Urol.**, 135: 586-588 (1986).
21. Nickols, W.W.; M. Evans; M.P.E. Slack and H.L. Walmsley, **J. Gen. Microbiol.**, 135: 1291-1303, (1989).
22. Qui, P.G. and K.K. Beleni, **J. Infect. Dis.**, 156: 543-7, (1987).
23. Reid, G.; S. Sharma, K. Advikolanu, C. Tieszer, R. Martin, and A. W. Bruce., **Antimicrob. Agents Chemother.**, 38: 1490-1495, (1994).
24. Rosser, B. T.; D. Taylor, P. A. Ciz, and R. Cluland, **Antimicrob. Agents Chemother.**, 31: 1502-1506, (1987).
25. Warren, J. W.; H.L. Mucie, E.J. Berquist and J. M. Hoppes, **J. Urol.**, 125:1-2, (1981).
26. Widmer, A.F.; A. Wiestner; R. Frei and W. Zimmerli, **Antimicrob. Agents Chemother.**, 35: 741-746, (1991).
27. Yasuda, H.; Y. Ajiki; T. Koga; H. Kawada and T. Yokota, **Antimicrob. Agents Chemother.**, 37: 1749-1755, (1993).
28. Yasuda, H.; Y. Ajiki, T. Koga and T. Yokota, **Antimicrob. Agents chemother.**, 38: 138-141, (1994).

دراسة تخيير الفيلم الحيوى للسودوموناس ايروجينوزا بواسطة الكينولونز على نموذج خارجى لقسطره الأوعيه المستعمرة بالميكروبات .

محمود ياسين ، على أحمدى ، سلوى رسمى ، محمد طعيمة

و* نانسى خاردورى

قسم الميكروبيولوچى - كلية الصيدلة جامعة القاهرة

* مدرسة الطب ، جامعة الينوى الجنوبية ، سبرنج فيلد- الينوى- أمريكا

تمت دراسة تأثير الكينولونز " السبروفلوكساسين ، نور فلوكساسين ، بيفلوكساسين والنور فلوكساسين " ، على التصاق البكتريا وكذلك الفيلم الحيوى سابق التكوين للسودوموناس ايروجينوزا على قسطرة الأوعية كنموذج خارجى بإستعمال جهاز روبن المعدل ، لقد تم تحديد على البكتيريا الحيه الملتصقة بعد إزالتها وزراعتها على أطباق الآجار بالدم ، وكذلك تم دراسة تكوين الجليكوكالكس وخاصيه الإلتصاق بإستخدام الميكروسكوب الألكترونى ، وهذه الدراسة أوضحت أنه بإستعمال تركيز تحت المحيط للكينولونز (½ ، ¼ ، ⅛ لأقل تركيز محيط) لوحظ غياب كلى أو نمو ضعيف حول قطع القسطره عند زراعتها وكذلك إختزال عدد الخلايا الملتصقة بها إلى ٢١ - ٢٣ ٪ ، ٤٠ - ٤٦ ٪ ، ٥٥ - ٧٠ ٪ بالنسبه للكونترول تدريجياً حسب التركيز السابق وكذلك لوحظ إختزال واضح لتكوين الجليكوكالكس والتصاق الخلايا بإستعمال الميكروسكوب الألكترونى ، وفى دراسة أخرى تم تعرض قطع لقسطره الأوعية المستعمره مسبقاً بالميكروبات لتركيزات ٥٠ ، ١٠٠ ميكروجرام / مل لمدة ساعتان شوهد غياب كلى أو نمو ضعيف حول قطع القسطره حسب التركيز المستعمل وذلك بالنسبه للقطع غير المعالجة ، وكذلك إختزال عدد البكتريا الملتصقة لأقل من ٥ ٪ بإستعمال تركيز ٥٠ ميكروجرام / مل وإزالة كامله عند تركيز ١٠٠ ميكروجرام / مل ، ولقد شوهدت كميه قليله من الجليكوكالكس وبعض المستعمرات الدقيقه المتفرقة بعد العلاج بـ ٥٠ ميكرون وإنعدام النمو بإستعمال ١٠٠ ميكرون ، وذلك بإستعمال الميكروسكوب الألكترونى ، ولقد أظهرت هذه النتائج أن التركيز تحت المحيط من الكينولونز المختلفه ذات تأثير محبط على إلتصاق السودوموناس ايروجينوزا " العامل الأول و الخطير لمصدو التلوث فى قسطرات الأوعيه والقسطرات الأخرى " على جدار قسطرات الأوعية وكذلك أن تركيز الكينولونز المعالج داخل الدم كاف لإزالة البكتريا المتراكمة على جدار هذه القسطرات .