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Antibacterial, Anti-biofilm Activity of Some Non-steroidal Anti-Inflammatory Drugs and N-acetyl Cysteine against Some Biofilm Producing Uropathogens

Ahmed Mohsen[1], Alyaa Gomaa[1], Fatma Mohamed[1], Roaa Ragab[1], mennatallah Eid[1], Al-Hussein Ahmed[2], Areej Khalaf[2], Mohamed Kamal[1], Safaa Mokhtar[1], Hadeer Mohamed[1], Islam Salah[1], Rania Abbas[1], Sameh Ali[3], Rehab Mahmoud Abd El-Baky[4,*]

[1]Undergraduate student, faculty of pharmacy, Minia University
[2]Undergraduate student, Faculty of science, Minia University
[3]Demonstrator of microbiology, Faculty of Pharmacy, Minia University
[4]Lecturer of microbiology, Faculty of Pharmacy, Minia University
*Corresponding author: dr_rehab010@yahoo.com

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Abstract Ureteral catheters are indispensable devices used in the management of ureteral obstruction. Although the stent is essential for treatment, it also has complications, which are encrustation, stone formation and biofilm formation. Biofilm infections result in a complication in the course of treatment, increasing the length of patients stay in hospital and overall cost. Catheter-associated infections are difficult to be treated with antibiotics and there is a need to change catheters due to the formation of biofilm on their surfaces. In this study, In this study, we examine the effect of some of prescribed drugs as NSAIDs and N-acetylcysteine on the adherence of *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *Proteus mirabilis* on the surface of catheters, and their effects on the preformed mature biofims. Also, we determine their antibacterial activity. The results showed that the tested agents had good antibacterial activity, a significant effect on the inhibition of adherence of the tested strains to plastic surfaces and a high disruptive effect on mature biofilms. In conclusion, the tested drugs can be used in the treatment of catheter-associated infections.

Keywords: *NSAIDs, biofilm, mucolytics, adherence*

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1. Introduction

Bacterial biofilms can be defined as microbe-derived sessile communities attached to a surface and embedded in a self produced matrix of polymers. These biofilm bacteria show a different metabolic state than planktonic bacteria, especially with respect to transcription and cell interactions [1,2]. Biofilm-forming organisms are often more resistant to high levels of antimicrobial agents than the planktonic cells, which makes bacterial eradication difficult to achieve despite the use of in vitro active antibiotics [3,4,5,6]. It seems that biofilm-embedded bacteria enter in a stationary state, driving to physicochemical interactions with production of slime, followed by lower diffusion of active molecules, and then a lesser susceptibility to antimicrobial killing [7]. Medical and environmental impact of microbial biofilm has led to an expanding investigation of the biology and regulatory mechanisms of biofilm formation and dispersal [8,9]. Strategies have been proposed to inhibit biofilm formation by using different drugs both in solution and applied to some medical devices [10,11,12,13].

Catheter-associated infections are difficult to be treated with antibiotics and there is a need to change catheters due to the formation of biofilm on their surfaces. In recent years, due to the increased resistance of many bacteria to the commonly used antimicrobial agents, attention has shifted to drugs belonging to different pharmacological classes for possible antimicrobial activity. Many studies showed that NSAIDs have antibacterial activity and decrease adherence and biofilm formation by bacteria. Unfortunately, one of the main side effects of NSAID administration is renal function damage. NSAID are accountable for 7% of all cases of acute renal failure and for 37% incidents of drug-associated acute renal failure [14]. Inhibition of prostaglandin synthesis by NSAID administration might lead to renal ischaemia, decline in glomerular hydraulic pressure and, consequently, to acute renal insufficiency [15,16,17]. Indeed, renal biopsies from patients with NSAID-induced acute renal failure disclose

signs of acute tubular necrosis [14]. N-acetylcysteine (NAC) has been shown to effectively prevent nephrotoxicity induced by contrast media, hypoperfusion or in toxin-induced renal failure in humans and experimental animals [18-23]. In addition, NAC is known to exert a vasodilatatory effect on renal microcirculation [24]. The operative mechanisms have not yet been fully elucidated. Thus far, the probability of NAC playing a role in prevention or attenuation of NSAID-induced acute renal failure has never been approached. NAC successfully attenuated the deterioration of renal function by inducing renal vasodilatation, decreasing oxidative stress via inhibition of intrarenal ROS content and, most importantly, restoration of intrarenal PGE2 release back to the normal levels [25]. So, we studied the effect of Three NSAIDs with and without N-acetylcysteine on the adherence and Biofilm formation of some pathogens which may cause urinary tract infection.

2. Mateirals and Methods

2.1. Microbial Strains

Standard strains of *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC 10031), *Ps. aeruginosa (ATCC 10145)*, were obtained from MIRCIN culture collection of the Faculty of Agriculture, Ain Shams University. *Proteus mirabilis* (clinical strain) was obtained from the department of microbiology and immunology, Faculty of Pharmacy, Minia university, Minia, Egypt.

2.2. Biofilm Production

The tested organisms were tested for their ability to form biofilm by Tissue Culture Plate method (TCP) [26].

2.3. Evaluation of the Antibacterial Effect of the Tested Agents and the Determination of Their MIC using Agar Well Diffusion Method

Microorganisms (0.5 ml) of 1.5×10^{8} CFU/ml (0.5 Mcfarland turbidity) were plated in sterile petri dishes then 20 ml of sterile, molten and cooled (45°C) Muller Hinton agar media was added to all petri dishes. The plates then were rotated slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched using a sterile cork bore. Two fold serial dilutions were performed on the tested NSAIDs and N-acetyl cysteine. Equal volumes of each dilution were applied separately to each well in three replicates using a micropipette. All plates were incubated overnight at 37°C, then collected and zones of inhibition that developed were measured. The average of the zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of each dilution against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value [27].

2.4. Testing the Effect of the Tested Drugs on the Adherence of the Tested Pathogens to Plastic Surfaces by Crystal Violet Assay Method

All strains were first streaked on Muller Hinton agar media then, incubated at 25°C for 48 h. A large loop of actively growing cells (for each strain) was transferred to sterile trypticase soy broth (TSB) (Difco Laboratories) containing 0.9% D-glucose. After incubation at 25°C for 24 h, the cells were centrifuged and washed twice with 0.5 ml PBS (phosphate buffered saline), followed by vortexing and centrifugation at 5000 *g* for 5 min. The washed cells were suspended in 1 ml TSB broth and adjusted to a final OD600 nm value of 1.0 with TSB broth. These cell suspensions were then used to grow biofilms.

100 µl of the suspension (OD600) was inoculated into individual wells of polystyrene 96-well plates (flat bottom; Nunc). TSB broth was used as a negative control. The plates were incubated at 25°C for 90 min (adhesion period). Supernatants including planktonic cells were discarded and wells were gently washed with PBS twice to remove any non-adherent cells. 100 µl of fresh TSB broth containing MIC concentrations of each of the following solutions: NSAIDs (Ketoprofen, Ibuprofen, Sodium Diclofenac) and N-acetyl cysteine each alone and in combination was added to each well. The plates were covered to prevent evaporation and incubated at 25°C for 24 h. Liquid media containing the non adherent cells were discarded through two rounds of washing with 200 µl sterile PBS buffer. Adherent cells to the plastic surfaces were quantified using Crystal violet assay. Experiment was performed in triplicate.

2.5. Testing Their Ability to Disrupt the Already Formed Mature Biofilms by Crystal Violet Assay Method

100 µl of the suspension (OD600) was inoculated into individual wells of polystyrene 96-well plates (flat bottom; Nunc). The plates were incubated at 25°C for 48 h. After the incubation period, the supernatants from each well were aspirated and the wells washed twice with PBS without disturbing the biofilms at the bottom of the wells, 100 µl of fresh TSB broth containing MIC concentrations of each of the following solutions: NSAIDs (Ketoprofen, Ibuprofen, Sodium Diclofenac) and N-acetyl cysteine each alone and in combination was added to each well. Normal saline without any agents was added to the control wells. The plates were incubated at 25°C for 24 h. Supernatants were discarded through two rounds of washing with 200 µl sterile PBS saline. Cells adherent to the plastic surfaces were quantified using Crystal violet assay [28]. Experiment was performed in triplicate.

2.6. Scanning Electron Microscopy (SEM)

The untreated *bacterial cells* (control) and *strains* treated with the tested drugs 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) [29].

2.7. Effect of MICs of the Tested Agents on Motility of *Ps. aeruginosa* and *Proteus mirabilis* Cells

Following 24-, 48- and 72-h incubation at 37 °C in the presence and absence of MICs of NSAIDs and NAC. Bacterial cells were centrifuged for 2 min at 2500 rpm to separate the cells. Bacterial cells were washed three times with PBS. Bacterial culture was inoculated into the tube with Motility Agar. Control cultures contained no drugs [30].

2.8. Statistical Analysis

One-Way ANOVA as 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, 17 statistical software (SPSS Inc., Chicago, IL).

3. Results

3.1. Biofilm Production

According to TCP method strains can be classified into weak adherent at OD <0.120, moderate 0.120-0.240 and strong >0.240. It was found that *S. aureus* ATCC 6538 and *Ps. aeruginosa* ATCC 10145 were strong biofilm producers

Table 1. Ability of the tested microorganisms to produce biofilm

Strains	Ability to adhere to TCP surface
***S. aureus* (ATCC 6538)**	Strong
***K. pneumoniae* (ATCC 10031)**	Moderate
***Ps. aeruginosa* (ATCC 10145)**	Strong
Proteus mirabilis	Moderate

3.2. Antibacterial Activity and MIC of the Tested Drugs against Microorganisms

Ibuprofen showed the highest antibacterial and the lowest MIC against *S. aureus, Ps. aeruginosa* and *Proteus mirabilis* while *Sodium diclofenac* showed the highest activity against *K. pneumoniae.* The tested NSAIDs showed higher antibacterial activity than N-acetylcysteine (Table 2).

Table 2. the Minimum inhibitory concentration of ketoprofen, Ibuprofen, Sodium diclofenac and N-acetyl cysteine

Microorganisms	MIC (µg/ml)			
	ketoprofen	Ibuprofen	sod Diclofenac	NAC
***S. aureus* (ATCC 6538)**	**1607.5**	**952**	**1465**	**20000**
***K. pneumoniae* (ATCC 10031)**	**1732.5**	**250**	**173.7**	**2500**
***Ps. aeruginosa* (ATCC 10145)**	**1756.7**	**1292.2**	**1675.7**	**2500**
Proteus mirabilis	**1635.3**	**1465.4**	**1769.5**	**2500**

3.3. Effect of the Tested Drugs on the Adherence of Microorganisms

Figure 1 and Figure 2 showed that N-acetyl cysteine had a higher inhibitory effect on the adherence of *S. aureus, K. pneumoniae* and *Proteus mirabilis* in comparison to ketoprofen (P<0.05) and Sodium diclofenac (P<0.01). On the other hand, Ketoprofen showed a higher effect (P<0.05) on *Ps. aeruginosa* adherence than NAC. Their combination significantly inhibit the adherence of (P<0.05) all tested organisms in comparison to each drug and NAC alone. Ibuprofen and NAC combination showed a significant inhibitory effect (P<0.05) on adherence of the tested organisms in comparison to Ibuprofen but not significant in comparison to NAC (Figure 3). In addition, Ketoprofen showed a higher inhibitory effect on *S. aureus, K. pneumoniae* and *Ps. aeruginosa* compared to NSAIDs (Figure 4).

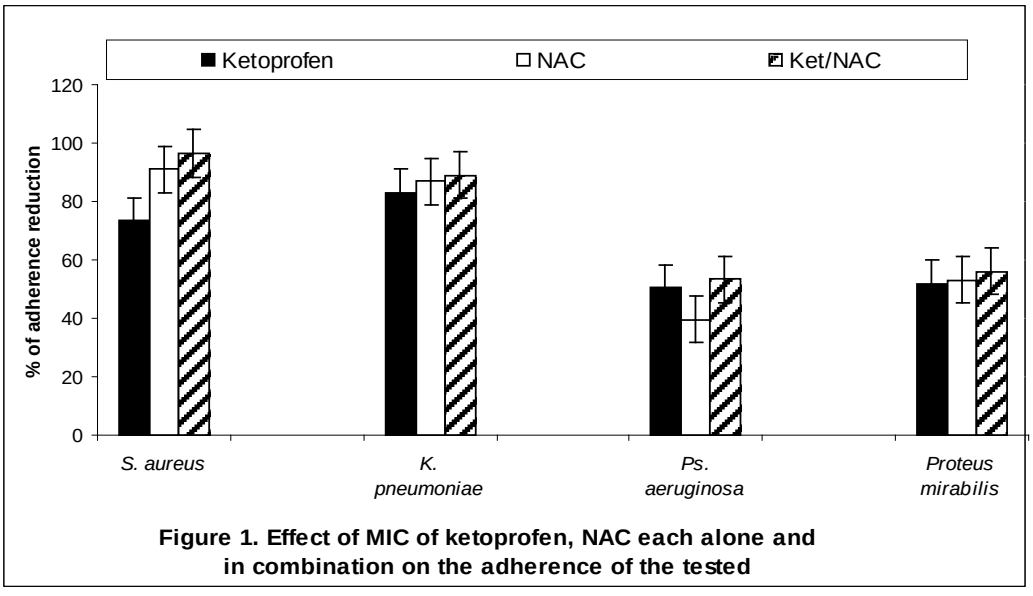

Figure 1. Effect of MIC of ketoprofen, NAC each alone and in combination on the adherence of the tested

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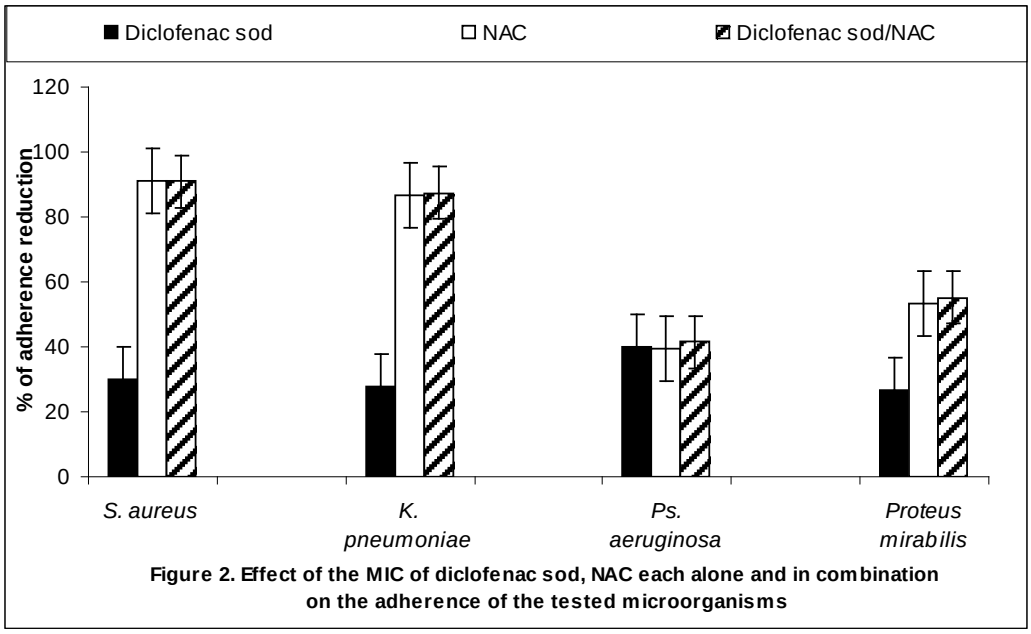

Figure 2. Effect of the MIC of diclofenac sod, NAC each alone and in combination on the adherence of the tested microorganisms

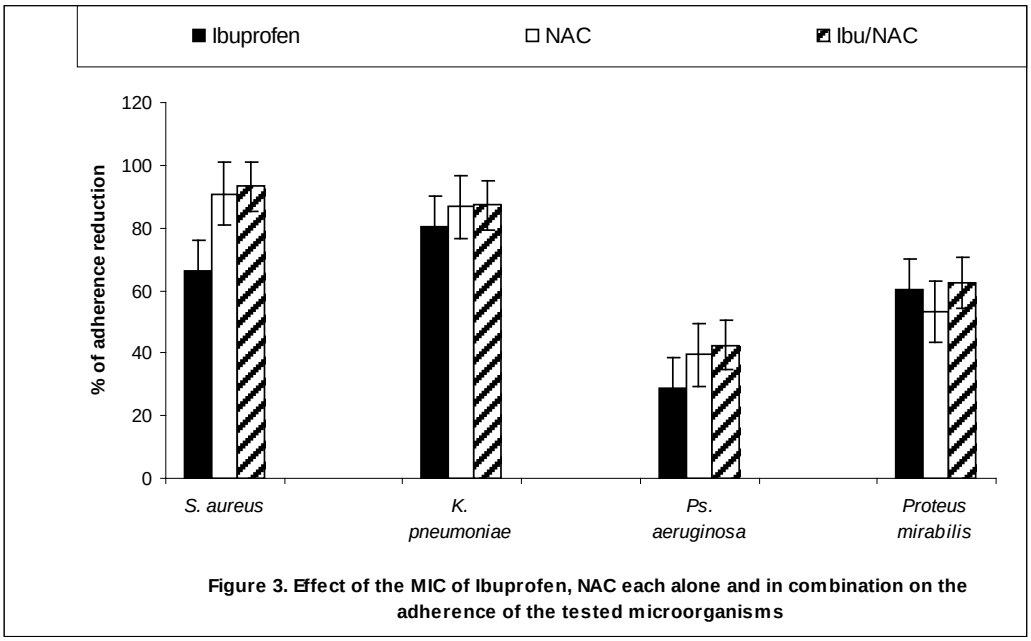

Figure 3. Effect of the MIC of ibuprofen, NAC each alone and in combination on the adherence of the tested microorganisms

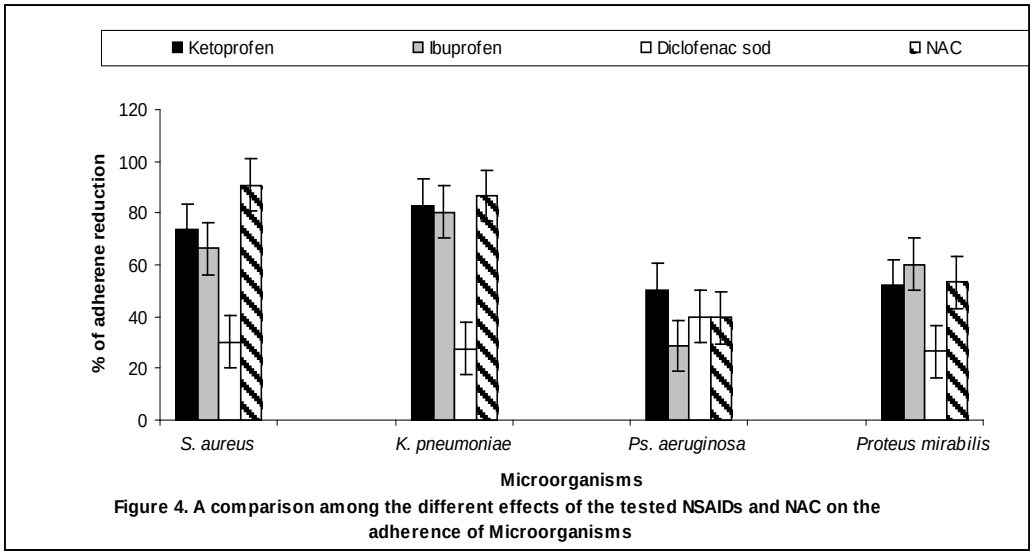

Figure 4. A comparison among the different effects of the tested NSAIDs and NAC on the adherence of Microorganisms

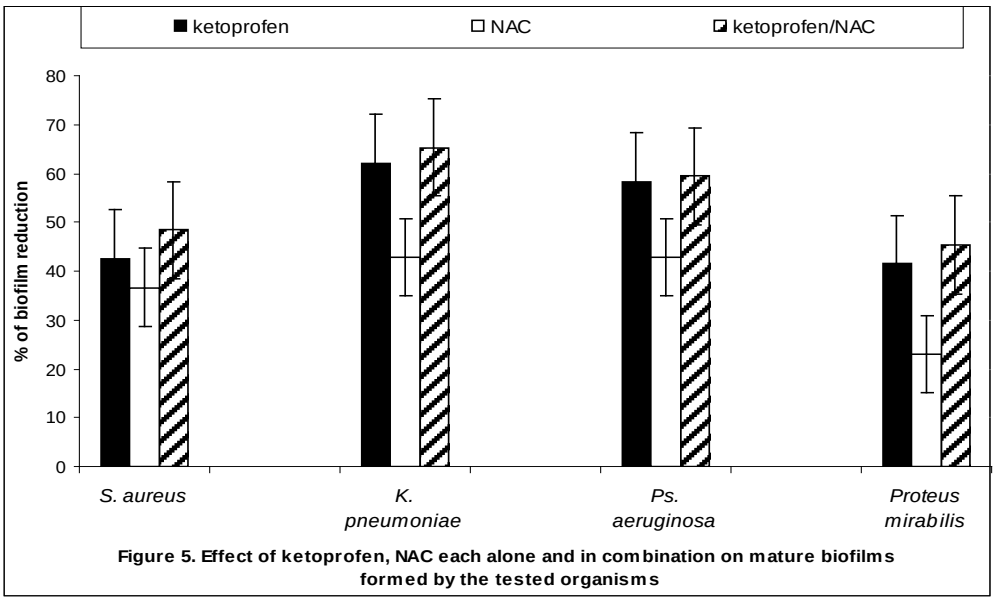

Figure 5. Effect of ketoprofen, NAC each alone and in combination on mature biofilms formed by tested organisms

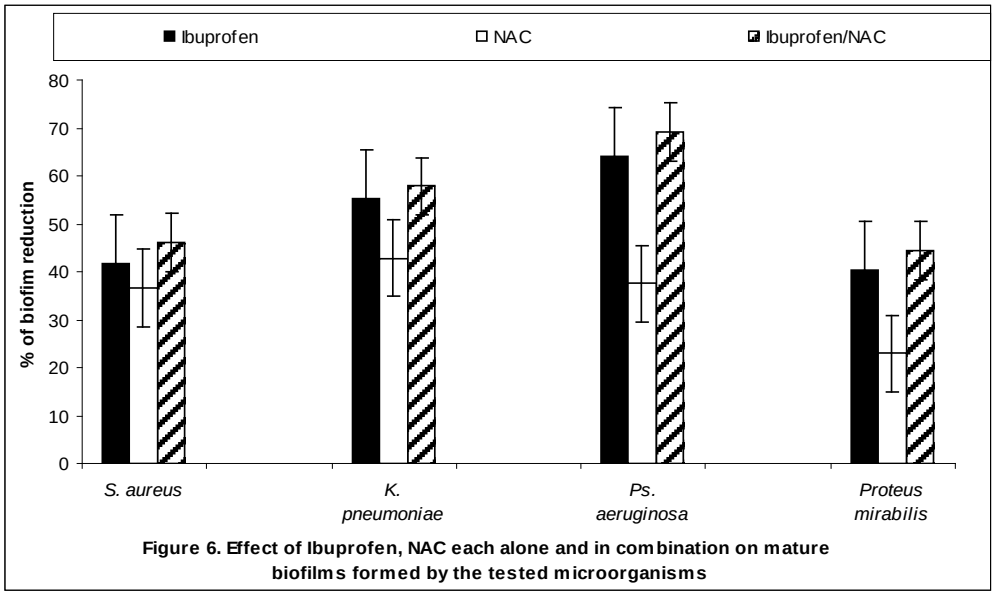

Figure 6. Effect of Ibuprofen, NAC each alone and in combination on mature biofilms formed by the tested microorganisms

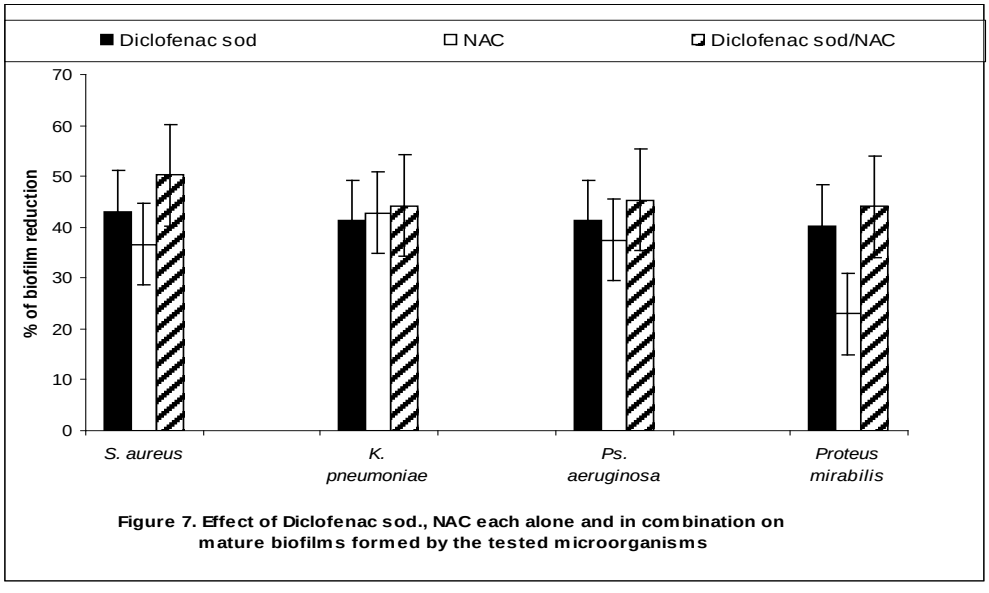

Figure 7. Effect of Diclofenac sod., NAC each alone and in combination on mature biofilms formed by the tested microorganisms

3.4. Effect of the Tested Compounds on Mature Biofilms Formed by the Tested Microorganisms

Combination between NSAIDs and NAC showed the highest disruptive effect on the mature biofilms formed by the tested microorganisms. Ketoprofen and Ibuprofen had a significant disruptive effect ($P<0.01$) on all mature biofilms formed by the tested microorganisms in comparison to controls and NAC (Figure 5 and Figure 6). Sodium diclofenac had the highest disruptive effect on biofilms formed by all tested microorganisms except for *K. pneumoniae* (Figure 7). A comparison among the tested drugs in their effects on mature biofilms formed showed that ketoprofen was more potent against biofilms formed by *S. aureus*, *K. pneumoniae* and *Proteus mirabilis* but Ibuprofen was more potent against biofilm formed by *Ps. aeruginosa* (Figure 8).

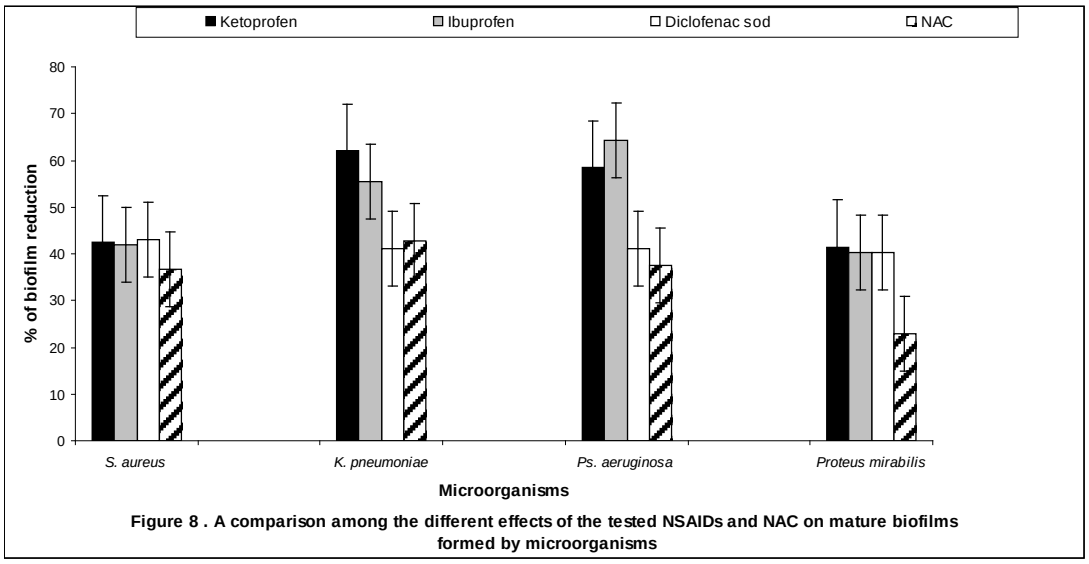

Figure 8. A comparison among the different effects of the tested NSAIDs and NAC on mature biofilms forms by microorganisms

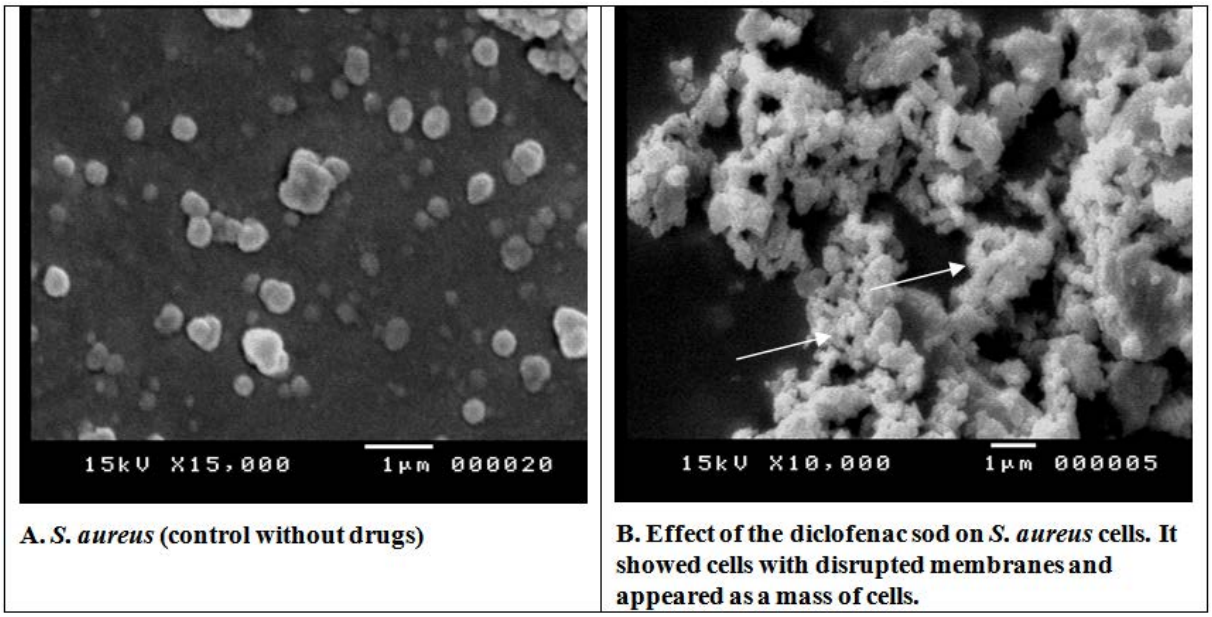

Figure 9. Scanning electron micrographs showed the morphological response of *S. aureus* to the effect of Sodium diclofenac

3.5. Effect of the Tested Drugs on the Morphology of Microorganisms Using SEM

Images showed a disturbance to membranes of bacteria in the presence of Sodium diclofenac.

3.6. Effect of the Tested Drugs on Motility of *Ps. aeruginosa* and *Proteus mirabilis*

The tested drugs showed no effect on the motility of the tested organisms.

4. Discussion

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used medicines for the management of pain and inflammation. Many studies showed that some NSAIDs have good antibacterial action especially Diclofenac sodium [31,32]. N-acetylcysteine (NAC) is a non-antibiotic drug that has antibacterial properties [34]. NAC affects several processes that are important for bacterial

biofilm formation on stainless steel surfaces medical devices, including a drastic reduction in extracellular polysaccharide production, its ability to detach mature biofilms and thus acts as an antibiofilm substance [35].

On the other hand, Efrati *et al.*, [25] demonstrated that NAC act as renoprotective agent against NSAIDs induced renal damage which may be due to the well-expected antioxidant effects and the augmentation of PGE2 release by renal tissue. Many studies reported that NAC increase the therapeutic activity of antibiotics. El-Rehewy et al., [36] and El-Feky *et al.* [37] investigated the effect of NAC (2 and 4mg/mL) in combination with ciprofloxacin (MIC and 2 × MIC) against biofilm cells of several bacteria including *S. aureus*, *S. epidermidis*, *E. coli*. and others. The authors concluded that NAC potentiate the therapeutic action of ciprofloxacin. This was due to the ability of NAC to degrade the extracellular polysaccharide matrix of biofilms. As general conclusion they suggest the potential use of ciprofloxacin-NAC combination as therapeutic strategy against bacterial infections mediated by biofilms. Efrati *et al.*, [25] reported that NAC was able of acting at the level of the matrix promoting the increasing therapeutic efficacy of vancomycin, which alone had no significant effect on cells embedded in biofilms. This activity of NAC can result in the detachment of cells, individually or in clusters, resulting in making biofilm and detached cells more susceptible to the action of other antimicrobial agents and to the immune system. Thus, the role of NAC and other antimicrobial agents targeting matrix and promoting this mechanism of detachment of cells can be an important help in the eradication of biofilms associated infections.

Our study showed that N-acetyl cysteine had a higher inhibitory effect on the adherence of *S. aureus*, *K. pneumoniae* and *Proteus mirabilis* in comparison to ketoprofen ($P<0.05$) and Sodium diclofenac ($P<0.01$). On the other hand, Ketoprofen showed a higher effect ($P<0.05$) on *Ps. aeruginosa* adherence than NAC while the tested NSAIDs were reported to have higher disruptive effect on mature biofilms more than NAC. In addition, ketoprofen had the highest effect on the inhibition of adherence of *S. aureus*, *K. pneumoniae* and *Ps. earuginosa* but ibuprofen had the highest effect against *Proteus mirabilis* in comparison to the tested agents. For mature biofilms, ketoprofen had the highest effect on *S. aureus*, *K. pneumoniae* and *Proteus mirabilis* but ibuprofen had the highest effect on *Ps. aeruginosa*. Many studies reported that Diclofenac sodium is a potent anti-inflammatory, analgesic, anti-pyretic agent and in reducing the post-operative endodontic pain with less gastrointestinal side effects [37,38,39,40,41]. Diclofenac was found to show antibacterial effect against both gram-positive and gram-negative bacteria and synergism with other antibiotics [42,43,44,45]. Hersh *et al.* [46] demonstrated the antibacterial activity of ibuprofen against six common periodontal pathogens. The exact mechanism of this antibacterial activity of diclofenac and ibuprofen is unclear. However, studies have proposed inhibition of bacterial DNA synthesis [47] or impairment of membrane activity that agree with results obtained by SEM in this study [32,43,46,48,50,51]. Dutta N.K. and his colleagues [45] had determinate the ability of diclofenac to protect mice from a virulent *Salmonella* infection. Their study had demonstrated that diclofenac (1.5-3 microg/g) protected animals from the lethality of *Salmonella [45]*. The time-kill curve study indicates of diclofenac comes in part, from its ability to inhibit the DNA synthesis of *E. coli* and *L. monocytogenes*. Diclofenac could protect murine listeriosis, salmonellosis, and tuberculosis at doses ranged within its maximum recommended human or non-toxic *ex-vivo* doses [48]. Although few studies found that ibuprofen and acetaminophen has significant effects to reduce some of body disorders after bacterial infection, antibacterial action of these agents are not clear for many species of pathogenic bacteria. Ibuprofen and acetaminophen were tested for antibacterial activity against seven isolates of bacteria including gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*) and gram negative bacteria *(E. coli*, *Enterobacter aerogene*, *E. cloacae*, *Salmonella typhi* and *Paracoccus yeei*) [52]. The *Staphylococcus aureus* and *Paracoccus yeei* strains were susceptible to lower concentration of ibuprofen and acetaminophen (MIC=1.25 mg/ml) and *Enterobacter* were resistant. The same strains were tested with diclofenac sodium, indomethacin and mefenamic acid. Diclofenac seems to be effective to inhibit the growth of bacteria in lower concentration (2.5-5 mg/ml). *Staphylococcus aureus* could be considered the most susceptible bacteria to diclofenac than other strains (according to disc diffusion method) [53]. Spectrophotometer assay gave much more valuable value about the inhibitory action of tested chemical agents. Diclofenac sodium also considered the powerful compound on tested bacteria. Comparing with control, the growth of all isolates was significantly reduced by 2.5mg/ml (MIC) of diclofenac sodium. Meanwhile, *Paracoccus yeei* tend to be the most susceptible strain to lower level of diclofenac (0.15-0.3mg/ml) followed by *B. subtillis* and *S. aureus* (0.6-1.25mg/ml) [54]. In another study, aspirin or ibuprofen was administered to mice undergoing treatment of tuberculosis infection (*Mycobacterium tuberculosis*) to determine if these non-steroidal anti-inflamatory drugs enhance pyrazinamide activity *in vivo* [55]. Simultaneous administration of either aspirin or ibuprofen with pyrazinamide resulted in a further decrease of about 0.4 $\log_{10}$ CFU in the lung and more than 1 $\log_{10}$ CFU in the spleen compared with mice receiving pyrazinamide alone. Aspirin and ibuprofen enhance the effect of pyrazinamide during the initial phase of tuberculosis treatment in the mouse model. The antimicrobial ability of diclofenac sodium, indomethacin and mefenamic acid to eliminate pathogenic organisms is not limited with direct inhibitory action of those organisms, but also includes indirect effects by using the main function of such compounds as anti-inflammatory to facilitate the destruction of affected organisms. In meningitis patients, diclofenac sodium and indomethacim reduce the inflammation resulted from infection with bacterial meningitis [56].

5. Conclusion

The impact of NSAIDs on adhesion and bacterial biofilm formation leads us to say that administration of the drugs in post-surgical period may lead to decrease the infection risk.

Many studies showed that NSAIDs act synergistically with antibiotics, so application of these drugs in perioperative period may increase the effectiveness of antibiotics

applied for prophylactic purposes NAC showed more significant effect on the adherence than the tested NSAIDs while NSAIDs showed more disruptive effect than NAC against mature biofilms. NSAIDs and NAC combination showed increased activity and a higher effect than the effect of each alone.

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References

[1] Pettit RK, Weber CA, Kean MJ, et al. Microplate Alamar Blue assay for *Staphylococcus epidermidis* biofilm susceptibility testing. *Antimicrob Agents Chemother*. 2005, 49: 2612-2617.

[2] Flemming K, Klingenberg C, Cavanagh JP, et al. High in vitro antimicrobial activity of synthetic antimicrobial peptidomimetics against staphylococcal biofilms. *J Antimicrob Chemother*. 2009, 63: 136-145.

[3] Lindsay D, von Holy A. Bacterial biofilms within the clinical setting: what healthcare professionals should know. *J Hosp Infect*. 2008, 64: 313-325.

[4] Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 2002, 15: 167-193.

[5] Chambers ST, Peddie B, Pithie A. Ethanol disinfection of plastic-adherent micro organisms. *J Hosp Infect*. 2006, 63: 193-196.

[6] Frank KL, Reichert EJ, Piper KE, Patel R. In vitro effects of antimicrobial agents on planktonic and biofilm forms of *Staphylococcus lugdunensis* clinical isolates. *Antimicrob Agents Chemother*. 2007, 51: 888-895.

[7] Gualtieri M, Bastide L, Villain-Guillot P, Michaux-Charachon S, Latouche J, Leonetti JP. In vitro activity of a new antibacterial rhodanine derivative against *Staphylococcus epidermidis* biofilms. *J Antimicrob Chemother*. 2006, 58: 778-783.

[8] Jefferson KK. What drives bacteria to produce a biofilm? FEMS Microbiol Lett 2004; 236: 163-173.

[9] Prüß BM, Besemann C, Denton A, Wolfe J. A complex transcription network controls the early stages of biofilm development by *Escherichia coli*. J Bacteriol. 2006, 188: 3731-3739.

[10] An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. *J Biomed Mater Res*. 1998, 43: 338-348.

[11] Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteriaematerials interactions. *Euro Cell Mater*. 2004, 8: 37-57.

[12] Cerca B, Martins S, Pier G, Oliveira R, Azevedo J. The relationship between inhibition of bacterial adhesion to a solid surface by sub-MICs of antibiotics and subsequent development of a biofilm. *Res Microbiol*. 2005, 156: 650-655.

[13] Kinnari TJ, Peltonen LI, Kuusela P, Kiviahti J, Kononen M, Jero J. Bacterial adherence to titanium surface coated with human serum albumin. *Otol Neurotol*. 2005, 26: 380-384.

[14] Kleinknecht D, Landais P, Goldfarb B. Analgesic and nonsteroidal anti-inflammatory drug-associated acute renal failure: a prospective collaborative study. *Clin Nephrol*. 1986, 25: 275-281.

[15] Oates JA, FitzGerald GA, Branch RA, Jackson EK, Knapp HR, Roberts LJ. Clinical implications of prostaglandin and thromboxane A2 formation (2). *N Engl J Med*. 1988, 319: 761-767.

[16] Patrono C, Dunn MJ. The clinical significance of inhibition of renal prostaglandin synthesis. *Kidney Int*. 1987, 32: 1-12.

[17] Huerta C, Castellsague J, Varas-Lorenzo C, Garcia Rodriguez LA. Nonsteroidal anti-inflammatory drugs and risk of ARF in the general population. *Am J Kidney Dis*. 2005, 45: 531-539.

[18] Efrati S, Averbukh M, Berman S et al. N-Acetylcysteine ameliorates lithium-induced renal failure in rats. *Nephrol Dial Transplant.* 2005, 20: 65-70.

[19] Efrati S, Dishy V, Averbukh M et al. The effect of N-acetylcysteine on renal function, nitric oxide, and oxidative stress after angiography. *Kidney Int*. 2003, 64: 2182-2187.

[20] Feldman L, Efrati S, Dishy V et al. N-acetylcysteine ameliorates amphotericin-induced nephropathy in rats. *Nephron Physiol*. 2005, 99: 23-27.

[21] DiMari J, Megyesi J, Udvarhelyi N, Price P, Davis R, Safirstein R. N-acetyl cysteine ameliorates ischemic renal failure. *Am J Physiol*. 1997, 272: F292-F298.

[22] Mazzon E, Britti D, De Sarro A, Caputi AP, Cuzzocrea S. Effect of N-acetylcysteine on gentamicin-mediated nephropathy in rats. *Eur J Pharmacol*, 2001, 424: 75-83.

[23] Marenzi G, Assanelli E, Marana I et al. N-acetylcysteine and contrast-induced nephropathy in primary angioplasty. *N Engl J Med 2006*; 354: 2773-2782.

[24] Heyman SN, Goldfarb M, Shina A, Karmeli F, Rosen S. N-acetylcysteine ameliorates renal microcirculation: studies in rats. *Kidney Int* 2003, 63: 634-641.

[25] Efrati S, Berman S, Siman-Tov Y, Lotan R, Averbukh Z, Weissgarten J and Golik A. *N*-acetylcysteine attenuates NSAID-induced rat renal failure by restoring intrarenal prostaglandin synthesis *Nephrol Dial Transplant*. 2007, 22: 1873-1881.

[26] Christensen G.D., W.A. Simpson, J.A. Younger, L.M. Baddour, F.F. Barrett, D.M. Melton, et al. Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. *J. Clin. Microbiol*. 1985, 22: 996-1006.

[27] Bennet J.V., Brodie J.L., Benner J.L., Kirby W.M.M., "*Simplified accurate method for antibiotic assay of clinical specimens". Appl Microbiol.* 1966, 14: p. 2170-2177.

[28] Xiaogang L, Zhun Y and Jianping X. Quantitative variation of biofilms among strains in natural populations of *Candida albicans. Microbiology* 2003, 149: 353-362.

[29] Soboh F, Khoury AE, Zamboni AC, Davidson D, Mittelman MW. Effects of ciprofloxacin and protamine sulfate combinations against catheter-associated *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother* 1995, 39: 1281-1286.

[30] Wojnicz D, Tichaczek-Goska D. Effect of sub-minimum inhibitory concentrations of ciprofloxacin, amikacin and colistin on biofilm formation and virulence factors of *Escherichia coli* planktonic and biofilm forms isolated from human urine. *BJM* 44, 1, 259-265 (2013).

[31] Wang WH, Wong WM, Dailidiene D, Berg DE, Gu Q, Lai KC et al. Aspirin inhibits the growth of Helicobacter pylori and enhances its susceptibility to antimicrobial agents. *Gut*. 2003 Apr; 52(4): 490-5.

[32] Hersh EV, Hammond BF, Fleury AA. An-timicrobial activity of flurbiprofen and ibupro-fen in vitro against six common periodontal pathogens. *J Clin Dent*. 1991, 3(1): 1-5.

[33] Olofsson AC, Hermansson M, Elwing H. N-acetyl-l-cysteine affects growth, extracellular polysaccharide production and bacterial biofilm formation on solid surfaces. *Appl. Environ. Microb*. 2003, 69: 4814-22.

[34] Perez-Giraldo C, Rodriguez-Benito A, Moran FJ, Hurtado C, Blanco MT, Gómez-García AC. Influence of N-acetylcysteine on the formation of biofilm by *Staphylococcus epidermidis*. *J Antimicrob chemoth*. 1997, 39: 643-6.

[35] Abbas Hisham A., Fathy M. Serry, Eman M. EL-Masry (2012) Combating *Pseudomonas aeruginosa* Biofilms by Potential Biofilm Inhibitors *Asian J. Res. Pharm. Sci*. 2012, Vol. 2: 66-72.

[36] El-Rehewy M S K, El-Feky M A, Hassan MA, Abolella H A, Abolyosr A, Abd El-Baky R M and Gad G F *In vitro* Efficacy of Ureteral Catheters Impregnated with Ciprofloxacin, N acetylcysteine and their Combinations on Microbial Adherence *Clinical Medicine: Urology* 2009, 21-8.

[37] El-Feky, M.A., El-Rehewy, M.S., Hassan, M.A., Abolella H.A., Abd El-Baky, R.M., and Gad, G.F. (2009). Effect of ciprofloxacin and N-acetylcysteine on bacterial adherence and biofilm formation on ureteral stent surfaces. *Pol. J. Microbiol*., 58(3), pp. 261-267.

[38] Scholer DW, Boettcher I, Ku EC, Schweizer A. Pharmacology of diclofenac so-dium (Voltaren). *Semin Arthritis Rheum*. 1985, 15(2 Suppl 1): 61-4.

[39] Zuniga JR, Malmstrom H, Noveck RJ, Campbell JH, Christensen S, Glickman RS, et al. Controlled phase III clinical trial of diclo-fenac potassium liquid-filled soft gelatin cap-sule for treatment of postoperative dental pain. *J Oral Maxillofac Surg*. 2010, 68(11): 2735-42.

[40] Kubitzek F, Ziegler G, Gold MS, Liu JM, Ionescu E. Analgesic efficacy of low-dose dic-lofenac versus paracetamol and placebo in postoperative dental pain. J Orofac Pain. 2003 Summer; 17(3): 237-44.

[41] Buyukkurt MC, Gungormus M, Kaya O. The effect of a single dose prednisolone with and without diclofenac on pain, trismus, and swelling after removal of mandibular third molars. *J Oral Maxillofac Surg*. 2006, 64(12): 1761-6.

[42] Saatchi M, Mosavat F, Razmara F, Soley-mani B. Comparison of the effect of Ibuprofenand slow-released Diclofenac Sodium in con-trolling post endodontic pain. *J Dent Tehran Uni Med Sci*. 2010, 22: 185-91.

[43] Negm MM. Effect of intracanal use of nonsteroidal anti-inflammatory agents on post-treatment endodontic pain. *Oral Surg Oral Med Oral Pathol*. 1994, 77(5): 507-13.

[44] Kristiansen JE, Hendricks O, Delvin T, Butterworth TS, Aagaard L, Christensen JB, et al. Reversal of resistance in microorganisms by help of non-antibiotics. J Antimicrob Che-mother. 2007, 59(6): 1271-9.

[45] Dutta NK, Mazumdar K, Seok SH, Park JH. The anti-inflammatory drug Diclofenac retains antilisterial activity in vivo. *Lett Appl Microbiol*. 2008, 47(2): 106-11.

[46] Dutta NK, Mazumdar K, Dastidar SG, Park JH. Activity of diclofenac used alone and in combination with streptomycin against *Mycobacterium tuberculosis* in mice. *Int J Anti-microb Agents*. 2007, 30(4): 336-40.

[47] Dutta NK, Kumar KA, Mazumdar K, Das-tidar SG. In vitro and in vivo antimycobacteri-al activity of antiinflammatory drug, diclofenac sodium. *Indian J Exp Biol*. 2004, 42(9): 922-7.

[48] Dutta NK, Annadurai S, Mazumdar K, Dastidar SG, Kristiansen JE, Molnar J, et al. Potential management of resistant microbial infections with a novel non-antibiotic: the anti-inflammatory drug diclofenac sodium. *Int J Antimicrob Agents*. 2007 Sep; 30(3): 242-9.

[49] Dastidar SG, Ganguly K, Chaudhuri K, Chakrabarty AN. The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. *Int J Antimicrob Agents*. 2000, 4(3): 249-51.

[50] Negm MM. Intracanal use of a corticosteroid-antibiotic compound for the management of post treatment endodontic pain. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2001, 92(4): 435-9.

[51] Ehrmann EH, Messer HH, Adams GG. The relationship of intracanal medicaments to postoperative pain in endodontics. *Int Endod J*. 2003, 36(12): 868-75.

[52] Mazumdar K. Dastidar S.G., Park J. H., et al. The anti-infl amatory non-antibiotic helper compound diclofenac: an antibacterial drug target. *Eur. J. Clin. Microbiol. Infect. Dis.* 2009. 28 (8) 881-891.

[53] AL-Janabi A.S. In vitro antibacterial activity of ibuprofen and acetaminophen. J. Global Infect. Dis. 2010. 2. 105-108.

[54] AL-Janabi A.S. Comparison of disc diffusion assay with spectrophotometer technique for antibacterial activity of diclofenac sodium, indomethacin and mefenamic acid. *Asian J. Pharm*. 2009. 3: 148-52.

[55] Byrne S.T., Denkin S.M., Zhang Y. Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. *J.Antimicrob. Chemother*. 2006, 59. 313-316.

[56] Van der Flier M., Geelen S.P., Kimpen J.L. Anti-infl ammatory adjuvant agents in bacterial meningitis. *Curr. Med. Chem.* 2002, 1. 55-62.