The Effect of Fluoroquinolone Antibacterials on the Adhesion of *E.coli* 078 to Buccal Epithelial cells

Asaad Mirza Mohamed* and Nidhal AK Mohammed Ali**

Ministry of Agriculture, Veterinary Medicine Division, Babylon*; Department of Pharmacology, College of Medicine, Hawler Medical University, Erbil**, Iraq.

nabdulqader@yahoo.com**

Article Info

Received:29th September 2012
Accepted:26 November 2012
Published online: 1st December 2012

ISSN: 2231-9123

Abstract

The effect of three fluoroquinolone antibacterial agent, ciprofloxacin, enrofloxacin, and flumequine on important virulence factors in the adhesion of *E.coli*to epithelial cells was investigated.
Specimens of buccal epithelial cells were obtained from healthy volunteers. The effects of MIC and sub-MICs of ciprofloxacin, enrofloxacin, and flumequine on the adhesion of *E.coli* 078 to buccal epithelial cells by *in vitro* method an bacterial morphology was studied using electron microscope.
The three antibacterial agents significantly reduced the *in vitro* bacterial adhesion at all concentrations and that ciprofloxacin produced the greatest inhibition. Morphological changes in *E.coli* shown by electron microscopy were observed with all antibiotics tested with the prominent changes was exhibited by ciprofloxacin.

Key words: **Ciprofloxacin, Enrofloxacin, flumequine, *E.coli*, Adhesion, epithelial cells**

1. Introduction

Following administration of any antimicrobial agent, the concentration of the drug in the body will fall to sub-inhibitory levels (Bidgood and Papich, 2005).Exposure of bacteria to subinhibitory concentration (sub-MIC) found to interfere with some important bacterial cell functions and host- bacteria interactions like the ability of bacteria to adhere to host cells, changes in cell morphology, rate of growth, and production of enzyme and toxin (Wojnicz*et al.*, 2007). It is postulated that sub-inhibitory antimicrobial concentrations may exert their anti-adhesive effects through suppression of formation and/or expression of the surface adhesion structures, or produce direct effect on the bacterial energy or motility (Dynes*et al*., 2009).

The fluoroquinolone antibacterial agents are highly potent, broad-spectrum agents which penetrate bacterial cell walls and inhibit DNA gyrase, a key enzyme in DNA replication (Boothe*et al*., 2006). Flumequine, a first-generation fluoroquinolone, is an older member of this group still used in veterinary medicine (Mevius, 1990). Due to the emergence of resistance and their considerable toxicity, it is replaced by newer fluoroquinolone compounds with more potent antibacterial activity, higher tissue distribution and wider spectrum of activity (Bolon,2011).Enrofloxacin, norfloxacin, ciprofloxacin,pradofloxacin, and marbofloxacinare the most commonly used fluoroquinolone antibioticsnow days(Pallo-Zimmerman *et al*., 2010). Of these, ciprofloxacin is among the most potent clinically, especially against members of the family Enterobacteriaceae and *Pseudomonas aeruginosa*, providing a standard therapy for these micro-organisms ((Bolon, 2011).

Escherichia coli, is the predominant enteric pathogen causing extraintestinal infections in man (Spurbeck et *al*., 2011). Fimbriae appendages (pili) are believed to mediate adhesion (Bavington and Page, 2005). The majority of uropathogenic *E. coli* carry pili thought to interact with specific receptors on the uroepithelium and found to recognize similar receptors present on human erythrocytes (Pompilio*et al*., 2010).

Among the investigations on the interaction of antimicrobial agents and microorganisms is the suppression of bacterial growth after exposure to an antibiotic for a short period of time (Anderson*et al*., 2010). The purpose of the present study was to compare the relative *in vitro* effects of different inhibitory concentration (MIC and sub-MICs) of ciprofloxacin, enrofloxacin and flumequine on the adhesion of *E.coli 078* to buccal epithelial surfaces.

2. Methodology

2.1. Drugs: the antibiotics ciprofloxacin, enrofloxacin and flumequine used in the study were purchased from Vapco, Jordan.

2.2. Bacterial strains and culture media: *E.coli* 078 was obtained from the department of pathology and poultry, College of Veterinary Medicine, University of Baghdad, Iraq. The bacterial strain was identified by the International *Escherichia* and *Klebsiella* status serum Center in Denmark. Culture media used were: Brain heart infusion (BHI) broth and agar, Mac Conky agar, Mueller Hinton broth and agar, Phosphate buffer saline (pH 7.2). Percoll (Pharmacia, Sweden).The sensitivity of the *E.coli* 078 to the tested antibiotic was performed according to the method of (Bauer*et al.*, 1966).

2.3. Determination of MIC and sub MIC: The MICs for each antibiotic were determined using the micro dilution broth method as instructed by Clinical and Laboratory Standards Institute (CLSI) (National Committee for clinical laboratory standards (2002).

From the MIC of each drug, sub MIC were prepared (**(½ - ⅛** MIC) in BHI broth. The concentration of the bacterial suspension was adjusted to the desired concentration according to the method of (Marth, 1988).

2.4. Adherence assay: The method of (Valentine-Weigand, 1987) was followed for the adherence assay. From normal healthy human volunteer (males and females) buccal epithelial

cells were collected by gentle scraping of buccal mucosa with a cotton tipped swab. The swabs were immersed in PBS buffer saline (pH7.2), vortexed and placed in incubator for ½ hour to prevent aggregation of the epithelial cells.

The cells were washed three times in PBS (pH7.2). The epithelial cell suspension was added to a test tube containing percoll (to obtain optimum separation of epithelial cells). The suspension was then centrifuged (8000rpm) and the epithelial cell were taken and diluted with PBS, centrifuged to get rid from percoll layer for two time. The epithelial cells were harvested and finally suspended in PBS to get a concentration of 2×10^5cells/ml.

The bacterial cells were suspended in PBS to get a concentration of 1×10^8cells/ml. 1 ml of bacterial culture was mixed with 1 ml of epithelial suspension (2×10^5cells) and incubated in shaking water bath (60 rpm) at 37˚C for three hours. The mixture was then washed three times and finally filtered. A slide was gently pressed against the filter, air dried, fixed with acetone, stained for 5 minutes with methylene blue, and washed with distilled water and air dried. The mean number of bacteria adhered to the first 50 epithelial cells was counted and the standard error of mean was calculated for each preparation.

Each test was performed for three times and included the determined MIC concentration and sub MICs (½, ¼ and ⅛) of each antibiotic. The bacterium was grown in BHI with or without (control) the different antibiotics for 18 hours at 37˚C and the adherence assay was performed as described above.

2.5. Electron microscopy: Scanning electron microscopy was performed according to (Reid *et al.,* 1994) on selected specimens to examine the effects of the three antibiotics on *E.coli* 078. The specimens with or without antibiotic were fixed in 1% osmium tetroxide for 2 hours , then placed in 0.1 M sodium phosphate buffer (pH7.2), processed and stained with uranyl acetate and lead citrate. The bacteria were then examined for fimbriae with scanning electron microscope.

Statistical analysis: The results were analyzed by SPSS version 18. The mean and standard deviation of each parameter was obtained at each treatment. ANOVA was used to compare between groups.

3. Results and Discussion

The MIC of ciprofloxacin, enrofloxacin and flumequine found in this study for *E.coli* 078 were 0.05, 0.06 and 0.6 µg/ml respectively (figure1). These MIC values are close to those reported by for *E.coli* strain (Cavaco and Arestrup, 2009) and less than the breakpoint of ciprofloxacin (≥ 4µg/ml), enrofloxacin ((≥8 µg/ml) and flumequine ((≥16 µg/ml) for resistant *E.coli*(Hombach *et al*., 2012) which indicates the sensitivity of this strain to these antibiotics.

The low MIC especially of ciprofloxacin (0.05 µg/ml) and enrofloxacin (0.06 µg/ml) found in this study indicates their high bactericidal activity versus *E.coli* owing to their property in penetrating cell membrane easily and inhibiting DNA synthesis of this microorganism

(Bolon, 2011). While the older member of quinolone class of antimicrobial agent, flumequine showed relatively higher MIC value (0.6 µg/ml) indicating its lower antibacterial activity which is related to its chemical structure that makes its penetration through bacterial cell membrane quite limited (Martinez *et al.*, 2006).

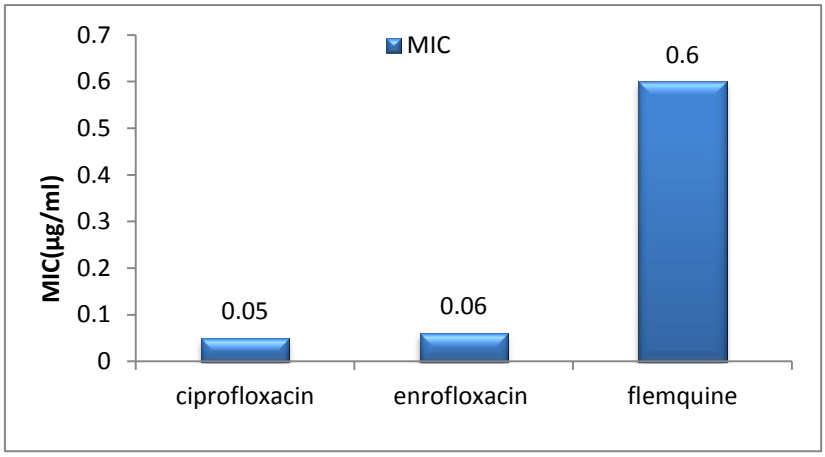

Figure 1.The minimum inhibitory concentration (MIC) of ciprofloxacin, enrofloxacin and flumequine for *E.coli* 078.

The inhibition of *E.coli* 078 adhesion to buccal epithelial cells in the presence of various concentrations (MICs and sub-MICs) of ciprofloxacin, enrofloxacin and flumequine are shown in figures (2). Compared to untreated control, which was considered as producing 100% adhesion property, a significant differences ($P<0.05$) were seen in the percentage of *E.coli* adhesion when the three antibiotics at MIC and sub- MIC vales were used and the greatest inhibition was shown at MIC values. This denotes that these antibacterial agents have the affinity to interfere with adhesion structures and consequently their ability to initiate infection and virulence (Vidya*et al*., 2005).

This anti-adhesive property found to be related to alteration in function of the surface adhesion factors such as fimbriae (Spurbeck*et al*., 2011) and that antibiotics at MIC and sub-MICs can impair bacterial adhesions through modifying the molecular structure of the external surface of bacteria and some of bacterial functions that aids in adhesion step to host cells and render them more susceptible to host defense mechanisms thus influencing bacterial virulence (Zalas-Wiecek*et al*., 2011).

Comparison between the effects of different concentrations of the three antibiotics showed that ciprofloxacin in MIC produced the most significant inhibition of adhesion than others (figure 2) with no statistical differences seen between enrofloxacin and flumequine. In ½ MIC, a significant difference ($P>0.05$) was observed between the three antibiotics whereas no statistical differences were seen between these three fluoroquinolones in ¼and ⅛ MIC (figure 2). This indicates that the modification in the chemical structure of quinolone molecule (4-quinolone) that led to the development of fluoroquinolones (6-fluoroquinolne) by addition of fluorine atom at C6 enhanced their bactericidal potency, improved their cell penetration and anti-adhesive activity (Riddle *et al*., 2000). Such characteristic for ciprofloxacin is being well documented (Martinez *et al*., 2006) and this modification even allowed for ciprofloxacin's increased bioavailability as it achieves concentrations at various

sites of infection above the minimum inhibitory concentrations (MIC's) of most pathogens affecting respiratory, urinary, prostate, bone, liver, bile, genital and inflammatory fluids (Bolon, 2011).

Scanning electron microscope (SEM) of the *E.coli* 078 used in this study was to confirm the presence of fimbriae (figure 3A; control untreated E.coli) and looking for any possible changes in bacterial morphology upon exposure to MIC of antibacterial agents has been suggested (Dynes, 2009). Indeed, on examination of electron micrographs, these adherence factors as pilli and fimbriae were disappeared and also distortion of plasma membrane, vacuolation of internal structures and bacterial elongation was observed (figure 3; B, C). These structural alterations in the adhesion factors will ultimately preclude the process of bacterial adhesion to cell membranes and infectivity. Such morphological and biochemical changes were also observed by (Deo*et al.*, 2010).

In conclusion, the overall results indicate that ciprofloxacin possess greater bactericidal and anti-adhesion activity than enrofloxacin and flumequine by interfering with the adhesiveness and hence the risk of colonization of *E.coli* to epithelial cells.

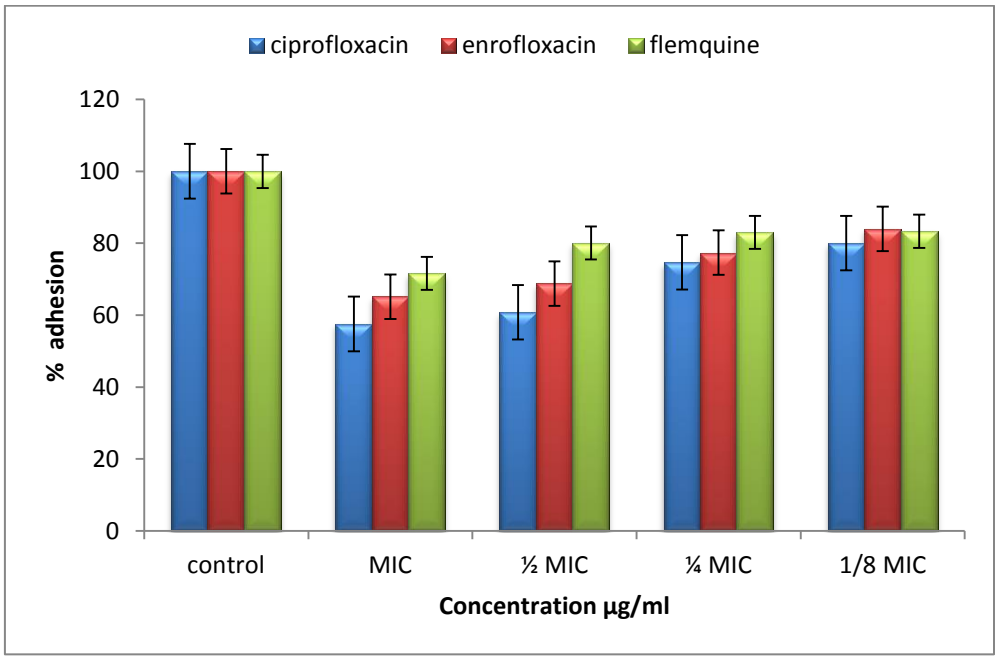

Figure 2. The effect of different minimum inhibitory concentration (MIC) of ciprofloxacin, enrofloxacin and flumequine on the adhesion of *E.coli* 078 to buccal epithelial cells.

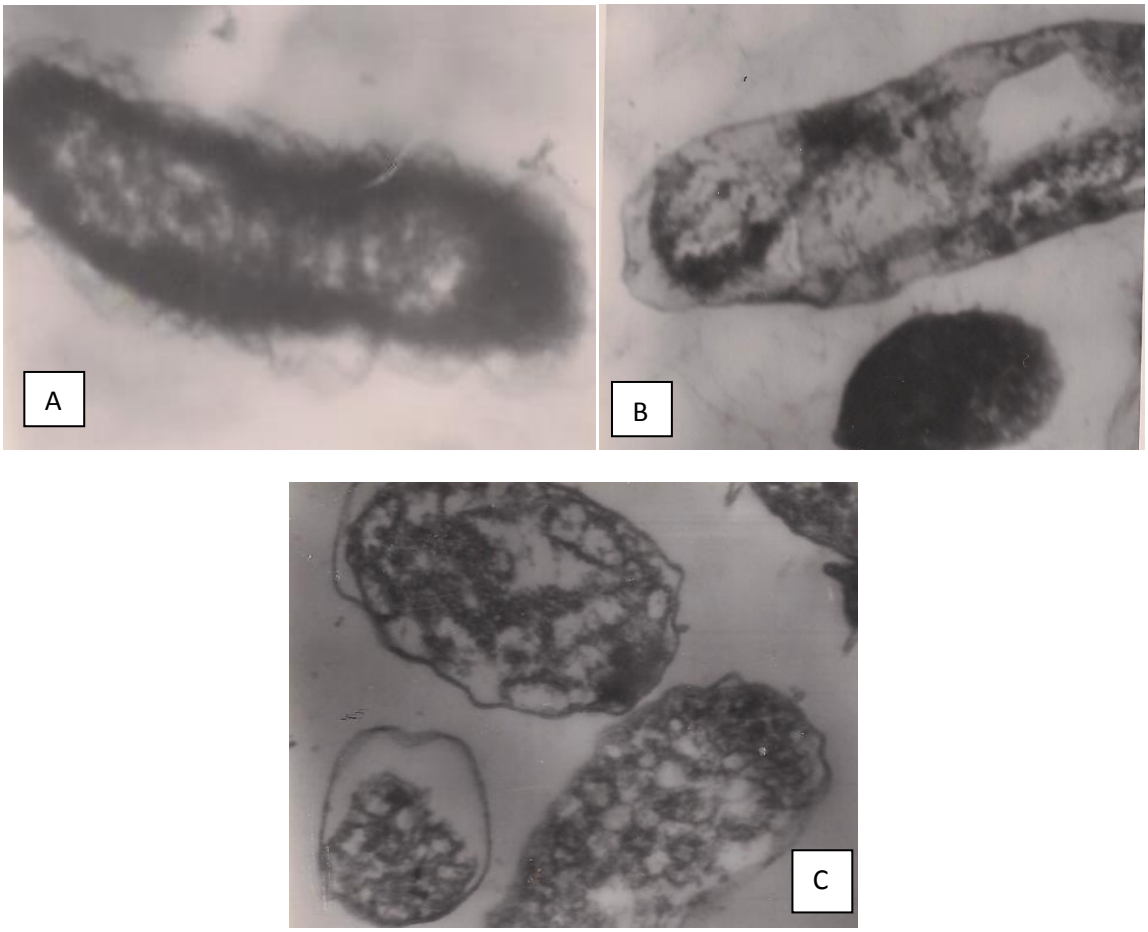

Figure 3. Scanning electron microscopy of *E*.coli 078 in untreated (A) and treated with MIC values of (B) ciprofloxacin and (C) flumequine. (X 94000)

References

Anderson, R.; Tintinger, G.; Cockeran, R.; Potjo, M. and Feldman, C. (2010). Beneficial and harmful interactions of antibiotics with microbial pathogens and the host innate immunity system. Pharmaceuticals. 3: 1694-1710.

Bauer, A.W.; Kirby, W.M.; Sherris, J.G. and Urk, M. (1966). Antibiotic susceptibility tests by a standardized single disk method. Am. J Clin Path.45:493-496. Bavington,C. and Page,C. (2005). Stopping bacterial adhesion: A noval approach to treating infections. Respiration. 72:335-344.

Bidgood, T.L,.andPapich, M.G.(2005). Plasma and interstitial fluid pharmacokinetics of enrofloxacin, its metabolite ciprofloxacin, and marbofloxacin after oral administration and a constant rate intravenous infusion in dogs. J Vet PharmacolTher 28: 329-41.

Bolon, M.K. (2011).The newer flouroquinolnes. Med. Clin Am. 95:793-817. Boothe, D.M.; Boeckh, A.; Simpson,B. and Dubose,K.(2006). Comparison of pharmacodynamics and pharmacokinetic indices of efficacy for 5 flouroquinolones toward pathogens of dogs and cats. J Vet Intern Med. 20:1297-1306.

Cavaco, L. M. and Arestrup, F. M. (2009). Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new

transmissible resistance mechanisms *qnrA*, *qnrB*, *qnrS*, and *aac*(6_)*Ib-cr*, in Escherichia *coli* and Salmonella entericaand determinations of wild-Type distributions. J. ClinMicrobiol. 47(9):2751-2758.

Deo, A.A.; Kulkarni, A.S. and Meshram, S.U. (2010). Monocationic surfactant induced ultra-structural changes in antibiotic resistant Escherichia coli.Indian J Med Res. 131:825-8.

Dynes, J.; Lawrence, J.R.; Korber D.R.; Swerhone, G.W.; Leppard, G. and Hitchcock, A.P. (2009).Morphological and biochemical changes in Pseudomonas fluorescensbiofilms induced by sub-inhibitory exposure to antimicrobial agents.Can. J. Microbiol. 55: 163-178.

Hombach, M.; Bloemberg G.V.; Erik C. and Bottger, E.C. (2012). Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli. J Antimicrob. Chemother. 67: 622–632

Marth, E. H.(1988). Standard methods for examination of dairy products.14th ed. A.M. Public health Association, Washington, DC.

Martinez, M.; McDermott, P. and Walker, R. (2006). Pharmacology of the Flouroquinolnes: a perspective for use in domestic animals. Vet. J. 172:10-28.

Mevius, D.J.; Breukink, H.J.andVan Miert, A.S. (1990). In vitro activity of flumequine in comparison with several other antimicrobial agents against five pathogens isolated in calves in the Netherlands. Vet Q. 12(4):212-20.

National Committee for Clinical and Laboratory Standard Institute (2002) .Performance standard for antimicrobial susceptibility, 14th informational supplement. M 100-S 14, NCCL, Wayne,PA, USA.

Pallo-Zimmerman, L.M. ,Byren JK., Graves, TK. (2010). Fluoroquinolnes: Then and now. Compendium: Continuing Education for Veterinarian, Vetlearn.com. CE articles.

Pompilio,A.; Catavitello,C.; Picciani, C.; Confalone,P.; Piccolomini, R.; Savini, v.; Fiscarelli,E.; D'Antonio, D. and Di Bonaventura, G. (2010). Sub-inhibitory concentration of moxifloxacin decrease adhesion and biofilm formation of Stenotrophomonasmaltophilia from cystic fibrosis.Med Microbial. 59: 76-81.

Reid, G.; Sharma, S.;Advikolanu, K.; Tieszer, C.; Martin, R.A.; and Bruce, A.W. (1994). Effects of Ciprofloxacin, Norfloxacin, and Ofloxacin on In Vitro Adhesion and Survival of Pseudomonas aeruginosa AK1 on Urinary Catheters.Antimicrob Agents Chemother. 38(7):1490-1495.

Riddle, C.; Lemons, C.L.; Papich, M.G., and Altier. C. (2000). Evaluation of Ciprofloxacin as a Representative of Veterinary Fluoroquinolones in Susceptibility Testing. J Clinical Microbiol. 38(4):1636–1637.

Spurbeck, R.R.; Stapleton, A.E.; Johnson, J.R.; Walk, S.T.; Hooton, T.M. andMobley, H.L. (2011). Fimbrial profiles predict virulence of uropathogenic Escherichia coli strains: contribution of ygi and yad fimbriae.Infect Immun. 79(12):4753-63.

Valentine- Weigand, P. (1987). A simple method for quantitative determination of bacterial adherence to human and animal epithelial cells. Microbiol.Immunology. 31(10):1017- 1023.

Vidya, K.C.; Mallya, P.S. and Rao, P.S. (2005). Inhibition of bacterial adhesion by sub-

inhibitory concentrations of antibiotics. Indian j. Medical Microbiology. 23:102-105.

Wojnicz, D.; Klak, M.; Adamski, R. and Jankowski, S. (2007). Influence of sub-inhibitory concentrations of amikacin and ciprofloxacin on morphology and adherence ability of uropathogenic strains. Folia Microbiol. 52:429-436.

Zalas –Wiecek, P.; G ospodarek, E. and Piecyk, K. (2011). Influence of Subinhibitory concentrations of cefotaxime, imipenem and ciprofoxacin on adhesion of *Escherichia coli* strains to polystyrene. Polish Journal of Microbiology. 60 (4): 345–349.