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Evaluation of antibacterial activity of some non-steroidal anti-inflammatory drugs against *Escherichia coli* causing urinary tract infection

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Extensive use of antibiotics for urinary tract infections has led to the emergence of drug-resistant microorganisms and one solution to this problem is to search for non-antibiotic compounds that exert anti-bacterial activity through different mechanisms such as non-steroidal anti-inflammatory drugs (NSAIDs). In this study, out of 100 urine samples; 48 *Escherichia coli* strains were detected, 47.9% were multi-drug resistant. The antibiogram resistance pattern of the strains was carried out by agar dilution method. Diclofenac sodium, indomethacin, aspirin and ibuprofen were tested against the *E. coli* isolates. Diclofenac sodium showed the lowest MIC_{50} and MIC_{90}; 8 and 256 μg/ml, respectively. Aspirin showed MIC_{50} of 64 μg/ml, while both indomethacin and ibuprofen showed MIC_{50} of 256 μg/ml. Indomethacin, aspirin and ibuprofen showed the same MIC_{90} of 1024 μg/ml. The combined effects of the four NSAIDs and five antibiotics (Amoxicillin, Augmentin, Cefotaxime, Ciprofloxacin and Gentamicin) were tested on five resistant clinical *E. coli* strains by checkerboard dilution technique. All the tested NSAIDs significantly reduced the minimum inhibitory concentrations (MICs) of antibiotics against the tested bacteria and fractional inhibitory concentration indices (FICIs) for this combination ranged from 0.03 to 0.5. In this study, leakage of intracellular components suggests that the effect of NSAIDs on *E. coli* could be the formation of pores in the plasma membrane and scanning electron microscopy (SEM) observations confirmed the damage to the structural integrity of the tested bacteria. In conclusion, NSAIDs showed antibacterial activity against *E. coli* causing urinary tract infections (UTIs), a combination of them and antibiotics exhibited good synergism and the mechanism of their action was by damaging the bacterial cell membrane.

Key words: Urinary tract infection (UTI), *Escherichia coli*, NSAIDs, antibacterial resistance, antibacterial activity, synergism.

INTRODUCTION

Urinary tract infections (UTIs) are the most common hospital acquired infection with a percentage of 35% of

nosocomial infections (Stamm, 2002; Weinstein et al., 1997). *Escherichia coli* is the major pathogen causing urinary tract infections (UTIs) and represents more than 85% of recurrent cystitis and about 35% of recurrent pyelonephritis (Barnett and Stephens, 1997). UTIs remain the most common human bacterial infection, despite the high spread of antibiotics. The massive and irrational use of antibiotics and antibacterial agents for long periods has led to the emergence of multi drug resistant (MDR) microorganisms, and it is currently advised that the clinical administration of antibiotics against the pathogenic bacteria be gradually prohibited (Ray and Rice, 2004; Chowdhary et al., 1994). Another solution for this problem is to search for non-antibiotic compounds that have antibacterial activity through different mechanisms (Mazumdar et al., 2009). Recent studies have shown that some medicines have antibacterial activity in addition to their main function such as antihistamines, antipsychotics, tranquillizers, anti-hypertensives and local anesthetics (Rani et al., 2005; Dastidar et al., 1995). All these drugs with moderate to powerful anti-microbial activities have been known as “non-antibiotics” (Dastidar et al., 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used medicines for pain and inflammation management and previous studies have revealed that some NSAIDs have antibacterial activity (Wang et al., 2003; Hersh et al., 1991). NSAIDs exhibited strong antimicrobial activity when tested against a large number of Gram-positive and negative bacteria and the MIC ranged from 50-200 µg/mL in most of the cases and even lower in some cases (Annadurai et al., 1998; Sukul et al., 2015; Obad et al., 2015). The antibacterial agents, whether bacteriostatic or bactericidal, might act by inhibition of microbial cell wall synthesis, alteration of membrane function or membrane damage, inhibition of nucleic acid synthesis or inhibition of protein synthesis (Mazumdar et al., 2006). The aim of this study was to detect antibacterial activity of some NSAIDs (diclofenac sodium, aspirin, indomethacin and ibuprofen) against *E. coli* isolates causing UTIs, examine the effect of their combination with different antibiotics and finally detect the possible mechanism of antibacterial action of these NSAIDs if present.

MATERIALS AND METHODS

Isolation of bacterial strains

One hundred urine samples were collected from UTI patients in Minia University Hospitals (MUH) in Minia, Egypt during the study period, from May 2014 to December 2015. Informed consent was obtained from all the subjects. Urine samples were inoculated on cysteine lactose electrolyte deficient (CLED) media (Lab, UK) (Winn and Koneman, 2006). All the samples were examined for the presence of *E. coli* by streaking them onto MacConkey agar (Lab, UK), EMB agar (Himedia, India) and incubating the plates at 37°C for 24 h. Identification of *E. coli* was based on fermentation of lactose giving pinkish colonies. Further identification was done by biochemical (citrate and triple sugar iron) tests. Bacteria were maintained by storage at -70°C on tryptone soy broth (TSB) medium (Himedia, India) enriched with 20% glycerol (Rusu et al., 2014; Nobmannn et al., 2010).

Drugs

The following NSAIDs were used: Diclofenac sodium (Glaxo, Egypt), Ibuprofen (Kahira/Abbott, Egypt), Aspirin and Indomethacin (Kahira, Egypt). The following antibiotics were used: Ampicillin, Amoxicillin (EIPCO, Egypt), Augmentin (Sedico, Egypt), Cephalexin (Glaxo, Egypt), Cephradin (Smithkline, Egypt), Cefotaxime (EIPCO, Egypt), Ciprofloxacin (Amriya, Egypt) and Gentamicin (Memphis, Egypt). Working solution concentrations ranged from 5-1.6 mg/ml. All the drugs were obtained as pure dry powder and stored at 4°C.

Susceptibility testing

Bacterial cultures were tested against some NSAIDS (diclofenac sodium, aspirin, indomethacin and ibuprofen) by agar dilution method (CLSI, 2005). Mueller-Hinton agar (MHA) plates (Lab, UK) contained two fold serial dilutions of NSAIDS from 0.25 to 1024 µg/mL. Bacterial suspensions of isolated bacteria were made in sterile saline and matched with McFarland index 0.5 tubes. Each bacterial suspension (1 µl) was inoculated (3×10^5 CFU/spot) on drug containing plates and incubated at 37°C for 24 h.

Determination of interaction between NSAIDs and antibiotics by checkerboard dilution technique

Two drugs combined effects were determined by the Checkerboard dilution technique to determine the fractional inhibitory concentration (FIC) indices. Definition of FIC is as follows: MIC of $substance_A$ tested in combination/MIC of $substance_A$ tested alone + MIC of $substance_B$ tested in combination/MIC of $substance_B$ tested alone. The FIC index (FICI) was calculated using the following formula:

$FIC\ index = FIC_A + FIC_B = [A]/\ MIC_A + [B]/MIC_B$.

Synergism is shown as FIC index of ≤0.5, while indifference is showed as an FIC index of >0.5 ≤4 and antagonism is shown as an FIC index of>4. FIC index was an average of two independent experiments (Lorian, 2005).

Membrane-permeability assay

Membrane-disruptive activity of Amoxicillin, diclofenac sodium, aspirin, indomethacin, ibuprofen and amoxicillin/aspirin on *in vitro* grown *E. coli* (ATCC 8739) was determined by measuring the fluorescence enhancement of ethidium bromide (Sigma) (Paixão et al., 2009). To this end, *E. coli* were grown in tryptone broth medium in the presence or absence of amoxicillin as positive control, NSAIDs and Amoxicillin combined with aspirin for 24 h. The bacterial culture was incubated with ethidium bromide for 20 min at room temperature in the dark. Membrane permeability was

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Table 1. Distribution of minimum inhibitory concentrations and the prevalence of antibiotics resistance among the isolated *E. coli.*

Antibiotics	MIC (μg/ml)													MIC_{50}	MIC_{90}	R	%
	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024				
Ampicillin	0	0	0	4	3	13	1	0	2	4	7	3	11	128	1024	27	56.3
Amoxicillin	0	0	0	10	1	5	0	2	2	1	1	3	23	512	1024	32	66.7
Augmentin	0	0	3	10	2	2	11	7	4	2	1	1	5	32	512	20	41.7
Cephalexin	0	0	0	7	6	11	3	7	2	2	2	4	4	8	512	21	43.8
Cephradin	0	0	2	7	1	6	12	2	4	6	3	1	4	16	256	20	41.7
Cefotaxime	0	2	19	17	5	0	3	0	0	0	0	1	1	2	4	10	20.8
Ciprofloxacin	2	11	23	3	3	4	0	0	0	0	1	1	0	1	8	9	18.8
Gentamicin	0	0	18	13	7	7	1	1	1	0	0	0	0	2	8	3	6.3

determined by measuring the ethidium bromide fluorescence (excitation at 518 nm, emission at 605 nm). Fluorescence values presented are corrected with those obtained from untreated bacteria (Bink et al., 2012).

Loss of 260 nm absorbing material

The release of UV-absorbing material concentrations were determined by UV spectrophotometer (Zhou et al., 2008). Loss of 260 nm absorbing material released from bacteria was measured by the technique performed by Devi et al. (2010).Overnight broth cultures of *E. coli* ATCC 43889 in tryptone broth medium were adjusted to OD 600. Cells were harvested by centrifugation at 400 rpm for 15 min, supernatant was discarded, and pellet was washed twice and re-suspended in phosphate buffer saline (PBS) at pH 7.4. Different concentrations of NSAIDs [1\2MIC, MIC, 2MIC and 4MIC] were added to the cell suspension. Amoxicillin (1.6 mg/ml) was used as positive control. The experiment was done in triplicates. Cells without NSAIDs treatment were used as control. All the samples were incubated at 37°C for 60 min. After treatment, the cell suspension was centrifuged at 13,400 rpm for 15 min and OD 260 value of the supernatant was taken as a percentage of the released extracellular UV-absorbing materials. All the measurements were done in triplicates in Jenway 7305 UV spectrophotometer (UK).

Scanning electron microscopy (SEM)

E. coli (ATCC 8739) cells were suspended in saline solution containing 0.2% Tween-80 and incubated at 37°C with Amoxicillin, diclofenac sodium, aspirin, indomethacin, ibuprofen and Amoxicillin/aspirin at 2× MIC at room temperature. After 24 h, the bacterial cells were centrifuged at 8000 rpm for 15 min. The bacterial cells were then washed with 0.1 mol/l tris-acetate buffer (PH 7.1), fixed in tris-acetate buffer containing 1.5% glutaraldehyde, and then freeze-dried. Each bacterial culture was observed by SEM (Hitachi, Japan) at magnifications of 10000, 7500 and 15000x. The bacterial cell suspension in saline with no NSAIDs treatment served as a negative control (Soboh et al., 1995).

Statistical analysis

Statistical analysis was performed using one way Anova test. P values of <0.05 were considered indicative of statistically significant differences.

RESULTS

Antibiogram pattern of the isolates

A total of 100 clinical samples were examined. All of these were urine samples from patients with UTI. Among the 100 patients samples, 48 *E. coli* strains were isolated (48%) and out of them; 23 strains (47.9%) were normally resistant to most of the antibiotics showing multi drug resistance (MDR). The antibiogram resistance pattern of the isolates, as shown in Table 1 was: amoxicillin (66.7%), ampicillin (56.3%), cephalexin (43.8%), augmentin (41.7%), cephradin (41.7%), cefotaxime (20.8%), ciprofloxacin (18.8%) and gentamicin (6.3%). *E. coli* (ATCC 8739) showed sensitive antibiogram pattern as illustrated in Table 1.

In vitro antimicrobial action of NSAIDs

NSAIDs were tested against a total of 48 isolates of *E. coli* as shown in Table 2. Diclofenac sodium showed the lowest MIC_{50} and MIC_{90}: 8 and 256 μg/ml, respectively. Aspirin showed MIC_{50} of 64 μg/ml, while both indomethacin and ibuprofen showed MIC_{50} of 256 μg/ml. Indomethacin, aspirin and ibuprofen showed the same MIC_{90} of 1024 μg/ml. But for the standard strain as illustrated in Table 3, indomethacin showed the lowest MIC: 128 μg/ml, followed by aspirin: 256 μg/ml. Diclofenac sodium and ibuprofen showed the same MIC: 1024 μg/ml.

Determination of interaction between NSAIDs and antibiotics by checkerboard dilution technique

The combined effects of the four NSAIDs (diclofenac sodium, indomethacin, aspirin and ibuprofen) and five antibiotics (amoxicillin, augmentin, cefotaxime, ciprofloxacin and gentamicin) were tested on five resistant clinical *E. coli* strains. All the tested NSAIDs significantly lowered the MICs of antibiotics against the tested bacteria. The synergistic effects of NSAIDs and five antibiotics combination are shown in Tables 4, 5, 6 and 7.

Table 2. Distribution of minimum inhibitory concentrations of NSAIDs among the isolated *E. coli.*

Drug	MIC (µg/ml)													MIC_{50}	MIC_{90}
	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024		
Diclofenac sodium	4	2	1	1	3	16	2	1	2	5	7	1	3	8	256
Indomethacin	0	0	0	5	4	6	2	2	1	2	3	9	14	256	1024
Aspirin	0	0	1	8	2	2	2	1	2	2	2	3	23	64	1024
Ibuprofen	0	1	1	5	3	8	2	0	1	2	2	13	10	256	1024

Table 3. Distribution of MICs of NSAIDS and antibiotics against the standard strain.

Drug	MIC (µg/ml)
Diclofenac sodium	1024
Indomethacin	128
Aspirin	256
Ibuprofen	1024
Ampicillin	8
Amoxicillin	8
Augmentin	16
Cephalexin	16
Cefotaxime	1
Gentamicin	≤0.25
Ciprofloxacin	≤0.25

FICIs for this combination ranged from 0.03 to 0.5 against the tested bacteria. All the examined *E. coli* showed high reduction in MIC values with NSAIDs and the five antibiotics. On the other hand, the combined effects of the four NSAIDs and the five antibiotics on standard *E. coli* strain are shown in Table 8. These results showed that NSAIDs have a synergistic effect when combined with antibiotics and this combination could effectively inhibit UTIs causing bacteria.

Membrane-permeability assay

These results suggested an effect of pretreatment of NSAIDs on the *E. coli* activity. It is hypothesized that NSAIDs affect membrane permeability of the tested *E. coli* cells, exhibited by the use of the fluorescent dye ethidium bromide. It is revealed that *E. coli* treated with different concentrations of NSAIDs during the growth phase resulted in a significantly increased membrane permeability of *E. coli* cells compared to the untreated ones as found by the significant increase in fluorescence of NSAIDS-treated cells (Figure 1). The presented fluorescence values are corrected with those obtained from untreated bacteria.

Effect of NSAIDs on leakage of 260 nm absorbing materials from *E. coli*

The measurement of release of UV-absorbing materials is an index of cell lysis (Zhou et al., 2008). The leakage of cytoplasmic membrane was analyzed by determining the release of cellular materials including nucleic acids, metabolites and ions, which were absorbed at 260 nm into the bacterial suspensions (Bajpai et al., 2014). After treatment with different concentrations of NSAIDs, the OD significantly increased up to 1.87 from 0.00 (P value < 0.05) as shown in Table 9. These results suggest that NSAIDs damage cytoplasmic membrane and cause subsequent leakage of intracellular constituents.

Scanning electron microscopy (SEM)

SEM images showed differences in cell structures between NSAIDs-treated bacteria and the non-treated control bacteria. Non-treated bacteria were intact (regular rod shaped) and showed smooth surfaces as seen in Figure 2A, while bacterial cells treated with the individual NSAIDs underwent considerable structural changes as shown in Figures 2B to G. SEM observations confirmed the damage to the structural integrity of the cells and considerable morphological alteration to the tested bacteria. In Figure 2G, combined NSAIDs treatments altered the outer membrane, the structures of the cells and made them more permeable.

DISCUSSION

E. coli is the major bacterial uropathogen in the world (Miragliotta et al., 2008). In the study on 100 urine samples, 48 (48%) *E. coli* strains were detected. This is similar to findings from studies done in other countries such as India (50, 59 and 68%) (Ranjini et al., 2015; Kothari and Sagar, 2008; Tambekar et al., 2006) and Madagascar (67%) (Randrianirina et al., 2007). Another study performed in Egypt reported that *E. coli* was in 36% of UTIs patients (Alabsi et al., 2014). A study performed in South Africa revealed that *E. coli* was present in 75% of uncomplicated and 59% of complicated UTIs and it was similar to this study (Agpaoa et al., 2015). In this study, 23 strains (47.9%) of *E. coli* isolates were normally resistant to most of the antibiotics showing multi-drug resistance (MDR). The antibiogram resistance pattern of the strains was: Amoxicillin (66.7%), Ampicillin (56.3%),

Table 4. Synergistic effect of diclofenac sodium combination with five antibiotics on resistant clinical *E. coli* strains.

Antibiotics	FIC_A	FIC_B	FIC_{index}	Synergistic
Amoxicillin	0.004	0.5	0.5	S
Augmentin	0.02	0.01	0.03	S
Cefotaxime	0.008	0.06	0.07	S
Gentamicin	0.25	0.01	0.3	S
Ciprofloxacin	0.004	0.5	0.5	S

S = Synergistic effect.

Table 5. Synergistic effect of indomethacin combination with five antibiotics on resistant clinical *E. coli* strains.

Antibiotics	FIC_A	FIC_B	FIC_{index}	Synergistic
Amoxicillin	0.1	0.1	0.2	S
Augmentin	0.03	0.1	0.1	S
Cefotaxime	0.03	0.3	0.3	S
Gentamicin	0.13	0.02	0.2	S
Ciprofloxacin	0.5	0.004	0.5	S

S = Synergistic effect.

Table 6. Synergistic effect of aspirin combination with five antibiotics on resistant clinical *E. coli* strains.

Antibiotics	FIC_A	FIC_B	FIC_{index}	Synergistic
Amoxicillin	0.02	0.01	0.03	S
Augmentin	0.02	0.06	0.1	S
Cefotaxime	0.004	0.03	0.03	S
Gentamicin	0.1	0.001	0.1	S
Ciprofloxacin	0.1	0.001	0.1	S

S = Synergistic effect.

Table 7. Synergistic effect of ibuprofen combination with five antibiotics on resistant clinical *E. coli* strains.

Antibiotics	FIC_A	FIC_B	FIC_{index}	Synergistic
Amoxicillin	0.01	0.3	0.3	S
Augmentin	0.03	0.02	0.05	S
Cefotaxime	0.1	0.02	0.1	S
Gentamicin	0.1	0.002	0.1	S
Ciprofloxacin	0.13	0.001	0.1	S

S = Synergistic effect.

Cephalexin (43.8%), Augmentin (41.7%), Cephradin (41.7%), Cefotaxime (20.8%), Ciprofloxacin (18.8%) and Gentamicin (6.3%). A study done in Egypt revealed the same percentage of MDR *E. coli*: 40% (Alabsi et al., 2014). A very high degree of MDR of 82.5% among *E. coli* isolates was reported by Ranjini et al. (2015). This

Table 8. Distribution of NSAIDs/antibiotics FIC_{index}against the *E. coli* standard strain.

	Diclofenac sodium	Indomethacin	Aspirin	Ibuprofen
Amoxicillin	0.03 (S)	2 (I)	0.03 (S)	0.03 (S)
Augmentin	0.3 (S)	0.5 (S)	0.02 (S)	0.02 (S)
Cefotaxime	0.3 (S)	0.3 (S)	0.3 (S)	0.3 (S)
Gentamicin	1 (I)	1 (I)	1 (I)	1 (I)
Ciprofloxacin	1 (I)	1 (I)	1 (I)	1 (I)

S = Synergistic effect, I = additive effect, A = antagonistic effect.

Table 9. Effects of NSAIDs at different concentrations on membrane integrity in *E. coli* standard strain measured by release of UV absorbing components at 260 nm.

Drug	1/4MIC		MIC		2MIC		4MIC	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Amoxicillin	0.592	±0.001	0.989	±0.002	1.199	±0.004	1.433	±0.001
Aspirin	0.422	±0.008	0.840	±0.012	1.335	±0.001	1.625	±0.010
Indomethacin	0.030	±0.015	1.103	±0.006	1.400	±0.000	1.662	±0.008
Diclofenac sodium	1.468	±0.005	1.565	±0.012	1.583	±0.003	1.746	±0.026
Ibuprofen	0.531	±0.000	1.083	±0.002	1.356	±0.002	1.529	±0.002
Aspirin/Amoxicillin	0.963	±0.003	1.342	±0.001	1.589	±0.018	1.642	±0.002
Indomethacin/Amoxicillin	1.644	±0.000	1.719	±0.017	1.714	±0.001	1.867	±0.017
Diclofenac/Amoxicillin	1.338	±0.003	1.548	±0.005	1.649	±0.027	1.671	±0.015
Ibuprofen/Amoxicillin	0.277	±0.027	0.807	±0.031	1.023	±0.006	1.536	±0.001

SD = Standard deviation.

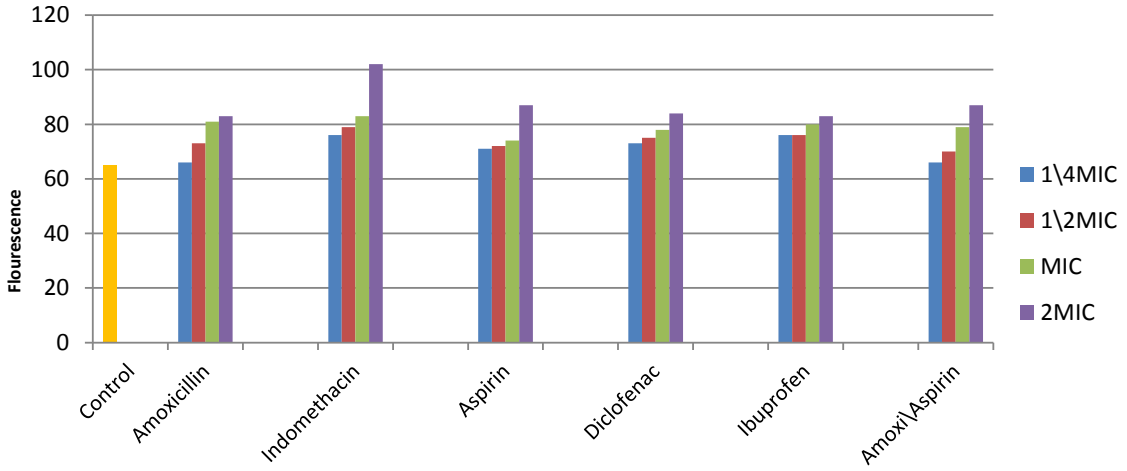

Figure 1. Membrane-disruptive activity of NSAIDs on *in vitro* grown *E. coli*. Data presented are the mean and standard error of the mean of 3 independent biological repeats. Statistical analysis was performed using one way ANOVA test and relative to untreated bacteria ($P < 0.05$).

study is in accordance with the study of Mazumdar et al. (2006) who reported the antibiogram resistance pattern of the *E. coli* strains as: ampicillin (74.4%), augmentin (59%), cefotaxime (38%), and these findings were similar to the results of Samsygina et al. (2000) and Khan et al. (2002). Alabsi et al. (2014) from Egypt reported 89 and 57% resistance among urinary *E. coli* isolates to ampicillin and gentamicin, respectively. NSAIDs are commonly used medicines for the treatment of pain and inflammation. Many studies found that some NSAIDs

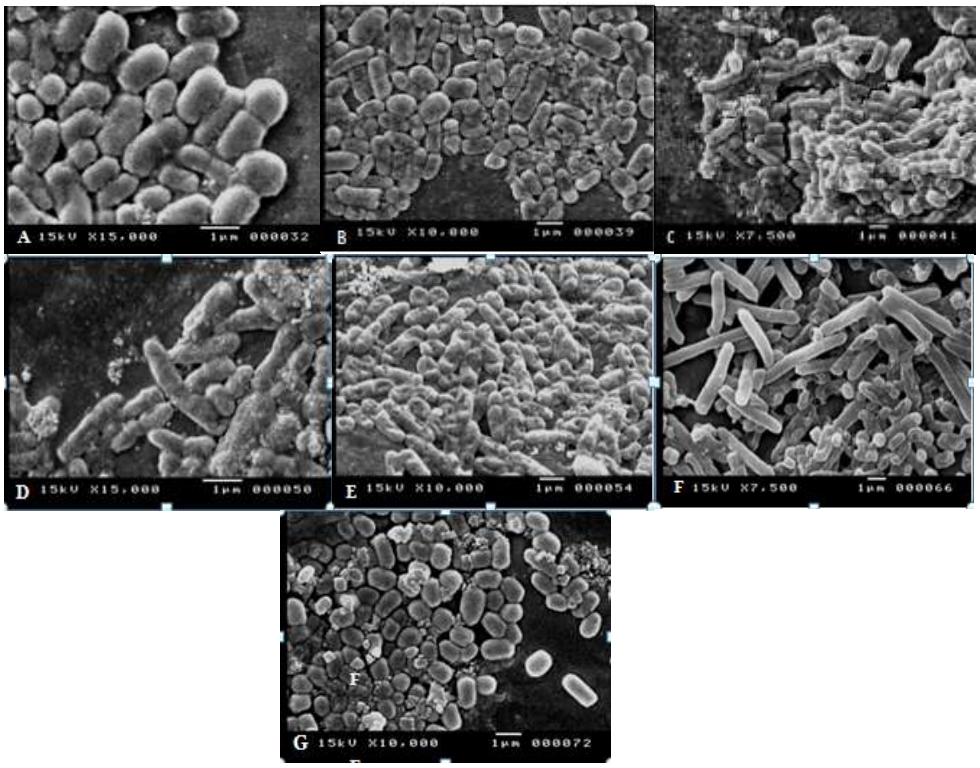

Figure 2. SEM images of (A) control, (B) amoxicillin treated cells, (C) aspirin treated cells, (D) indomethacin treated cells, (E) diclofenac sodium treated cells, (F) ibuprofen treated cells and (G) aspirin/amoxicillin treated cells.

have good antibacterial activity especially diclofenac sodium (Wang et al., 2003). In this study, some NSAIDs (diclofenac sodium, aspirin, indomethacin and ibuprofen) were tested against a total of 48 isolates of *E. coli*. Diclofenac sodium showed the lowest MIC_{50} and MIC_{90}; 8 and 256 µg/ml, respectively. Annadurai et al. (1998) reported that the MIC in most of the cases ranged from 50-200 µg/ml and even lower in some cases and diclofenac was bactericidal in its action. Dutta et al. (2007a) studied 32 isolates of *E. coli*, 8 were inhibited at 50 µg/ml diclofenac, 9 at 100 µg/ml, 5 at 400 µg/ml and the remaining isolates of *E. coli* were resistant to diclofenac (MIC≥ 800 µg/ml). In this study, aspirin showed MIC_{50} of 64 µg/ml, while both indomethacin and ibuprofen showed MIC_{50} of 256 µg/ml. Indomethacin, aspirin and ibuprofen showed the same MIC_{90} of 1024 µg/ml. Wang et al. (2003) tested the MICs of aspirin for 66 *H. pylori* isolates and the MIC_{50} of aspirin was 256 µg/ml, MIC_{90} was 512 µg/ml, and the range of MIC values was 256 to 512 µg/ml and this finding is close to the current study results. Activity of ibuprofen on *E. coli* was proximally studied by Al-Janabi (2010) and showed susceptibility to the tested agent at MIC of 2.5 mg/ml, which is higher than this results. There is an ongoing trial in Germany evaluating reduction of the use of antibiotics for uncomplicated UTI by giving initial management with ibuprofen (Gágyor et al., 2012). NSAID is equally effective as an antibiotic, and this may lead to a reduction in the use of antibiotics and reduce antibiotic resistance. This is good to the environment and will reduce the costs in health services internationally (Vik et al., 2014). The combined effects of the four NSAIDs (diclofenac sodium, indomethacin, aspirin and ibuprofen) and five antibiotics (amoxicillin, augmentin, cefotaxime, ciprofloxacin and gentamicin) were tested on five resistant clinical *E. coli* strains by checkerboard dilution technique. All the tested NSAIDs significantly reduced the MICs of antibiotics against the tested bacteria and FICIs for this combination ranged from 0.03 to 0.5 with respect to synergism. Dutta et al. (2007a) used the checkerboard technique giving a FIC index for *E. coli* of 0.49 for diclofenac and streptomycin, thereby showing a synergistic effect and another study showed that the combination effect of diclofenac with gentamicin/ampicillin which was examined by using checkerboard technique yielded FIC index ranging from 0.4 to 0.5 for diclofenac + gentamicin and values >1 for diclofenac + ampicillin (Dutta et al., 2009). In the present study, NSAIDs alone recorded antimicrobial activity, but NSAIDs in combination with antibiotics exhibited significant synergistic effect and the drugs were bactericidal. These data suggested that NSAIDs in combination with antibiotics could be useful for the treatment of complicated bacterial infections. In addition

to yielding these synergistic effects, the combinations of two or more compounds are important to prevent or suppress the developing of resistant strains, to decrease dose toxicity and to perform a broad spectrum activity (Eliopoulos and Moellering, 1996). The bacterial membrane is a structural component which may be damaged during a bactericidal challenge. Therefore, release of intracellular components is an indicator of membrane integrity. Small ions such as potassium and phosphate when treated with a suitable antimicrobial agent leach out first, followed by large molecules such as DNA, RNA and other materials. These substances have strong UV absorption at 260 nm, they are known as "260-nm absorbing materials" and this method is widely used in the determination of membrane integrity parameters (Denyer, 1990; Hugo and Snow, 1981). In this study, leakage of intracellular components suggests that the NSAIDs effect on *E. coli* can be through pores formation in the bacterial plasma membrane. The bacterial surface morphology alteration and cell damage could be confirmed thoroughly by SEM (Benli et al., 2008). In this study, SEM images showed differences in cell structures between NSAIDs-treated bacteria and the non-treated control bacteria. In addition, combined NSAIDs treatments altered the outer membrane as the structures of the cells made them more permeable. Thus, the mode of bactericidal action of NSAIDs against *E. coli* is through membrane disruption and so blocking the bacterial growth. The exact mechanism of antibacterial activity of diclofenac and ibuprofen is unclear. However, studies have suggested inhibition of bacterial DNA synthesis (Dutta et al., 2004) or impairment of membrane activity that agree with results obtained by SEM in this study (Hersh et al., 1991; Dutta et al., 2007a, b; Mohsen et al., 2015; Sikkema et al., 1995).

In conclusion, diclofenac sodium, aspirin, indomethacin and ibuprofen showed antibacterial activity against *E. coli* causing UTIs. This study results indicate that a combination of these NSAIDs and antibiotics exhibited good synergism against *E. coli* associated with UTIs, and the mechanism of their action was by damaging the bacterial cell membrane. This new finding of combination treatment with NSAIDS and antibiotics might provide an alternative way to overcome antibacterial drug resistance. However, further *in vivo* and clinical studies will be required to support this suggestion.

Conflict of interest

The authors declare that there is no conflict of interest.

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