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Antibacterial Activity of Some Non-steroidal Anti-inflammatory Drugs against Bacteria Causing Urinary Tract Infection

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Abstract This study aims to evaluate the effect of NSAIDs on the activity of some antibiotics against urinary tract pathogens. Urine samples were collected and cultured on cysteine lactose electrolyte deficient (CLED) media and MICs for some antibiotics and NSAIDs were determined using Agar dilution method. The combined effects of some NSAIDs and some β-lactam antibiotics were tested on standard strains by checkerboard dilution technique. Out of 100 samples (63 female patients and 37 male patients suffering from UTIs), 122 bacterial strains were isolated. *E. coli* and Coagulase negative Staphylococci (CoNS) were the most common (39.3% and 26.2%, respectively), followed by *S. aureus* (9.8%), *Klebsiella spp*, *Enterococcus faecalis* (7.4% each), *P. aeruginosa* (3.2%), Streptococci, *Proteus spp*. (2.5% each) and *Bacillus spp.* (1.6%). Most strains showed high resistance against the tested antibiotics. Diclofenac sodium and indomethacin showed the lowest MIC_{90} against the tested strains. All the tested NSAIDs significantly lowered the MICs of antibiotics against the tested bacteria and FICIs for these combinations ranged from 0.004 to 0.5. In conclusion, NSAIDs significantly increased the therapeutic activity of the tested antibiotics showing good synergistic effect.

Keywords: *UTI, NSAIDs, antibacterial resistance, synergism*

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1. Introduction

Urinary tract infections (UTIs) exhibit an increasing point of concern for investigations. They have ranked as one of the most common hospital-acquired infection as well as occupying the second place in the list of causes of bacteremia in hospitalized patients [1,2]. Nevertheless, UTI causes significant distress to the individual and is associated with high healthcare and social costs. Acute uncomplicated infection is the most common form of symptomatic UTI, affecting 40% of women at some point in their life. One-third of patients who develop a UTI will go on to have recurrent infections. Symptomatic infection is less common in men. Asymptomatic bacterial colonization of the urinary tract is a common finding in women and the elderly [3]. Studies clearly demonstrate increasing antibiotic resistance in uropathogens causing both community- and nosocomial acquired UTIs [4]. WHO and the European Union (EU) have recognized the importance of studying the emergence and causes of antibiotic resistance and the need for strategic development to control this public health issue [5,6].

The rapid and global dissemination of antibiotic-resistant bacteria has resulted in the decrease of therapeutic options for many infectious diseases, highlighting the urgent need for new therapies [7]. Moreover, the toxic side effects produced by antibiotics reducing their demand. Recently, remarkable antimicrobial action was observed by several compounds [8], belonging to various pharmacological categories, such as the antihistamines [9], tranquilizers [10], the antihypertensive [11], the antipsychotics [12] and the anti-inflammatory agents [13].

The anti-inflammatory, analgesic and anti-pyretic properties of non-steroidal anti-inflammatory drugs (NSAIDs) are particularly useful in treating rheumatic and other musculoskeletal disorders [14]. These non-steroidal anti-inflammatory drugs have demonstrated strong antimicrobial property when tested against a large number of Gram-positive and Gram-negative bacteria [15] and are now referred to as "non-antibiotics" [16]. In this study; we aimed to isolate bacteria causing UTIs, detection of antibacterial activity of some NSAIDs (diclofenac sodium, aspirin, indomethacin, and ibuprofen) against these bacteria and finally; examine the combination effect of NSAIDs with β-lactam antibiotics against standard *P. aeruginosa* (ATCC 10145) and *K. pneumoniae* (ATCC 10031) strains.

2. Patients, Materials and Methods

From May 2014 until December 2015, a total number of 100 hospitalized patients suffering from UTIs of different ages and gender were enrolled into the study. They were attending the Urology Department at Minia University Hospital (MUH). All patients were subjected to clinical examination and a sheet was filled for each patient and included: date, name, age, sex, diagnosis, and symptoms. Patients were 37 males and 63 females of different ages from 5 months to 78 years. As 19 patients were in the age group of 1 to 20 years, 29 patients were in the age group of 21 to 40 years, 33 patients were in the age group of 41 to 60 years**,** and 15 patients were in the age group of >60 years.

2.1. Isolation of Bacterial Strains

Urine samples were inoculated on cysteine lactose electrolyte deficient (CLED) media (Lab M, UK) [17]. All the samples were examined for presence of bacteria by streaking them onto nutrient agar (Lab M, UK), MacConkey agar (Lab M, UK), EMB agar (Himedia, India) and mannitol salt agar (Becton, USA). Further identification was done by conventional biochemical tests. All media were prepared according to the manufacturers' instructions. Standard strains were obtained from MIRCIN center, Faculty of Agriculture, Ain Shams University, Egypt. Bacteria were maintained by storage at -70°C on tryptone soy broth (TSB) medium (Himedia, India) enriched with 20% glycerol [18,19].

2.2. Drugs

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). The following NSAIDs were used: Diclofenac Sodium (Glaxo, Egypt), Ibuprofen (Kahira/Abbott, Egypt), Aspirin and Indomethacin (Kahira, Egypt). All the drugs were obtained as pure dry powder and stored at 4°C.

2.3. Antimicrobial Susceptibility Testing

Stock solutions of the tested NSAIDs and antibiotics were prepared at a concentration of 2.5 mg/ml. MICs were determined using Agar dilution method according to Clinical laboratory standard institute (CLSI). Bacterial suspensions of isolated bacteria were prepared in 2ml sterile saline and turbidity was adjusted to 0.5 McFarland ($1\text{-}2x10^8$ CFU/ml). Serial dilutions for the tested compounds were performed using Muller Hinton Agar plates. Then, the tested strains were inoculated on the surface of agar using multi-inoculator device.

2.4. Determination of the Combined Effect 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. As *E. coli* was the major pathogen; combination between NSAIDs and antibiotics against resistant *E. coli* strains was mentioned in previous publication [20].

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 showed as FIC index of ≤0.5, while indifference is showed as an FIC index of >0.5 ≤4 and antagonism is showed as an FIC index of>4. FIC index was an average of two independent experiments [21].

2.5. Statistical Analysis

Statistical analysis was done using SPSS one way Anova test and paired t test. P values of <0.05 were considered with statistically significant differences.

3. Results

3.1. Prevalence of UTIs in Relation to Age and Gender

Patients were classified into different age groups from 5 months to 78 years. Figure 1 shows that higher prevalence of UTIs was observed in female patients (63%) than male that represented 37%. In female patients; the highest incidence of infection was in age group of 21 to 40 (39.7%) while the lowest incidence was in the age group above 60 years (11.1%). On contrary, in male patients; the highest incidence of infection was observed in the age group above 60 years (40.54%) while the lowest incidence of infection was in the age group from 21 to 40 (21.6%).

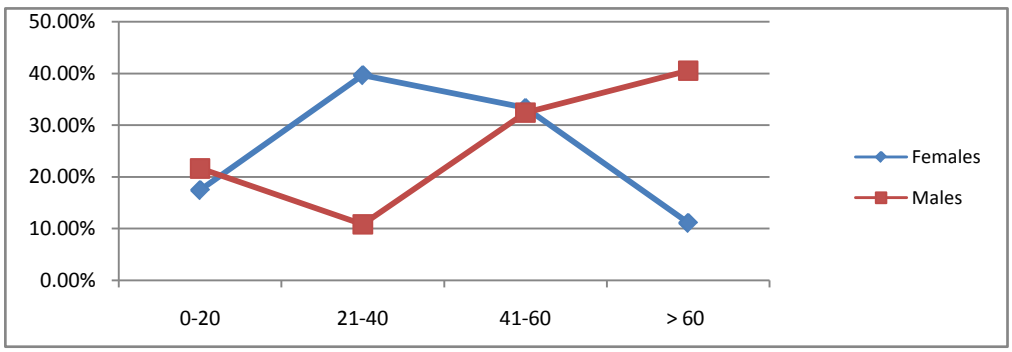

Figure 1. Prevalence of UTIs in Relation to Age and Gender

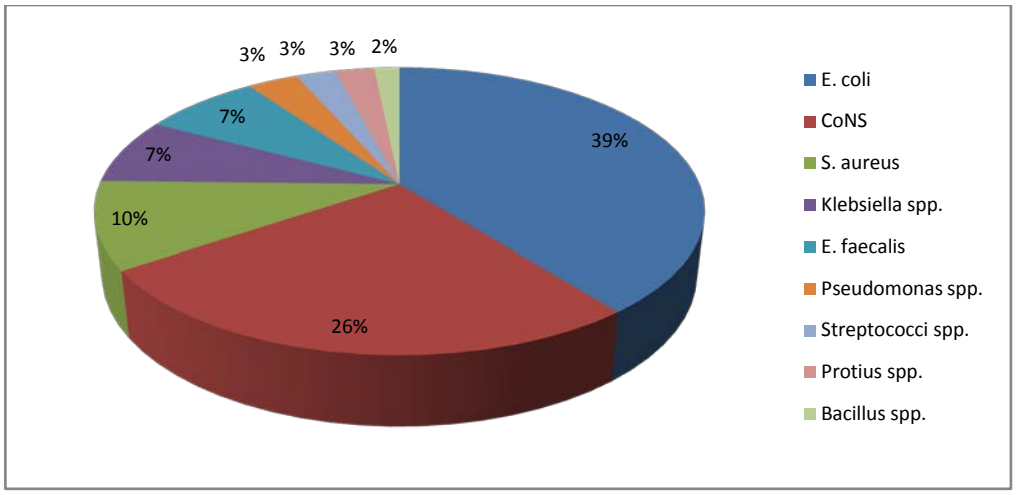

Figure 2. Distribution of isolated bacteria from UTIs

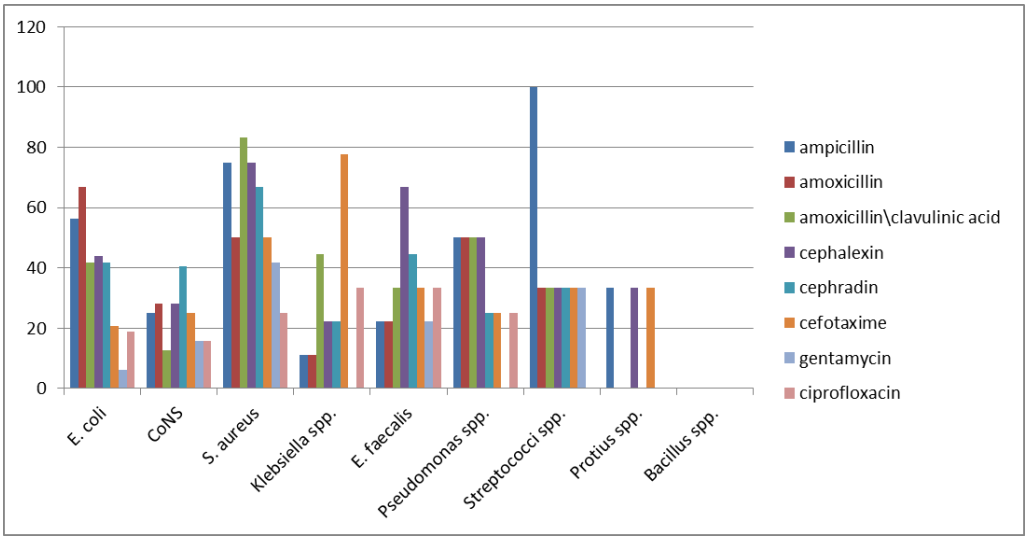

Figure 3. Prevalence of antibiotics resistance among the isolated microorganisms

3.2. Isolation and Identification of Isolates

A total of 122 isolates of 100 patients were retrieved, in which 48 isolates (39.3%) of *E. coli* and 32 isolates of coagulase negative Staphylococci (CoNS) (26.2%) were the most common isolates. The other identified pathogens were 12 (9.8%) *S. aureus*, 9 (7.4%) *Klebsiella spp.*, 9 (7.4%) *Enterococcus faecalis*, 4 (3.2%) *P. aeruginosa*, 3 Streptococci (2.5%), 3 *Proteus spp.* (2.5%) and 2 *Bacillus spp.* (1.6%) (Figure 2).

3.3. Antimicrobial Susceptibility Testing

The different UTI isolated species were screened for their susceptibility to different antibiotics. The minimum inhibitory concentration (MIC) values of each of the tested antibiotic against the different pathogens isolated from UTI samples were determined and for better comparison, MIC_{90} and MIC_{50} and percentage of resistance of different UTI isolates to each antibiotic were recorded (Figure 3).

3.4. In Vitro Antimicrobial Action of NSAIDs

As shown in Tabe1, MIC_{90} of diclofenac sodium against *Bacilli* and *Streptococci spp.* were 0.5μg/ml and 1μg/ml, respectively. MIC_{90} values against *Proteus spp.* was 64μg/ml. MIC_{90} values against *E. coli, Klebsiella spp., E. faecalis and Pseudomonas aeruginosa* were the same (256μg/ml). Diclofenac sodium showed the highest MIC_{90} against CoNS and *S. aureus* (512μg/ml and 1024μg/ml, respectively). Regarding MIC_{90} of aspirin against all isolates; the lowest MIC_{90} value against aspirin was 4μg/ml against Bacilli. MIC_{90} of aspirin was 512μg/ml against *E. faecalis, Proteus* and *Pseudomonas spp.*, while, MIC_{90} against *E. coli, Klebsiella, S. aureus,* CoNS and Streptococci was 1024μg/ml (Table 2). Regarding MIC_{90} of indomethacin against all isolates; *Bacilli* exhibited the lowest MIC_{90} value against indomethacin (1μg/ml), while; *Pseudomonas spp., E. coli,* and *S. aureus* showed the highest MIC_{90} against indomethacin (1024μg/ml). MIC_{90} against *E. faecalis* and *Proteus* were 128μg/ml and against Streptococci was 256μg/ml (Table 3). Data of MIC_{90} values of ibuprofen against all isolates showed that MIC_{90} for Bacilli was 2μg/ml represented the lowest value against ibuprofen, on contrary, *S. aureus, E. coli,* CoNS, *Pseudomonas spp.* and Streptococci revealed the highest and the same MIC_{90} value (1024μg/ml). For *E. faecalis, Proteus* and *Klebsiella,* MIC_{90} values were the same; 512μg/ml (Table 4).

Table 1. Distribution of minimum inhibitory concentrations of diclofenac sodium, MIC_{90} and MIC_{50} among the isolated bacteria

Micro-organisms	No.	MIC (μg/ml)													MIC_{90}	MIC_{50}	p value*
		0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**			
E. coli	48	4	2	1	1	3	16	2	1	2	5	7	1	3	256	8	0.0001<
CoNS	32	0	2	2	4	0	8	1	4	1	1	2	5	2	512	8	0.0001<
S. aureus	12	0	0	0	0	0	4	0	0	0	1	4	0	3	1024	256	0.0001<
Klebsiella spp.	9	0	0	0	1	1	1	0	1	0	0	4	0	1	256	32	0.0001<
E. faecalis	9	0	0	0	2	0	3	0	0	0	1	3	0	0	256	8	0.002
Pseudomonas spp.	4	0	0	0	0	1	0	0	0	0	2	1	0	0	256	128	0.0001<
Streptococci spp.	3	0	0	1	2	0	0	0	0	0	0	0	0	0	2	1	0.06
Proteus spp.	3	0	0	0	1	0	0	0	1	1	0	0	0	0	64	32	0.0006
Bacillus spp.	2	0	1	0	1	0	0	0	0	0	0	0	0	0	2	0.5	NA*

* One-Way Anova test was used for statistical analysis, NA: Not Applicable.

Table 2. Distribution of minimum inhibitory concentrations of aspirin, MIC_{90} and MIC_{50} among the isolated bacteria

Micro-organisms	No.	MIC (μg/ml)													MIC_{90}	MIC_{50}	p value*
		0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024			
E. coli	48	0	0	1	8	2	2	2	1	2	2	2	3	23	1024	64	0.0001<
CoNS	32	0	0	0	5	3	7	3	2	0	0	0	3	9	1024	16	0.0001<
S. aureus	12	0	0	0	0	0	3	1	0	1	1	1	2	3	1024	128	0.0001<
Klebsiella spp.	9	0	0	0	1	1	1	0	0	1	0	0	1	4	1024	512	0.0001<
E. faecalis	9	0	0	0	0	2	2	2	0	0	1	0	1	1	512	16	0.0001<
Pseudomonas spp.	4	0	0	0	0	0	1	1	0	0	0	0	2	0	512	16	0.0001<
Streptococci spp.	3	0	0	0	2	0	0	0	0	0	0	0	0	1	1024	2	0.0003
Proteus spp.	3	0	0	0	0	1	0	0	0	0	0	1	1	0	512	256	0.0026
Bacillus spp.	2	0	0	0	1	1	0	0	0	0	0	0	0	0	4	2	0.1

* One-Way Anova test was used for statistical analysis.

Table 3. Distribution of minimum inhibitory concentrations of indomethacin, MIC_{90} and MIC_{50} among the isolated bacteria

Micro-organisms	No.	MIC (μg/ml)													MIC_{90}	MIC_{50}	p value*
		0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024			
E. coli	48	0	0	0	5	4	6	2	2	1	2	3	9	14	1024	256	0.0001<
CoNS	32	0	0	0	10	0	5	4	1	2	1	2	5	2	512	16	0.0001<
S. aureus	12	0	0	0	0	1	4	2	0	1	1	0	0	3	1024	16	0.0001<
Klebsiella spp.	9	0	0	0	3	0	1	0	0	0	1	1	2	1	512	8	0.0001<
E. faecalis	9	0	1	0	2	0	2	0	1	1	1	0	1	0	128	8	0.0001<
Pseudomonas spp.	4	0	0	0	2	0	0	0	0	0	0	0	0	2	1024	2	0.0001<
Streptococci spp.	3	0	0	0	2	0	0	0	0	0	0	1	0	0	256	2	0.0001<
Proteus spp.	3	0	0	0	1	0	0	0	0	0	2	0	0	0	128	128	0.0001<
Bacillus spp.	2	0	0	1	1	0	0	0	0	0	0	0	0	0	2	1	0.3

* One-Way Anova test was used for statistical analysis.

Table 4. Distribution of minimum inhibitory concentrations of ibuprofen, MIC_{90} and MIC_{50} among the isolated bacteria

Micro-organisms	No.	MIC (μg/ml)													MIC_{90}	MIC_{50}	p value*
		0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**			
E. coli	48	0	1	1	5	3	8	2	0	1	2	2	13	10	1024	256	0.0001<
CoNS	32	0	0	0	9	9	1	0	1	2	0	1	4	5	1024	4	0.0001<
S. aureus	12	0	0	0	0	0	3	0	0	2	0	0	2	5	1024	512	0.0001<
Klebsiella spp.	9	0	0	0	2	1	1	1	0	1	0	0	3	0	512	16	0.0001<
E. faecalis	9	0	0	0	1	2	2	0	0	1	0	0	2	1	512	8	0.0001<
Pseudomonas spp.	4	0	0	0	2	0	0	0	0	0	0	0	1	1	1024	2	0.0001<
Streptococci spp.	3	0	0	0	2	0	0	0	0	0	0	0	0	1	1024	2	0.0001<
Proteus spp.	3	0	0	0	1	0	0	0	0	0	0	0	2	0	512	2	0.0001<
Bacillus spp.	2	0	0	0	2	0	0	0	0	0	0	0	0	0	2	2	NA*

* One-Way Anova test was used for statistical analysis, NA: Not Applicable.

3.5. Distribution of Minimum Inhibitory Concentrations of Different Antibiotics and NSAIDS against Standard Strains

Data in Table 5 represented the MIC values of some widely used antibiotics and NSAIDS against standard strains. MIC values of nearly all antibiotics against *P. aeruginosa* were the highest among all species. Finally, MIC of ciprofloxacin against tested standard strains was ≤0.25μg/ml. Indomethacin showed the lowest MIC; 128μg/ml against *K. pneumoniae* but *P. aeruginosa* was more sensitive and revealed MIC of 64μg/ml. MIC of diclofenac sodium and ibuprofen were 1024μg/ml against *K. pneumonia*. Ibuprofen recorded MIC of 512μg/ml against *P. aeruginosa*, while diclofenac sodium showed MIC; 1024μg/ml. MIC of aspirin against *K. pneumoniae* was 256μg/ml which was doubled that against *P. aeruginosa* (128μg/ml).

3.6. Determination of Interaction between NSAIDs and Antibiotics by Checkerboard Dilution Technique

The combined effects of NSAIDs with the beta-lactam antibiotics on standard *P. aeruginosa* (ATCC 10145) and *K. pneumoniae* (ATCC 10031) strains were shown in Table 6 and Table 7. All the tested NSAIDs significantly lowered the MICs of antibiotics against the tested bacteria and fractional inhibitory concentration indices (FICIs) for this combination ranged from 0.004 to 0.5. These results showed that NSAIDs have a synergistic effect when combined with antibiotics and this combination could effectively inhibit growth of bacteria.

Table 5. Distribution of minimum inhibitory concentrations of different antibiotics and NSAIDs against the standard strains

Drug	MIC (μg/ml)	
	P. aeruginosa (ATCC 10145)	*K. pneumonia* (ATCC 10031)
Ampicillin	1024	16
Amoxycillin	1024	16
Amoxy/clav	512	8
Cephalexin	1024	32
Cefotaxime	8	≤0.25
Gentamicin	1	≤0.25
Ciprofloxacin	≤0.25	≤0.25
Diclofenac sodium	1024	1024
Indomethacin	64	128
Aspirin	128	256
Ibuprofen	512	1024

Table 6. Combinations effect of NSAIDs with antibiotics against *P. aeruginosa* (ATCC10145)

Tested NSAID	Antibiotic	MIC (μg/mL) of antibiotic		FIC_{index}	Outcome	p value*
		Alone	Combination			
Diclofenac sodium	Ampicillin	1024	4	0.008	Synergistic	0.02
	Amoxicillin	1024	2	0.004	Synergistic	
	Amox/Clav	512	32	0.06	Synergistic	
	Cephalexin	1024	8	0.02	Synergistic	
	Cefotaxim	8	0.25	0.03	Synergistic	
Aspirin	Ampicillin	1024	2	0.02	Synergistic	0.024
	Amoxicillin	1024	32	0.3	Synergistic	
	Amox/Clav	512	16	0.1	Synergistic	
	Cephalexin	1024	8	0.07	Synergistic	
	Cefotaxim	8	0.25	0.03	Synergistic	
Indomethacin	Ampicillin	1024	32	0.5	Synergistic	0.023
	Amoxicillin	1024	32	0.5	Synergistic	
	Amox/Clav	512	4	0.07	Synergistic	
	Cephalexin	1024	32	0.5	Synergistic	
	Cefotaxime	8	0.25	0.04	Synergistic	
Ibuprofen	Ampicillin	1024	16	0.05	Synergistic	0.024
	Amoxicillin	1024	4	0.01	Synergistic	
	Amox/Clav	512	4	0.02	Synergistic	
	Cephalexin	1024	16	0.05	Synergistic	
	Cefotaxime	8	0.25	0.03	Synergistic	

* Paired t test was used for statistical analysis.

Table 7. Combinations effect of NSAIDs with antibiotics against *K. pneumoniae* (ATCC10031)

Tested NSAIDs	Antibiotic	MIC (μg/mL) of antibiotic		FIC_{index}	Outcome	p value*
		Alone	Combination			
Diclofenac sodium	Ampicillin	16	2	0.1	Synergistic	0.04
	Amoxicillin	16	0.25	0.02	Synergistic	
	Amox/Clav	8	4	0.5	Synergistic	
	Cephalexin	32	8	0.3	Synergistic	
	Cefotaxim	0.25	0.25	1	Additive	
Aspirin	Ampicillin	16	0.25	0.02	Synergistic	0.04
	Amoxicillin	16	0.25	0.02	Synergistic	
	Amox/Clav	8	0.25	0.03	Synergistic	
	Cephalexin	32	0.25	0.01	Synergistic	
	Cefotaxim	0.25	0.25	1	Additive	
Indomethacin	Ampicillin	16	8	0.5	Synergistic	0.18
	Amoxicillin	16	16	1	Additive	
	Amox/Clav	8	4	0.5	Synergistic	
	Cephalexin	32	8	0.3	Synergistic	
	Cefotaxime	0.25	0.25	1	Additive	
Ibuprofen	Ampicillin	16	8	0.5	Synergistic	0.03
	Amoxicillin	16	0.25	0.02	Synergistic	
	Amox /Clav	8	0.25	0.03	Synergistic	
	Cephalexin	32	16	0.5	Synergistic	
	Cefotaxime	0.25	0.25	1	Additive	

* Paired t test was used for statistical analysis.

4. Discussion

In the present study, the selected age group was comparable to two studies conducted by Gupta and Bhadelia, 2014 and Kiffer *et al.*, 2007 who selected age group from 1 to > 60 years with mean age 35.5 [7,22]. A study performed in Egypt made by Ghonemy *et al.* consisted of 62.2% males and 37.8% females and the mean age of the patients was 52.03 ± 14.67years. The highest proportion of patients (31.9%) was aged between 50 and 60 years in both males and females [23] and this was the same as our result, but Kiffer *et al.* revealed that among the positive cultures, 88.8% belonged to female and 11.2% to male patients [22]. Das *et al.* revealed that elderly (61 years or more) males had a higher incidence of UTI (49.23%) compared to the elderly females (21.75%) [24] and that was similar to our result regarding male patients.

Akhter *et al.* revealed that bacteria isolated from urine samples were *E. coli* 30%, CoNS 26%, *S. aureus* 20%, *E. faecalis* 10%, *Proteus spp.* 6%, *Pseudomonas spp*. 6% and *Klebsiella spp*.2% [25] and this was similar to our result regarding *E. coli* and CoNS. A study done in Philippine reported that *E. coli* was the most common organism isolated (28.8%) which was lower than ours, followed by *Staphyloccocus spp.* (13.5%), *Klebsiella pneumoniae* (9.6%) which were close to ours, *Enterococcus faecalis* (2.9%), and *P. aeruginosa* (2.9%) which were lower than ours [26]. Mubanga *et al.* revealed that the top five cultured uropathogens were *E. coli* (61.5%), *S. aureus* (14%), *Pseudomonas* species (6.5%), *E. faecalis* (5.5%) and *Streptococcus agalactiae* (5%) and this was higher than our result [27].

In the present study; diclofenac sodium showed the lowest MIC_{90} against most strains; while indomethacin showed the lowest MIC against standard strains. A study done by Mazumdar on clinical strains of *E. coli* in hospitals have indicated that diclofenac sodium has shown antibacterial activity against many strains of bacteria from 5-50 µg/ml and was effective in treating UTIs and this was lower than ours [28]. A study on *E. faecalis* was done to evaluate antibacterial effect of diclofenac in comparison with ibuprofen, calcium hydroxide and amoxicillin. The results have depicted significant antibacterial activity of diclofenac and ibuprofen at 50µg/ml and above concentrations [29], but this was lower than our result, as in ours; MIC_{90} of ibuprofen against *E. faecalis* was 512µg/ml and MIC_{90} of diclofenac sodium was 256µg/ml.

Akhtar *et al.* determined the antibacterial effect of aspirin against different bacterial strains isolated from UTI. Aspirin was effective at 500 µg/ml concentrations as similar to ours. Antibacterial effect of aspirin on isolates of diabetic foot infection showed that 100µg/ml concentrations of aspirin were mostly inhibitory for *S. aureus* and other isolates and this was lower than our result [25]. Muller *et al.* and Polonio *et al.* revealed that aspirin has effect on isolates of UTI; that *S. aureus* and *E. faecalis* indicated 100% inhibition at 100, 500,1000 µg/ml concentrations [30,31]. Al-Bakri *et al.* showed that aspirin possessed a broad spectrum antimicrobial activity against *E. coli* and *P. aeruginosa* [32]. All the previous studies were the same as ours. Another studies have reported that

aspirin and other NSAIDs interfere with growth of both *Gram* negative and *Gram* positive bacteria in *vitro* [33] in contrast to ours.

Obad *et al.* concluded that ibuprofen may be responsible for the broad spectrum of activity, both antibacterial and antifungal activity [34]. Al- Janabi studied activity of ibuprofen on *E. coli* and showed susceptibility to tested agent at MIC of 2.5 mg/ml [35] which is higher than our results. NSAID is equally effective as an antibiotic, and this may lead to a reduction in the use of antibiotics and reduce antibiotic resistance [36].

Annadurai *et al.*, exhibited that diclofenac sodium has shown significant antibacterial effect in synergism with aminoglycosides both in *vitro* and in *vivo* studies [37] and agreed with our result. The non-antibiotic drug; Diclofenac was found to protect mice from *Salmonella* infection more effectively when combined with streptomycin than used alone [38]. The synergism between diclofenac and streptomycin against *S. aureus* NCTC 6571 and *E. coli* K12 C600 was found to be statistically significant ($p<0.01$), when compared with their individual effects [39], and this agrees with our results which showed significant synergism between diclofenac sodium and β-lactam antibiotics. Ronser revealed that sodium salicylate and related compounds such as aspirin are known to have a variety of effects on microorganisms. *E. coli*, for example, exhibits increased resistance to chloramphenicol, ampicillin, nalidixic acid, and tetracycline after such treatment [40], but that study disagrees with our result, as aspirin showed $FIC_{index}<0.5$ showing synergism. On the other hand; Aumercier *et al.*, revealed that *E. coli* cells grown in the presence of salicylate are more sensitive to aminoglycosides [33]. Del Prado *et al.* revealed that animals receiving amoxicillin combined with ibuprofen showed a more pronounced reduction in bacterial counts even than those receiving the antibiotic alone and this was the same as our result [41]. Dutta *et al.* used the checkerboard technique giving a FIC_{index} for *E. coli* of 0.49 for diclofenac and streptomycin, there by showing a synergistic effect [42] as ours 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 [43], but that FIC_{index} was higher than ours regarding ampicillin which was <0.5. In the present study NSAIDs alone recorded antimicrobial activity, but NSAIDs in combination with antibiotics exhibited significant synergistic effect when used together and the drugs were bactericidal in addition to the synergistic effect and prevented the bacterial regrowth.

5. Conclusion

In conclusion, diclofenac sodium, aspirin, indomethacin, and ibuprofen showed *in vitro* antibacterial activity against bacteria associated with UTIs. Our results indicate that a combination of these NSAIDs and antibiotics exhibited good synergism against standard strains. This new finding might provide a new way to overcome antibacterial resistance. However, *in vivo* and clinical studies will be required to support this finding.

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