The Pharma Innovation Journal 2022; 11(12): 5045-5053

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 5045-5053

www.thepharmajournal.com
Received: 08-11-2022
Accepted: 12-12-2022

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Study on interaction of steroidal and non-steroidal anti-inflammatory drugs with enrofloxacin and doxycycline in *Escherichia coli* and *Staphylococcus aureus*

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Abstract
NSAIDs and steroids are non-antibiotic drugs which may exhibit antibacterial activity when used in combination with antibiotics. The present study was designed to assess the *in-vitro* interaction of ketoprofen, meloxicam (NSAIDs) and betamethasone (steroid) with enrofloxacin and doxycycline in *E. coli* and *S. aureus*. Methods-The minimum inhibitory concentration (MIC) of ketoprofen, meloxicam, betamethasone, enrofloxacin and doxycycline was determined for *E. coli* and *S. aureus* isolates by micro-broth dilution method. The combination effect was estimated by calculating the fractional inhibitory concentration index (FICI). Results-The minimum inhibitory concentration (MIC) of enrofloxacin for *E. coli* ranged from 2 µg/ml to 32 µg/ml, and of doxycycline 32 µg/ml to 64 µg/ml. The MIC of enrofloxacin and doxycycline resistant *E. coli* isolates stood constant at 128 µg/ml for ketoprofen, 125 µg/ml of meloxicam, 125 µg/ml of betamethasone, respectively. The MIC of enrofloxacin for *S. aureus* ranged from 1 µg/ml to 128 µg/ml, and of doxycycline 0.25 µg/ml to 128 µg/ml. The MIC of ketoprofen, meloxicam and betamethasone for *S. aureus* ranged from 128 µg/ml to 512 µg/ml, 125 µg/ml to 500 µg /ml,and 125 µg/ml to 500 µg/ml, respectively. The enrofloxacin resistant *E. coli* isolates showed partial synergy (33%), additive (56.6%) and indifferent effect (10%) when enrofloxacin was used in combination with any of the three chosen non-antibiotics (ketoprofen, meloxicam and betamethasone). The combination effect of enrofloxacin with ketoprofen, meloxicam, and betamethasone were either indifferent (87%) or partial synergy (13%) for enrofloxacin resistant *S. aureus* isolates. About 96% of the doxycycline resistant *E. coli* and *S. aureus* isolates showed indifferent effect and 4% of the doxycycline resistant *E. coli* and *S. aureus* isolates showed partial synergy when doxycycline was used in combination with any of the chosen non-antibiotics (ketoprofen, meloxicam and betamethasone).The difference in interaction of NSAIDs and steroid with enrofloxacin and doxycycline among two bacteria indicated that mechanism of interaction may be bacteria specific and needs more elaborate studies.

Keywords: NSAIDs, steroid, drug-repurposing, tetracyclines, quinolones, *E. coli*, *S. aureus*

Introduction
Antibiotics are being widely used in the treatment against bacterial infections. However, indiscriminate use of antibiotics led to emergence and spread of AMR worldwide, hampering access to the essential antibiotics in many low- and middle-income countries [2]. An estimated 1.27 million deaths were attributed to bacterial AMR globally in 2019 [7]. Resistance to common antibiotics and development of multidrug resistance limits the therapeutic options available [16].

The novel concept of drug repurposing is gaining traction to re-sensitize multidrug resistant bacteria by combining the resistant antibiotic with approved non-antibacterial drugs. The non-antibiotics including anthelmintics, anticancer drugs, antipsychotics, antidepressant drugs, antiplatelets, NSAIDs, steroids, and herbal anti-bacterials are known to have antibacterial activity. NSAIDS are reported to exhibit synergistic interaction with antibiotics. They reduce inflammation, pain and fever mainly by decreasing the production of pro-inflammatory pathways. Some of the NSAIDs such as acetaminophen, acetyl salicylic acid, diclofenac, ibuprofen and flurbiprofen have no antibacterial activity at therapeutical plasma concentrations but in combination with antibiotics they broaden the spectrum of antibiotic activity through mechanisms that are different from those of existing antibiotics [11, 17].

Materials and Methods

1. Revival, isolation and identification of *E. coli* and *S. aureus* isolates

Bacterial strains used in the study- A total of 61 *E. coli* isolates from different clinical and para clinical samples preserved in glycerol stocks in clinical epidemiology laboratory, ICAR-IVRI, Izatnagar, India were revived (Table 1). The isolates were inoculated on MacConkey agar plates (Hi-media), incubated at 37 °C for 24h and selected pink colored lactose fermenting colonies were streaked on EMB agar (Hi-media) and incubated at 37 °C for 24h to look for the greenish metallic sheen. The *E. coli* isolates were further confirmed through biochemical tests such as IMViC (Indole, Methyl red, Voges Proskauer and Citrate utilization), catalase, oxidase tests and Gram's staining [10].

About 80 samples comprising mastitic milk (n=55), wound (n=20) and street food (n=5) were collected from different regions of Bareilly, UP (Table 2). The samples were processed for isolation of *S. aureus* within 6 hours of collection. Following enrichment in tryptic soy broth containing 10% Naclat 37 °C for 6-10 h, a loopful of enriched broth was streaked on blood agar plates and incubated at 37 °C for 12-18h. Based on the type of hemolysis, 2-3 colonies selected were streaked on mannitol salt agar (Hi-media), and incubated at 37 °C for 12-18 h for preliminary characterization. The characteristic yellow-colored colonies from mannitol salt agar were subjected for DNAse, catalase, oxidase, and coagulase activity, methyl red test, Voges Proskaeur test for acetoin production, urease test and Gram's staining to confirm their identity [5]. The *S. aureus* isolates were further confirmed through PCR targeting *nuc* gene [6] and 23SrRNA sequences [15] using the control strains ATCC 43300 and ATCC 29213 available in the division of Epidemiology, ICAR-IVRI, Izatnagar, India.

Table 1: Details of *E. coli* isolates recovered from different samples of animal origin

Source of *E. coli*	Disorder associated	Type of sample tested	Number of isolates
Cattle	Diarrhoea	Fecal swab	14
Cattle/buffalo	Mastitis	Milk	06
Cattle	Osteomyelitis	Pus	01
Wild animals	Apparently healthy	Fecal swab	19
Cattle	Apparently healthy	Fecal swab	04
Dog	Urinary tract infection	Urine	02
Dog	Metritis	Vaginal swab	02
Dog	Diarrhoea	Fecal swab	01
Dog	Wound	Pus swab	04
Gharial	Death	Stomach contents	02
Gharial	Death	Intestinal contents	01
Gharial	Death	Heart blood	01
Turkey	Death	Heart blood	04
		Total no of isolates	61

Table 2: Details of samples collected for isolation of *Staphylococcus aureus* from animal origin

Source of *S. aureus*	Disorder associated	Type of sample tested	Number of isolates
Cattle / buffalo	Mastitis	Milk	69
Steet food	-	Aloo tikki	02
Street food	-	chhole	01
Street food	-	Veg momos	01
Street food	-	Finger chips	01
Dog	Abscess	Pus swab	10
Horse	Abscess	Nostril swab	02
Cattle/ buffalo	Abscess	Pus swab	03
Cat	Abscess	Pus swab	01
Unknown	Abscess	Pus swab	06
		Total no of isolates	96

2. Antimicrobial resistance profiling of *E. coli* and *S. aureus* isolates

Antibiotic susceptibility testing (AST) was carried out for all biochemically and phenotypically confirmed *E. coli* and *S. aureus* isolates against different commercially available antibiotic discsusing Kirby-Bauer disk diffusion assay [9]. The antibiotics used for the susceptibility testing of *E. coli* included, imipenem (IPM- 10 µg), meropenem (MRP-10 µg), ertapenem (ETP-10 µg), doripenem (DOR-10 µg), enrofloxacin (ENO-5 µg), ciprofloxacin (CIP-5 µg), doxycycline (DO-30 µg), minocycline (MO), tetracycline (TE-30 µg), ceftriaxone (CTR-30 µg), cefoperazone (CFP-30 µg), gentamicin (GEN-10 µg), chloramphenicol (C-30 µg), trimethoprim/sulfamethoxazole (COT-25 µg), cefepime (FEP-30 µg), azithromycin (AZM-15 µg), cefoxitin (CX- 30 µg), nitrofurantoin (NIT-300 µg) and amoxycillin-clavulanic acid (AMC-30 µg). Similarly, *S. aureus* isolates were tested against doxycycline (DO-30 µg), tetracycline (TE- 30 µg), ciprofloxacin (CIP-5 µg), enrofloxacin (ENO-5 µg), gentamicin (GEN-10 µg), azithromycin (AZM-15 µg), chloramphenicol (C-30 µg), nitrofurantoin (NIT-300 µg), trimethoprim/sulfamethoxazole (COT-25 µg), cefoxitin (CX-30 µg), oxacillin (OX-1 µg), vancomycin (VA-30 µg), rifampicin (RF-5 µg), clindamycin (CD- 2 µg), linezolid (LZD- 30 µg) and mupirocin (MU-200 µg). Each culture was inoculated in sterile LB broth and kept for overnight incubation at 37 °C. The turbidity of the inoculum was compared with 0.5 McFarland standard. Broth culture of each isolate was spread on to the Muller-Hinton agar plates with 2% NaCl and kept for drying. Antibiotic discs were placed on the agar surface about 2cm apart. The plates were incubated at 37 °C overnight in inverted position. Thereafter zone of inhibition was measured as diameter in mm and the data was compared with CLSI guidelines to grade the isolates as resistant, sensitive and intermediate for respective antibiotics (7). The *E. coli* (GenBank accession number KU318701, KU318691, KU382501) and *S. aureus* (ATCC 43300 and ATCC 29213) reference strains used in this study were retrieved from the division of epidemiology repository, ICAR-Indian Veterinary Research Institute, Izatnagar, India.

3. Determination of minimum inhibitory concentration (MIC) of antibiotics, NSAIDs & steroidal drug

The minimum inhibitory concentration (MIC) of enrofloxacin, doxycycline, ketoprofen, meloxicam & betamethasone was evaluated using micro-broth dilution method in 96 well micro-titre plate for all the enrofloxacin and doxycycline resistant *E. coli* and *S. aureus* isolates [9].

All the media were procured from BBL Difco and chemicals/drugs used were procured from Sigma Aldrich (USA). The stock solutions for all the antimicrobial agents were prepared with appropriate diluent [9, 20]. The stock solutions of all the drugs were prepared to obtain a solution 4-fold more concentrated than the highest concentration of the drug to be tested.

Preparation of stock solutions

1. Enrofloxacin- was dissolved in ethanol at concentration of 1024 µg/ml
2. Doxycycline hyclate was dissolved in sterile distilled water at concentration of 1024 µg/ml
3. Ketoprofen was dissolved in DMSO at concentration of 5120 µg/ml
4. Meloxicam was dissolved in ethanol at concentration of 4 mg/ml
5. Betamethasone crystalline was dissolved in ethanol at concentration of 4 mg/ml

Preparation of broth culture- 10 µl of overnight broth culture was diluted in 990 µl of fresh LB broth to maintain the final concentration of broth in the micro-titre plate at 5×10^5 CFU/ml [4].

For broth of the dilution methods, 100 µL of fresh LB broth was added to all the 96 wells of the micro-titre plate. A 100 µL of antibiotic solution was added to eachwell of first column and serial dilution was made up to 11th column and 12th column was kept as growth control. Inoculum of 1.5 microliters of 1:100 diluted broth culture was added to all the wells of micro-titre plate. Plates were incubated overnight at 37 °C. The last dilution at which the bacterial growth was inhibited (reduced by >80%) was considered as the MIC of the drug. Similarly, the MIC assay was performed for NSAIDs (meloxicam and ketoprofen) and steroid (betamethasone) [9, 16].

4. Determination of fractional inhibitory concentration (FIC)

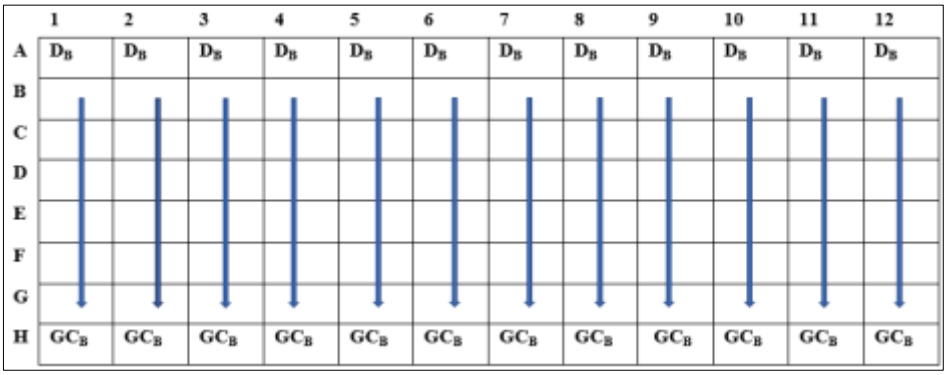

Fig 1: Dilution of drug B (ketoprofen/meloxicam/betamethasone) in 96-well micro-titre plate

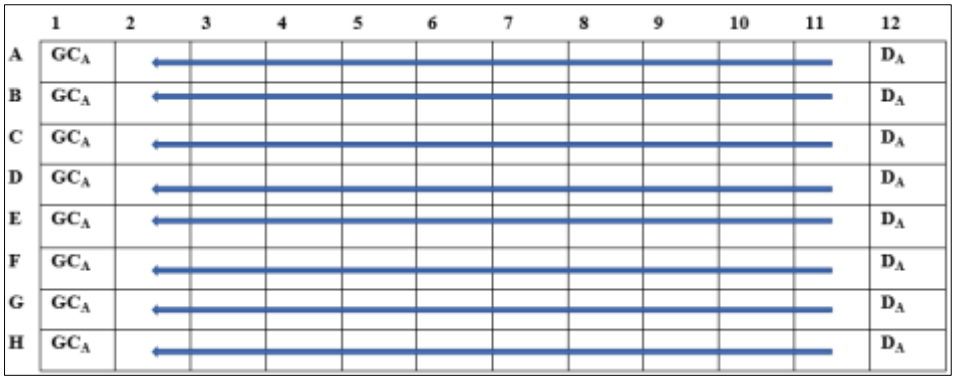

Fig 2: Dilution of drug A (enrofloxacin/doxycycline) in 96-well micro-titre plate

1. A 100 µL of fresh LB broth was added to each well of the micro-titre plate.
2. RowA (A1-A11) contains 100 µL of drug B (meloxicam/ketoprofen/betamethasone)
3. Row A12 contains 100 µL of 2×drug B (to keep constant the concentration of drug during the next serial dilution)
4. Serial dilution of drug B from row A to Row G using multichannel pipette set to 100 µL.
5. Row H (H1 – H12) serve as a growth control for drug B
6. Column 12 contains 100 µL of drug A in each well.
7. Serial dilution of drug A from column 12 to column 2 using multichannel pipette set to 100 µL
8. Column 1 serves as a growth control for drug A
9. An Inoculum of 1.5 microliters of 1:100 diluted broth culture was added to all the wells of micro-titre plate
10. The micro-titre plate with its cover was incubated at 35±2 °C for 18±2 h and optical density (OD) was read at 600nm in microplate reader.
11. The percentage of growth in each well was calculated using the formula

$OD_{drug\ combination\ well}$- $OD_{background}$/$OD_{drug\ free\ well}$- $OD_{background}$

12. Fractional inhibitory concentration (FIC) was calculated with the formula mentioned below.

FIC= MIC of drug in combination/MIC of drug alone

13. Fractional inhibitory concentration index (FICI) of drug combinations were calculated using the formula mentioned below.

$FICI_{A+B} = FIC_A + FIC_B$

The FIC indices were recorded as synergistic effect when FIC indices <0.5: partial synergy when FICI > 0.5 but < 1.0: additive when FICI=1.0: indifferent when FICI >1.0 but <4.0: and antagonistic when FICI >4.0. [1, 8]

Results and Discussion

Tetracyclines and quinolones are the two most commonly used groups of antimicrobials in clinical practice for treatment of bacterial infections of large animals and poultry in India [21, 23]. There are limited reports on impact co-administration of antibiotics and steroidal or non-steroidal anti-inflammatory drugs on antimicrobial efficacy of the combination [16]. The concurrent use of NSAIDs with antibiotics for treating bacterial infections, though common is not studied so far. Therefore, the present study was designed to understand the *in-vitro* interactions between antibiotics (enrofloxacin and doxycycline) with steroidal (betamethasone) and non-steroidal anti-inflammatory drugs (ketoprofen and meloxicam) against *E. coli* and *S. aureus*, the two most common causes of infections [12].

In the present study, a total of 61 *E. coli* isolates from clinical and non-clinical sources revived from repository and confirmed by biochemical characteristics [10] and 96 phenotypically confirmed *S. aureus* isolates (from mastitic milk, wounds and food samples) were included in the study. Of the 96 phenotypically confirmed *S. aureus* isolates, 77 were positive for *nuc* gene and 73 for 23S rRNA sequences, the two genes use to confirm identity of *S. aureus* [22]. A similar variation in results of phenotypic and genotypic identification of *S. aureus* was reported by [22].

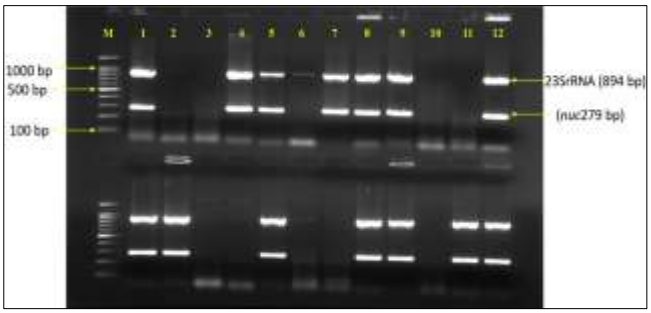

Fig 3: Duplex PCR for *nuc* and *23S rRNA* genes of *S. aureus*

In the study, 78.68% *E. coli* isolates were resistant to tetracycline followed by sulpha-trimethoprim (72.13%), ceftriaxone (65.57%), ciprofloxacin (49.18%) and doxycycline (40.98%) (Table 3). Similar results of tetracycline and ciprofloxacin resistance were reported earlier in the same region of the study [18]. Antibiogram study on 77 *S. aureus* showed that 49% were resistant to cefoxitin, 43% to ciprofloxacin and 14% to doxycycline. However, resistance to chloramphenicol and nitrofurantoin was not that common (Table 4). Similar results of tetracycline resistance (27.9%) were reported by Ou and co-workers [13]. Shah and co-workers [14] reported 18.2% ciprofloxacin resistance and 32% tetracycline resistance in *S. aureus* isolates. Zehra and co-workers [23] reported ciprofloxacin resistance (61.80%) and tetracycline resistance (45.14%) in *S. aureus* isolates of meat samples in India.

About 80.32% of the *E. coli* isolates and 49.3% of the *S. aureus* isolates were resistant to more than two classes of screened antibiotics and classified as MDR. However, no of antibiotics to which *E. coli* and *S. aureus* isolates were resistant did not differ significantly between isolates from different types of sources (p<0.05).

Table 3: Antimicrobial resistance of *E. coli* isolates from clinical and non-clinical samples

Antimicrobial agent	No of isolates resistant (n=61)	No of isolates resistant from clinical samples (n=38)							No of isolates resistant from non-clinical samples (n=23)
		FS	MM	P/W	U	VS	HB/SC/IC	Total	FS
AMC	30	03	00	00	01	00	07	11	19
AZM	16	02	01	01	01	00	01	06	10
C	05	04	00	00	00	00	00	04	01
CFP	39	10	02	02	01	00	04	19	20
CIP	30	03	02	02	01	00	06	14	16
COT	44	11	02	03	02	00	08	26	18
CRO	40	12	02	02	02	00	05	23	17
DOR	17	00	01	02	01	00	04	08	09
DOX	25	08	03	02	01	00	03	17	08
ERT	17	00	01	02	01	00	04	08	09
F/M	14	01	02	02	00	00	00	05	09
FEP	41	12	02	02	01	00	04	21	20
FOX	27	02	02	02	01	00	06	13	14
GEN	17	00	01	00	02	00	04	07	10
IMI	25	03	02	02	01	00	04	12	13
MEM	21	03	01	02	01	00	05	12	09
MI	14	05	01	01	01	00	00	08	06
TET	48	14	03	03	02	00	08	30	18

AMC, amoxycillin-clavulanic acid: AZM, azithromycin: C, chloramphenicol: CFP, cefoperazone: CIP, ciprofloxacin: COT, trimethoprim/sulfamethoxazole: CRO, ceftriaxone: DOR, doripenem: DOX, doxycycline: ERT, ertapenem: F/M, nitrofurantoin: FEP, cefepime: FOX, cefoxitin: GEN, gentamicin: IMI, imipenem: MEM, meropenem: MI, minocycline: TET, tetracycline: FS, fecal swab: MM, mastitic milk: P/W, pus/wound: U, urine: VS, vaginal swab: HB/SC/IC, heart blood/stomach contents/intestinal contents.

Table 4: Antimicrobial resistance of *S. aureus* isolates from mastitic milk, wound and street food samples

Antimicrobial agent	No of isolates resistant (n= 77)	No of isolates resistant from wound swab (n= 17)	No of isolates resistant from mastitic milk (n= 55)	No of isolates resistant from street food samples (n= 05)
AZM	20	05	13	02
FOX	38	11	23	04
C	07	04	03	00
CIP	33	13	15	04
CD	12	04	08	00
DOX	11	05	06	00
GEN	17	05	11	01
MU	13	08	02	03
F/M	07	05	02	00
OXA	23	05	18	00
RIF	10	05	05	00
COT	20	09	08	03
TET	19	06	12	01
VAN	18	06	12	00
LZ	08	03	05	00

AZM, azithromycin: FOX, cefoxitin: C, chloramphenicol: CIP, ciprofloxacin: CD, clindamycin: DOX, doxycycline: GEN, gentamicin: MU, mupirocin: F/M, nitrofurantoin: OXA, oxacillin: RIF, rifampicin: COT, trimethoprim/sulfamethoxazole: TET, tetracycline: VA, vancomycin: LZ, linezolid.

The minimum inhibitory concentration (MIC) of enrofloxacin for *E. coli* ranged from 2 µg/ml to 32 µg/ml and of doxycycline from 32 µg/ml to 64 µg/ml. The MICs of enrofloxacin and doxycycline resistant *E. coli* isolates for ketoprofen, meloxicam, and betamethasone were 128 µg/ml, 125 µg/ml and 125 µg/ml, respectively. The MIC of enrofloxacin for *S. aureus* ranged from 1 µg/ml to 128 µg/ml, and of doxycycline from 0.25 µg/ml to 128 µg/ml. The MIC of ketoprofen, meloxicam and betamethasone for *S. aureus* ranged from 128 µg/ml to 512 µg/ml, 125 µg/ml to 500 µg/ml, and 125 µg/ml to 500 µg/ml, respectively (Table 5, 6, 7, 8).

The fractional inhibitory concentration index (FICI) assessment used to measure the interaction between two drugs used in combination revealed that enrofloxacin against resistant *E. coli* isolates had partial synergy (33%), additive (56.6%) and indifferent effect (10%) when enrofloxacin was used in combination ketoprofen, meloxicam and betamethasone (Table 5). The combinations of enrofloxacin with ketoprofen, meloxicam and betamethasone were either indifferent (87%) or partial synergy (13%) for enrofloxacin resistant *S. aureus* isolates (Table 7). About 96% of the doxycycline resistant *E. coli* and *S. aureus* isolates showed indifferent effect and 4% of the doxycycline resistant *E. coli* and *S. aureus* isolates showed partial synergy when doxycycline was used in combination ketoprofen, meloxicam and betamethasone (Table 6 and Table 8). Similar variations in interactions of doxycycline and minocycline are reported recently with combination of acetylsalicylic acid and acetaminophen for different strains of *E. coli* and other bacteria [16]. Singh and co-workers [17] reported that NSAIDS may not be used as antimicrobials in therapeutically achievable systemic concentrations of the drugs within biologically safety limits and the findings were relatively similar to the present study. Our observations are in concurrence of report by Altaf and co-workers [1] studying the interaction of antibiotics and NSAIDS on *S. aureus* and reported partial synergistic, synergistic, additive and indifferent outcomes of the drug combinations. Chan and co-workers [8] conducted study on interaction of NSAIDS such as ibuprofen, mefenamic acid, diclofenac, aspirin with selected antibiotics against both gram positive and gram-negative bacteria. Artiniand co-workers [3] and Thangamani and co-workers [19] reported that antibiotic-steroid combination therapy is superior to antibiotic-alone in treatment to impair bacterial growth in *E. coli*. The difference in combination effect in present study with other studies indicates that drug interactions may be strain and species dependant.

Table 5: FIC Indices for combination of enrofloxacin with ketoprofen, meloxicam and betamethasone for enrofloxacin resistant *E. coli* isolates

Sl.no	Isolates	MIC				MIC in combination				FIC				FICI			Effect
		E (µg/ml)	K (µg/ml)	B (mg/ml)	M (mg/ml)	E_a	K_b	B_c	M_d	E_1	K_2	B_3	M_4	E & K	E & B	E & M	
1.	3008DUNH	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
2.	GITP	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.0	1.0	1.0	Additive
3.	GHBP	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.0	1.0	1.0	Additive
4.	418MFSP	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
5.	418MFSO	8	128	0.125	0.125	2	64	0.0625	0.0625	0.5	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
6.	GSCHLY	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
7.	GHBHLY	32	128	0.125	0.125	4	64	0.0625	0.0625	0.125	0.5	0.5	0.5	0.63	0.63	0.63	Partial Synergy
8.	TURKEY OL	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
9.	TURKEY O	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
10.	7758CCFP1	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
11.	L6	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
12.	A45	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
13.	A5	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
14.	HYBRID LION	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
15.	SAVITHRI	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
16.	6	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
17.	1	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
18.	SUHELI	16	128	0.125	0.125	4	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
19.	1874D	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
20.	KURKUM	1	128	0.125	0.125	1	64	0.0156	0.0156	1	0.5	0.12	0.12	0.12	0.12	0.12	Indifferent
21.	STIFY	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
22.	JACKAL	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
23.	L3	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
24.	ALI	4	128	0.125	0.125	4	64	0.0625	0.0625	1	0.5	0.5	0.5	1.50	1.50	1.50	Indifferent
25.	RRS1	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
26.	T24	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
27.	FOX	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive
28.	RC	8	128	0.125	0.125	2	64	0.0625	0.0625	0.25	0.5	0.5	0.5	0.75	0.75	0.75	Partial Synergy
29.	IZ	4	128	0.125	0.125	4	64	0.0625	0.0625	1	0.5	0.5	0.5	1.50	1.50	1.50	Indifferent
30.	DS 58	8	128	0.125	0.125	4	64	0.0625	0.0625	0.5	0.5	0.5	0.5	1.00	1.00	1.00	Additive

E_a- MIC of Enrofloxacin when used in combination with ketoprofen, betamethasone and meloxicam respectively: K_b-MIC of ketoprofen when used incombination with enrofloxacin:B_c-MIC of betamethasone when used in combination with enrofloxacin: M_d-MIC of meloxicam when used in combination with enrofloxacin: E_1-FIC of Enrofloxacin with ketoprofen, betamethasone and meloxicam respectively: K_2- FIC of ketoprofen when used in combination with enrofloxacin: B_3-FIC of betamethasone when used in combination with enrofloxacin:M_4- FIC of meloxicam when used in combination with enrofloxacin

Table 6: FIC Indices for combination of doxycycline with ketoprofen, meloxicam and betamethasone for doxycycline resistant *E. coli* isolates

Sl. No.	Isolates	MIC				MIC in combination				FIC				FICI			Effect
		D (µg/ml)	K (µg/ml)	B (mg/ml)	M (mg/ml)	D_a	K_b	B_c	M_d	D_1	K_2	B_3	M_4	D & K	D & B	D & M	
1.	300DUNH	32	128	0.125	0.125	32	16	0.0156	0.0625	1	0.125	0.1248	0.5	1.125	1.5	1.1248	Indifferent
2.	418MFSP	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
3.	A5	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
4.	SAVITHRI	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
5.	1	32	128	0.125	0.125	32	16	0.0156	0.0625	1	0.125	0.1248	0.5	1.125	1.5	1.1248	Indifferent
6.	T24	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
7.	RRS1	64	128	0.125	0.125	32	16	0.0156	0.0156	0.5	0.125	0.1248	0.1248	0.625	0.6248	0.6248	Partial Synergy
8.	DORI	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
9.	ALI	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
10.	4	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
11.	205FFSPM	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
12.	77QMFSPM	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
13.	2498FFSPM	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
14.	747MFSPM	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
15.	747MFSP	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
16.	RC	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
17.	IZ	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
18.	895	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
19.	NKUHLY	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
20.	7758CCFP1	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
21.	GHBHLY	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent

22.	GITP	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
23.	TURKEY O	32	128	0.125	0.125	32	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
24.	4070COSTEO	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
25.	418MFSO	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent

D_a- MIC of Doxycycline when used in combination with ketoprofen, betamethasone and meloxicam respectively: K_b- MIC of ketoprofen when used in combination with Doxycycline: B_c- MIC of betamethasone when used in combination with Doxycycline: M_d- MIC of meloxicam when used in combination with Doxycycline: D_1- FIC of Doxycycline with ketoprofen, betamethasone and meloxicam respectively: K_2- FIC of ketoprofen when used in combination with Doxycycline: B_3- FIC of betamethasone when used in combination with Doxycycline: M_4- FIC of meloxicam when used in combination with Doxycycline

Table 7: FIC Indices for combination of enrofloxacin with ketoprofen, meloxicam and betamethasone for enrofloxacin resistant *S. aureus* isolates

Sl. No.	Isolates	MIC				MIC in combination				FIC				FICI			Effect
		E (µg/ml)	K (µg/ml)	B (mg/ml)	M (mg/ml)	E_a	K_b	B_c	M_d	E_1	K_2	B_3	M_4	E & K	E & B	E & M	
1.	MS28	4	256	0.25	0.25	4	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
2.	MS38	8	256	0.25	0.25	4	16	0.0156	0.0156	0.5	0.0625	0.0624	0.0624	0.5625	0.5624	0.5624	Partial Synergy
3.	MS26	128	128	0.125	0.125	64	16	0.0156	0.0156	0.5	0.125	0.1248	0.1248	0.625	0.6248	0.6248	Partial Synergy
4.	MS84	4	256	0.125	0.25	8	16	0.0156	0.0156	2	0.0625	0.1248	0.0624	2.0625	2.1248	2.0624	Indifferent
5.	MS10	8	256	0.25	0.25	8	32	0.0156	0.03	1	0.125	0.0624	0.12	1.125	1.0624	1.12	Indifferent
6.	MS66	4	128	0.125	0.125	8	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
7.	MS43	8	256	0.25	0.25	8	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
8.	MS17	8	256	0.25	0.25	8	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
9.	MS06	8	256	0.25	0.25	8	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
10.	MS13	8	256	0.25	0.25	8	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
11.	MS83	8	128	0.25	0.25	8	16	0.0156	0.0156	1	0.125	0.0624	0.0624	1.125	1.0624	1.0624	Indifferent
12.	62	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
13.	60	4	128	0.125	0.125	2	16	0.0156	0.0156	0.5	0.125	0.1248	0.1248	0.625	0.6248	0.6248	Partial Synergy
14.	59	8	256	0.25	0.25	8	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
15.	R5BH	1	128	0.125	0.125	1	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
16.	RK5BH	4	128	0.125	0.125	8	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
17.	A5H	4	128	0.125	0.125	4	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
18.	A6H	2	128	0.125	0.125	4	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
19.	R7CH	4	128	0.125	0.125	4	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
20.	K5BLH	4	128	0.125	0.125	4	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
21.	K3	8	128	0.125	0.125	4	32	0.03	0.03	0.5	0.25	0.24	0.24	0.75	0.74	0.74	Partial Synergy
22.	565C	1	128	0.125	0.125	2	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
23.	SSGB3H	4	256	0.25	0.25	8	16	0.0156	0.0156	2	0.0625	0.0624	0.0624	2.0625	2.0624	2.0624	Indifferent
24.	895N	4	256	0.25	0.25	4	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
25.	RD7CH	2	256	0.25	0.25	2	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
26.	763	8	512	0.5	0.5	8	16	0.0156	0.0156	1	0.03125	0.0312	0.0312	1.03125	1.0312	1.0312	Indifferent
27.	74/12F	2	512	0.5	0.5	2	16	0.0156	0.0156	1	0.03125	0.0312	0.0312	1.03125	1.0312	1.0312	Indifferent
28.	18BMHLY	2	512	0.5	0.5	2	16	0.0156	0.0156	1	0.03125	0.0312	0.0312	1.03125	1.0312	1.0312	Indifferent
29.	3 EAHLY L	16	512	0.5	0.5	16	16	0.0156	0.0156	1	0.03125	0.0312	0.0312	1.03125	1.0312	1.0312	Indifferent
30.	DS9	64	256	0.25	0.25	64	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	1.0624	1.0624	Indifferent
31.	DS10	64	128	0.125	0.125	64	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent

E_a- MIC of Enrofloxacin when used in combination with ketoprofen, betamethasone and meloxicam respectively: K_b- MIC of ketoprofen when used in combination with enrofloxacin: B_c- MIC of betamethasone when used in combination with enrofloxacin: M_d- MIC of meloxicam when used in combination with enrofloxacin: E_1- FIC of Enrofloxacin with ketoprofen, betamethasone and meloxicam respectively: K_2- FIC of ketoprofen when used in combination with enrofloxacin: B_3- FIC of betamethasone when used in combination with enrofloxacin:M_4- FIC of meloxicam when used in combination with enrofloxacin

Table 8: FIC Indices for combination of doxycycline with ketoprofen, meloxicam and betamethasone for doxycycline resistant *S. aureus* isolates

Sl. No	Isolates	MIC				MIC in combination				FIC				FICI			Effect
		D (µg/ml)	K (µg/ml)	B (mg/ml)	M (mg/ml)	Da	Kb	Bc	Md	D1	K2	B3	M4	D & K	D & B	D & M	
1.	GDH	2	128	0.125	0.125	2	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
2.	DS2	0.5	128	0.125	0.125	0.5	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
3.	DS1	8	128	0.125	0.125	8	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
4.	DS7	8	128	0.125	0.125	16	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	1.1248	1.1248	Indifferent
5.	DS6	1	128	0.125	0.125	2	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
6.	DS5	4	256	0.25	0.25	2	16	0.0156	0.0156	0.5	0.0625	0.0624	0.0624	0.5625	0.5624	0.5624	P. Synergy
7.	MS43	1	256	0.25	0.25	1	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	2.0624	2.0624	Indifferent
8.	MS17	0.25	128	0.125	0.125	0.25	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
9.	A41	1	128	0.25	0.25	1	16	0.0156	0.0156	1	0.125	0.0624	0.0624	1.125	2.0624	2.0624	Indifferent
10.	R7CH	2	128	0.125	0.125	2	16	0.0156	0.0156	1	0.125	0.1248	0.1248	1.125	1.1248	1.1248	Indifferent
11.	B2H	1	128	0.125	0.125	2	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
12.	6R1	0.5	256	0.125	0.125	0.5	16	0.0156	0.0156	1	0.0625	0.1248	0.1248	1.0625	1.1248	1.1248	Indifferent
13.	DS 09	128	256	0.25	0.25	256	16	0.0156	0.0156	2	0.0625	0.0624	0.0624	2.0625	1.0624	1.0624	Indifferent
14.	DS 10	32	128	0.125	0.125	64	16	0.0156	0.0156	2	0.125	0.1248	0.1248	2.125	2.1248	2.1248	Indifferent
15.	B2D	1	256	0.25	0.25	1	16	0.0156	0.0156	1	0.0625	0.0624	0.0624	1.0625	2.0624	2.0624	Indifferent
16.	1199H1	16	128	0.125	0.125	32	16	0.0156	0.0156	2	0.1248	0.1248	0.1248	2.1248	2.1248	2.1248	Indifferent
17.	1105LF	16	128	0.125	0.125	16	16	0.0156	0.0156	1	0.1248	0.1248	0.1248	1.1248	1.1248	1.1248	Indifferent

D_a- MIC of Doxycycline when used in combination with ketoprofen, betamethasone and meloxicam respectively: K_b- MIC of ketoprofen when used in combination with Doxycycline: B_c- MIC of betamethasone when used in combination with Doxycycline: M_d- MIC of meloxicam when used in combination with Doxycycline: D_1- FIC of Doxycycline with ketoprofen, betamethasone and meloxicam respectively: K_2- FIC of ketoprofen when used in combination with Doxycycline: B_3- FIC of betamethasone when used in combination with Doxycycline: M_4- FIC of meloxicam when used in combination with Doxycycline

Conclusion

The present study revealed rampant occurrence of resistance for tetracycline and ciprofloxacin, two most commonly used antibiotics in veterinary therapeutics. The study indicated synergistic and additive effect on enrofloxacin combination with NSAIDs and steroid suggesting utility of giving NSAIDs with enrofloxacin for treatment of infections with enrofloxacin resistant *E. coli* infections. However, enrofloxacin interactions with NSAIDs and steroid for *S. aureus* were of insignificant value. Further the doxycycline had indifferent interaction with NSAIDs and steroid for both *S. aureus* and *E. coli.* This study warrants more studies on interaction of antibiotics with NSAIDs at plasma concentrations levels of the drugs and biomolecular aspect of NSAIDs and antibiotics for possible use of knowledge of interaction of two groups of drugs in therapeutics.

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