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Research Article

Comparative Antimicrobial Activity of Aspirin, Paracetamol, Flunixin Meglumine, Tolfenamic Acid, Diclofenac Sodium and Pheniramine Maleate

Bhoj R Singh*, BS Pruthvishree, Akanksha Yadav, Ravichandran Karthikeyan, Obli R Vinodhkumar and Dharmendra K Sinha

Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar, India

***Corresponding Author:** Bhoj R Singh, Head, Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar, India.

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Abstract

The study was conducted to evaluate the antimicrobial activity of aspirin, paracetamol, flunixin meglumine, tolfenamic acid, diclofenac sodium and pheniramine maleate on 499 strains of microbes of 117 species belonging to 26 genera of Gram-negative (G-ve), nine genera of Gram-positive (G+ve) bacteria and four strains of two *Candida* species. A total of 92.79%, 44.09%, 54.91% and 30.26% bacterial strains were sensitive to 2.56 mg/mL aspirin, 3.2 mg/mL flunixin, 2.56 mg/mL diclofenac and 1.28 mg/mL meloxicam, respectively. For paracetamol and pheniramine maleate only one strain of *Aerococcus* species was sensitive at ≤ 3.2 mg/mL concentration of these drugs and none of the strains was susceptible to tolfenamic acid even at 10.28 mg/mL. The analysis indicated that G+ve bacteria had significantly lower susceptibility to aspirin (OR = 0.30; CI_{99} = 0.12 - 0.78) but higher susceptibility to flunixin (OR = 7.22; CI_{99} = 4.12-12.50) and diclofenac (OR = 1.91; CI_{99} = 1.15 - 3.15) than G-ve bacteria. There was no significant difference in meloxicam susceptibility of G+ve and G-ve bacteria. The study concluded that NSAIDs and pheniramine maleate may not be used as antimicrobials in therapeutically achievable systemic concentrations of the drugs within biological safety limits of plasma concentration. However, the scope of use of NSAIDs still exists in form of non-antibiotic topical antimicrobial preparations.

Keywords: NSAIDs; Antihistaminics; Antibacterials; Antibiotics; Drug-repurposing; Alternative-antimicrobials

Introduction

Antibiotics are common in treatment against deadly bacterial and mycotic infections among man and animals. However, choices of antibiotics are getting narrower due to fast emergence of antimicrobial resistance (AMR). By 2030, approximately 24 million people would be affected by AMR and as a consequence of extreme poverty especially in low-income countries; millions will lose the life [1]. Resistance to frequently used antibiotic drugs among bacterial strains is right now a worldwide problem, in the meantime, the number of strains that have developed resistance to multiple antibiotics has risen at an increasing rate and has spread globally to most nations irrespective of antibiotic uses in therapeutics [2-4]. Due to the increase in resistance and delay in the development of newer antibiotics, the efficient treatment of bacterial diseases is affected [5,6]. Currently, research has been aimed at the investigation of non-steroidal anti-inflammatory drugs and other molecules for their antibacterial action [3].

"Drug repurposing" (or drug re-profiling), is a new idea in which the non-antibacterial drugs such as NSAIDs and others are

studied for their antimicrobial properties in order to minimize the time and economic costs associated with new drug discovery processes [7,8]. A variety of non-antibiotic drugs with varying degree of broad-spectrum antimicrobial activity have been acknowledged viz., herbal antimicrobials, anthelmintics, anticancer drugs, antipsychotics, antidepressant drugs, antiplatelets and NSAIDs [2,9-11]. Some of the drugs such as acetaminophen, acetylsalicylic acid, diclofenac and ibuprofen, flurbiprofen and similar NSAIDs hardly have any antibiotic activity in therapeutically achievable concentrations [12] but when jointly administered with the antibiotic drug they either inhibit bacterial growth or alter underlying resistance mechanism or amplifies those factors of antibiotics which adversely affect the survival of infectious bacteria and majorly they amplify the inhibitory capability of the administered drug [5]. From earlier observations, it is well understood that in therapeutically achievable plasma concentrations most of the drugs targeted in the present study may not be used systematically. However, antibiotics used for topical applications (many of which are too toxic to systematic use) may be replaced with non-antibiotic antimicrobials if found effective in acceptable concentrations. Hence, the present study was conducted with an objective to assess the comparative antimicrobial activity of commonly used NSAIDs including acetylsalicylic acid (aspirin), acetaminophen (paracetamol), flunixin meglumine, tolfenamic acid and diclofenac, and pheniramine maleate (antihistamine) against several strains of pathogenic bacteria to map their spectrum of activity with the possibility of their use in topical antimicrobial formulations.

Materials and Methods

Microbial strains used in the study

A total of 499 strains including previously isolated from different clinical and paraclinical samples (475) and reference (24) strains (Table 1) available as glycerol stocks in Clinical Epidemiology Laboratory, ICAR-Indian Veterinary Research Institute, Izatnagar, India were revived and tested for identity and purity using growth, morphology and biochemical characteristics [13-15]. Test strains belonging to 117 species falling under 26 genera of Gram-negative (G-ve) bacteria (322 strains), nine genera of Gram-positive (G+ve) bacteria (173 strains) and four strains of two *Candida* species (Table 2) were included in the study. After revival, all strains were kept on nutrient agar slant till tested within 15 days of revival.

Microbial geneus	Bacterial source											Total
	Air	Poultry birds	Domestic animals	Fish	Human	Plants	Milk	Reference strains	Water	Wild animals	Wild birds	
Escherichia	0	11	41	0	7	9	0	3	0	21	9	101
Hafnia	0	6	1	0	3	5	0	0	0	1	1	17
Klebsiella	0	4	14	3	2	3	0	0	0	5	2	33
Proteus	0	3	10	0	0	0	0	0	0	2	0	15
Pseudomonas	0	2	6	0	2	1	1	0	1	2	0	15
Salmonella	0	5	1	0	0	0	0	17	0	1	0	24
Staphylococcus	1	7	29	0	31	0	0	3	0	9	3	83
Streptococcus	0	1	11	0	4	0	1	0	0	3	2	22
Paenibacillus	12	0	2	0	0	2	0	0	0	1	0	17
Enterococcus	0	1	4	0	3	2	0	0	0	12	2	24
Other genera with less than 15 isolates tested	7	11	46	6	7	44	0	1	1	20	5	148
Total	20	51	165	9	59	66	2	24	2	77	24	499

Table 1: Sources of major bacterial genera in the study (isolates ≥15) tested for antimicrobial activity of NSAIDs.

Bacteria	Strains tested	Aspirin	Meloxicam	Paracetamol	Flunixin meglumine	Diclofenec	Tolfenamic acid	Pheniramine maleate
Acinetobacter alcaligenes	1	1.28	1.28	5.12	1.6	2.56	>12.8	6.4
Acinetobacter calcoaceticus	3	1.28	>12.8	5.12-10.24	0.4-6.4	5.12	>12.8	6.4->12.8
Acinetobacter lwoffii	3	0.32-1.28	1.28	5.12	1.6	2.56	>12.8	6.4
Aerococcus spp.	3	0.32	0.64	0.16-10.24	0.1-0.8	2.56	>12.8	3.2-12.8
Aeromonas bestiarum	5	1.28	0.64-1.28	10.24	0.2-12.8	2.56	>12.8	12.8
Aeromonas caviae	2	0.08-0.64	>1.28	10.24	0.1-0.8	5.12	>12.8	>12.8
Aeromonas eucranophila	2	1.28	0.64	10.24	0.8	2.56	>12.8	12.8
Aeromonas hydrophila	2	0.64-1.28	>1.28	10.24	0.4-6.4	5.12	>12.8	>12.8
Aeromonas media	2	0.08-0.32	0.01-0.08	10.24	0.05-0.8	2.56	>12.8	12.8
Aeromonas popoffii	4	1.28	0.64-1.28	10.24	0.8-12.8	2.56	>12.8	12.8
Aeromonas salmonicida	2	1.28	0.64	10.24	0.2-12.8	2.56	>12.8	12.8
Aeromonas schubertii	3	0.64-2.56	0.64->1.28	10.24	0.8	2.56	>12.8	12.8
Aeromonas trota	4	0.64-1.28	0.64->1.28	10.24	0.8-12.8	2.56	>12.8	12.8
Alcaligenes faecalis	1	0.64	1.28	10.24	1.6	2.56	>12.8	NT
Arsenophonus nasoniae	1	1.28	>1.28	10.24	0.8	1.28	>12.8	12.8
Bacillus amyloliquifaciens	1	1.28	1.28	10.24	12.8	0.64	>12.8	6.4
Bacillus badius	1	1.28	1.28	10.24	12.8	5.12	>12.8	NT
Bacillus brevis	1	0.08	1.28	10.24	0.05	5.12	>12.8	NT
Bacillus cereus	5	1.28	1.28	10.24	0.1-12.8	1.28-10.24	>12.8	NT
Bacillus licheniformis	3	1.28	0.32-1.28	5.12	0.4-0.8	0.16-0.64	>12.8	6.4
Bacillus megaterium	2	1.28-2.56	1.28	10.24	0.1-3.2	0.16	>12.8	6.4
Bacillus mycoides	2	1.28->5.12	1.28	10.24	0.2-0.4	0.64	>12.8	6.4
Bacillus sphaericus	1	1.28	1.28	10.24	0.2	0.64	>12.8	6.4
Burkholderia cepacia	1	>5.12	>1.28	>10.24	12.8	>10.24	>12.8	>12.8
Candida albicans	3	*1.28->5.12*	>1.28	10.24	1.6-3.2	1.28	>12.8	12.8
Candida famata	1	2.56	>1.28	10.24	3.2	1.28	>12.8	12.8
Cedecea lapagiae	1	1.28	>1.28	10.24	6.4	5.12	>12.8	12.8
Citrobacter freundii	1	1.28	>1.28	10.24	12.8	5.12	>12.8	12.8
Edwardsiella hoshniae	1	1.28	>1.28	10.24	12.8	5.12	>12.8	12.8
Edwardsiella tarda	2	1.28	1.28->1.28	10.24	12.8	5.12	>12.8	12.8
Enterobacter agglomerans	13	1.28-2.56	>1.28	10.24	0.4-12.8	5.12	>12.8	12.8
Enterobacter gregoviae	1	1.28	>1.28	10.24	12.8	5.12	>12.8	12.8
Enterococcus durans	2	2.56	>1.28	10.24	0.025-3.2	2.56-5.12	>12.8	NT
Enterococcus faecalis	8	0.64-5.12	>1.28	10.24	1.6-12.8	5.12	>12.8	NT
Enterococcus faecium	9	1.28-5.12	>1.28	10.24	0.8-12.8	2.56-5.12	>12.8	NT
Enterococcus malodoratus	1	1.28	>1.28	10.24	12.8	5.12	>12.8	NT
Enterococcus solitarius	4	0.32-2.56	>1.28	10.24	0.4-12.8	2.56-5.12	>12.8	NT
Erwinia amylovora	2	1.28	>1.28	10.24	1.6-12.8	2.56-5.12	>12.8	NT
Erwinia aphidicola	1	1.28	>1.28	10.24	12.8	2.56	>12.8	NT

Erwinia carotovora	1	1.28	>1.28	10.24	3.2	5.12	>12.8	NT
Erwinia cyperipedii	1	1.28	1.28	10.24	12.8	2.56	>12.8	NT
Erwinia nimipressuralis	1	1.28	>1.28	10.24	12.8	2.56	>12.8	NT
Erwinia stewartii	1	0.64	0.64	10.24	0.4	2.56	>12.8	NT
Erwinia tasmaniensis	1	1.28	>1.28	10.24	12.8	5.12	>12.8	NT
Escherichia coli	96	0.08->51.2	0.64->1.28	5.12-10.24	0.1-12.8	1.28->10.24	>12.8	6.4->12.8
Escherichia fergusonii	4	0.32-2.56	1.28->1.28	10.24	3.2-12.8	5.12	>12.8	12.8
Escherichia hermanii	1	1.28	>1.28	10.24	1.6	5.12	>12.8	12.8
Gallibacterium anatis	3	1.28	>1.28	10.24	0.1-1.6	>10.24	>12.8	12.8
Gardnerella spp.	1	1.28	>1.28	10.24	1.6	>10.24	>12.8	12.8
Geobacillus stearothermophilus	6	0.64-1.28	1.28->1.28	10.24	0.025-12.8	0.64-5.12	>12.8	6.4->12.8
Hafnia alvei	17	0.64-2.56	>1.28	10.24	0.4-12.8	1.28-5.12	>12.8	>12.8
Klebsiella oxytoca	2	2.56	>1.28	10.24	3.2-12.8	2.56	>12.8	>12.8
Klebsiella pneumoniae ssp. *pneumoniae*	31	1.28-2.56	0.64>1.28	10.24	3.2-12.8	2.56->10.24	>12.8	12.8->12.8
Kocuria rosea	1	0.32	1.28	>10.24	0.4	0.32	>12.8	12.8
Micrococcus luteus	1	0.32	1.28	>10.24	0.4	0.32	>12.8	12.8
Moelerella wisconsensis	1	1.28	1.28	10.24	1.6	2.56	>12.8	>12.8
Moraxella bovis	1	0.32	0.32	10.24	1.6	0.32	>12.8	>12.8
Moraxella osloensis	1	1.28	0.64	10.24	3.2	0.64	>12.8	>12.8
Moraxella ovis	2	1.28	1.28	10.24	1.6	2.56	>12.8	>12.8
Moraxella phenylpyruvica	1	1.28	0.64	10.24	3.2	0.64	>12.8	>12.8
Paenibacillus lactis	1	0.64	0.32	10.24	3.2	0.64	>12.8	6.4
Paenibacillus larvae	2	0.64-1.28	0.08	10.24	0.05	5.12	>12.8	6.4
Paenibacillus pantothenticus	14	<0.01-0.64	1.28	10.24	0.05-3.2	5.12	>12.8	12.8
Pasteurella canis	7	0.32-0.64	0.64->1.28	10.24	0.4-12.8	0.32-1.28	>12.8	12.8
Pasteurella multocida	2	0.32-0.64	0.64	10.24	12.8	1.28	>12.8	12.8
Proteus mirabilis	12	1.28-5.12	>1.28	10.24	6.4-12.8	2.56	>12.8	12.8->12.8
Proteus penneri	2	1.28-2.56	>1.28	10.24	6.4-12.8	2.56	>12.8	12.8->12.8
Proteus vulgaris	1	2.56	>1.28	10.24	12.8	2.56	>12.8	>12.8
Providencia stuartii	1	1.28	>1.28	5.12	6.4	2.56	>12.8	12.8
Providencia rustigianii	1	1.28	>1.28	10.24	12.8	5.12	>12.8	12.8
Pseudomonas aeruginosa	9	1.28>5.12	0.64->1.28	5.12-10.24	6.4-12.8	0.64->10.24	>12.8	12.8
Pseudomonas alcaligenes	1	>5.12	>1.28	5.12	12.8	2.56	>12.8	12.8
Pseudomonas diminuta	1	>5.12	>1.28	5.12	0.8	2.56	>12.8	12.8
Pseudomonas paucimobilis NDM	1	1.28	0.64	5.12	6.4	2.56	>12.8	12.8

Pseudomonas pseudoalcaligenes	2	1.28-2.56	0.64->1.28	5.12-10.24	6.4-12.8	2.56	>12.8	12.8
Pseudomonas stutzeri	1	>5.12	>1.28	5.12	0.2	0.64	>12.8	6.4
Raoultella terrigena	12	0.64-2.56	1.28	10.24	0.2-12.8	1.28-2.56	>12.8	12.8
Salmonella enterica ssp. *enterica*	23	1.28-2.56	0.32->1.28	5.12-10.24	0.2-12.8	1.28-5.12	>12.8	6.4-12.8
Salmonella enterica ssp. *indica*	1	2.56	>1.28	10.24	12.8	2.56	>12.8	12.8
Serratia fonticola	1	2.56	>1.28	10.24	12.8	2.56	>12.8	12.8
Serratia grimaceae	4	2.56	0.32->1.28	5.12-10.24	0.1-12.8	1.28-2.56	>12.8	6.4-12.8
Serratia marcescens	3	2.56	>1.28	10.24	12.8	2.56	>12.8	12.8
Serratia odorifera	3	2.56	>1.28	10.24	12.8	2.56	>12.8	12.8
Serratia plymuthica	1	1.28	>1.28	10.24	12.8	2.56	>12.8	12.8
Serratia proteomaculans	2	2.56	0.64	5.12	0.1	0.64	>12.8	12.8
Serratia rubidaea	1	2.56	>1.28	10.24	12.8	2.56	>12.8	12.8
Staphylococcus arlettae	3	1.28	>1.28	10.24	1.6-3.2	2.56	>12.8	12.8
Staphylococcus aureus	11	0.64->5.12	1.28->1.28	1.28-2.56	0.2-12.8	1.28-2.56	>12.8	12.8
Staphylococcus capitis ssp. *capitis*	5	1.28->51.2	>1.28	5.12-10.24	0.1-3.2	2.56	>12.8	12.8
Staphylococcus capitis ssp. *urealyticus*	1	0.64	>1.28	10.24	0.4	2.56	>12.8	12.8
Staphylococcus caseolyticus	1	1.28	>1.28	10.24	12.8	2.56	>12.8	12.8
Staphylococcus chromogenes	3	1.28-5.6	>1.28	10.24	0.2-12.8	1.28-2.56	>12.8	12.8
Staphylococcus delphini	5	1.28	>1.28	10.24	0.2-3.2	1.28	>12.8	12.8
Staphylococcus epidermidis	13	1.28->5.12	>1.28	5.12-10.24	0.1-6.4	1.28-10.24	>12.8	6.4-12.8
Staphylococcus equorum	3	1.28	>1.28	10.24	0.1-12.8	2.56->10.24	>12.8	12.8
Staphylococcus gallinarum	1	1.28	>1.28	10.24	3.2	2.56	>12.8	12.8
Staphylococcus haemolyticus	12	1.28->5.12	>1.28	5.12-10.24	0.1-12.8	1.28-10.24	>12.8	12.8
Staphylococcus hominis	6	1.28->5.12	>1.28	10.24	0.0125-3.2	1.28-2.56	>12.8	12.8
Staphylococcus hyicus	2	1.28-2.56	>1.28	10.24	0.1-0.4	2.56	>12.8	12.8
Staphylococcus intermedius	4	1.28-5.12	>1.28	10.24	0.2-12.8	1.28-2.56	>12.8	12.8
Staphylococcus lentus	4	1.28-2.56	>1.28	10.24	0.2-12.8	1.28-2.56	>12.8	12.8
Staphylococcus lugdunerisii	4	1.28	>1.28	10.24	0.2-12.8	2.56	>12.8	12.8
Staphylococcus schleiferi ssp. coaggulans	1	1.28	>1.28	10.24	0.05	2.56	>12.8	12.8
Staphylococcus sciuri	1	0.32	>1.28	10.24	0.2	2.56	>12.8	12.8
Staphylococcus simulans	1	1.28	>1.28	10.24	0.8	2.56	>12.8	12.8

Staphylococcus xylosus	2	0.04-1.28	>1.28	10.24	0.2	2.56	>12.8	12.8
Streptococcus equi ssp. *zooepidemicus*	1	2.56	>1.28	5.12	3.2	5.12	>12.8	6.4
Streptococcus milleri	8	1.28-2.56	1.28->1.28	10.24	0.8-12.8	2.56->10.24	>12.8	12.8
Streptococcus phocae	1	5.12	1.28	10.24	12.8	>10.24	>12.8	12.8
Streptococcus pnemoniae	3	1.28-2.56	1.28->1.28	10.24	0.4-12.8	2.56-5.12	>12.8	12.8
Streptococcus porcinus	1	2.56	>1.28	10.24	3.2	5.12	>12.8	12.8
Streptococcus pyogenes	1	0.01	>1.28	10.24	3.2	2.56	>12.8	12.8
Streptococcus salivaris	2	1.28-2.56	1.28	10.24	0.8-12.8	2.56	>12.8	12.8
Streptococcus suis	5	2.56	0.64->1.28	10.24	0.2-12.8	0.04->10.24	>12.8	12.8
Xenorhabdus bovienii	3	1.28	0.64	5.12-10.24	3.2-12.8	1.28	>12.8	6.4
Yersinia enteocolitica	1	1.28	0.01	5.12	0.2	1.28	>12.8	6.4
Yersinia ruckeri	2	1.28	0.01-0.64	5.12	0.2-3.2	0.64-1.28	>12.8	6.4

Table 2: The minimum inhibitory concentration (in mg/ mL) of different NSAIDs and pheniramine maleate on different species of microbes.

Determination of minimum inhibitory concentration (MIC) of test drugs

All strains were tested for determining MIC of aspirin, paracetamol, flunixin meglumine, tolfenamic acid, diclofenac sodium and pheniramine maleate using micro-broth dilution method as described earlier [12]. Briefly pure compounds were solubilised in Mueller Hinton broth (MHB, BBL Difco) to the required concentration viz. aspirin 51.2 mg/mL, meloxicam 1.28 mg/mL, paracetamol 10.24 mg/mL, flunixin meglumine 12.8 mg/mL, diclofenac sodium 10.24 mg/mL, tolfenamic acid 12.8 mg/mL and pheniramine maleate 12.8 mg/mL. Serial dilutions of drug solutions were made aseptically in sterile MHB from column one to 11 of 96 well microdilution plates, the last column was kept as no-drug control. Thereafter, each well of row 'A to G' were was inoculated with the test culture using 2 µL of 6 h grown broth culture of test organism in MHB containing about 10^5 CFU, the 'H' row was kept as no bacteria (negative) control. After applying the lid, plates were incubated at 37°C for 24h and then visible growth was read using a plate reader at 600 nm wavelength. An increase in optical density (OD) by 50% above the negative control was read as positive growth. The last dilution inhibiting the growth was considered as the MIC of the drug for the test strain. For conformity, the test was repeated twice. All media were procured from BBL Difco and chemicals/drugs used were from Sigma (USA). All bacteria included in the study were tested for their susceptibility to imipenem in similar way at a fixed concentration of 64 µg/mL to have the control of the working of the MIC assays.

Statistical analysis

The sources of the isolates were categorized into 11 meaningful groups (Table 1). The MIC data for all strains were entered in Microsoft Excel(R) sheet. The generic groups of microbes having more than 15 isolates were further analysed with respect to the MIC of flunixin, diclofenac and paracetamol using one-way variance of analysis (ANOVA) with Tukey HSD at $P < 0.05$. The MICs of meloxicam, tolfenamic acid, pheniramine maleate were measured qualitatively and the categorical data was analysed using chi-square or Fischer's exact test. Gram staining characteristic was analyzed with multivariate technique using Principal Component analysis with MissMDA package with the Kfold method and FactoMineR package in R software (R statistical platform 4.0.3). To compare MICs of different drugs for different types of bacteria (only for those species where the number of strains was ≥ 10) odds ratio and chi-square or Fischer's exact tests were used [13,14].

Results

The MIC results for different bacteria (Table 2) revealed that 92.79%, 44.09%, 54.91% and 30.26% bacterial strains were sensitive to 2.56 mg/mL aspirin, 3.2 mg/mL flunixin, 2.56 mg/mL diclofenac and 1.28 mg/mL meloxicam, respectively. For paracetamol and pheniramine maleate, only one strain of *Aerococcus* species isolated from *Azadirachta indica* leaves was sensitive at ≤ 3.2 mg/mL % concentration of these drugs. None of the tested strains was susceptible to tolfenamic acid even at 12.8 mg/mL. The distribution of strains as per their MIC depicted in table 3 indicated the variability in MIC of five different drugs. Table 4 depicted the susceptibility of strains of 11 major species (with > 10 strains) of bacteria (*Enterobacter agglomerans, Escherichia coli, Hafnia alvei, Klebsiella pneumoniae* ssp. *pneumoniae*, *Paenibacillus pantothenticus, Proteus mirabilis, Raoultella terrigena, Salmonella enterica* ssp. *enterica, Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus haemolyticus*) to aspirin, flunixin, diclofenac and meloxicam. The analysis indicated that G+ve bacteria had significantly lower susceptibility to aspirin (OR = 0.30; CI_{99} = 0.12-0.78) than G-ve bacteria, while G+ve had higher susceptibility to flunixin (OR = 7.22; CI_{99} = 4.12-12.50) and diclofenac (OR = 1.91; CI_{99} = 1.15-3.15). There was no significant difference in meloxicam susceptibility of G+ve and G-ve bacteria. Both G+ve and G-ve bacteria had significantly ($p < 0.03$) higher susceptibility to aspirin than *Candida* strains but with respect to susceptibility to other drugs difference was insignificant ($p > 0.05$).

Among major G-ve bacteria species (*E. agglomerans, E. coli, H. alvei, K. pneumoniae*, *P. mirabilis, R. terrigena* and *S. enterica*) compared for sensitivity to aspirin, there was no significant difference. However, among strains of G+ve species, strains of *S. epidermidis* were significantly more often aspirin resistant than strains of *P. pantothenticus* ($p = 0.004$) and *S. haemolyticus* ($p = 0.04$). *Staphylococcus aureus* strains were significantly more often resistant to aspirin than strains of *E. coli* ($p = 0.008$) and *S. enterica*, but strains of *S. epidermidis* were significantly more often resistant than strains of *E. agglomerans* (p, 0.005), *E. coli* ($P < 0.001$), *P. mirabilis* ($p = 0.04$), *R. terrigena* ($p = 0.007$) and *S. enterica* ssp. *enterica* ($p = 0.0004$).

Susceptibility to flunixin varied significantly among different G-ve bacterial species, as strains of *E. coli, P. mirabilis* and *K. pneumoniae* were significantly ($p < 0.05$) more often resistant than strains of *R. terrigena* and *S. enterica* to flunixin. None of the strains of *P. mirabilis* was susceptible to flunixin. Among all the G+ve bacterial species strains compared, *P. pantothenticus* strains were the most sensitive to flunixin (MIC ≤ 3.2 mg/mL). All the G+ve species strains were significantly ($p = 0.01$) more often susceptible to flunixin than G-ve species strains.

On the basis of susceptibility to diclofenac sodium, G-ve bacteria could be divided into two distinct groups, one often resistant and other sensitive. Strains of *E. agglomerans, E. coli* and *K. pneumoniae* were significantly ($p < 0.03$) more often resistant to diclofenac sodium than strains of *H. alvei, P. mirabilis, R. terrigena* and *S. enterica*. Similarly, among G+ve bacterial species compared, two distinct groups were there on the basis of their susceptibility to

MIC in mg/mL	Number of isolates with the specified MIC			MIC in mg/mL	Number of isolates with the specified MIC	
	Aspirin	Diclofenac	Meloxicam		Flunixin meglumine	Pheniramine maleate
0.005	4	0	0	0.0125	1	0
0.01	1	0	2	0.025	2	0
0.02	0	0	0	0.05	19	0
0.04	1	1	0	0.1	19	0
0.08	4	0	3	0.2	34	0
0.16	0	4	0	0.4	35	0
0.32	17	5	8	0.8	32	0
0.64	28	14	41	1.6	26	0
1.28	266	46	97	3.2	52	1
>1.28	178	429	348	6.4	62	38
2.56	146	204	NA	12.8	217	337
5.12	6	202	NA	>12.8		83
>5.12	26	NA	NA	NA	NA	NA
10.24	0	6	0	NA	NA	NA
>10.24	NA	17	NA	NA	NA	NA
Not tested	0	0	0	0	0	40

Table 3: Distribution of 499 bacterial isolates tested for minimum inhibitory concentration (MIC) of NSAIDs and pheniramine maleate.

Bacteria	Isolates tested	Number of strains inhibited by			
		0.256% Aspirin	0.32% Flunixin	0.256% Diclofenac	0.128% Meloxicam
Enterobacter agglomerans	13	13	4	0	0
Escherichia coli	96	94	12	4	23
Hafnia alvei	17	17	3	11	0
Klebsiella pneumoniae ssp. *pneumoniae*	31	31	3	9	3
Paenibacillus pantothenticus	14	14	14	0	14
Proteus mirabilis	12	11	0	12	0
Raoultella terrigena	12	12	6	12	12
Salmonella enterica ssp. *enterica*	23	23	7	22	7
Staphylococcus aureus	11	9	7	11	1
Staphylococcus epidermidis	13	7	12	11	0
Staphylococcus haemolyticus	12	11	11	10	0
G+ve	173	152	127	112	50
G-ve	322	309	89	158	101
Candida	4	2	4	4	0
All isolates tested	499	463	220	274	151
Percent sensitive		92.79	44.09	54.91	30.26

Table 4: Susceptibility of some major bacteria tested for NSAIDs.

diclofenac. *Paenibacillus pantothenticus* strains were all resistant while those of *Staphylococcus* species were mostly sensitive to diclofenac sodium.

With respect to susceptibility to meloxicam, similar to diclofenac, two distinct groups existed among G-ve and G+ve bacteria. Among G+ve bacteria, all *P. pantothenticus* strains were susceptible to meloxicam, and most of the staphylococci were resistant to meloxicam. Among G-ve bacteria, a significantly ($p \leq 0.05$) more meloxicam resistant group had strains of E. agglomerans, *H. Alvei, K. pneumoniae* and *P. mirabilis* while most of the strains of *R. terrigena, S. enterica* and many of the *E. coli* were sensitive to meloxicam.

For detailed descriptive analysis, sources of the isolates were categorized into 11 groups. The frequency distribution of different bacterial pathogens is given in table 1. For this purpose genera having ≥ 15 stains in the study were included (a total of 205 G-ve and 146 G+ve). As most of the strains were sensitive to aspirin at MIC ≤ 5.12 mg/mL and resistant to pheniramine maleate (MIC ≥ 1.28 mg/mL), tolfenamic acid (MIC ≥ 1.28 mg/mL) and meloxicam (MIC ≥ 1.28 mg/mL), variability was insignificant among different types of bacteria thus descriptive analysis was not done. The descriptive analysis (Table 5) showed no statistical difference in the MIC of paracetamol for G+ve and G-ve bacteria ($p > 0.05$). However, flunixin and diclofenac showed a significant difference ($p < 0.01$) between G+ve and G-ve bacteria. The bacterial genus wise analysis showed that *Pseudomonas* (mean 6.48 ± 34) differ significantly from other bacterial genera for the MIC value of paracetamol ($p < 0.05$). For flunixin, the *Paenibacillus* and *Staphyloccocus* showed no significant difference in the MIC. However, the MIC of diclofenac had a significant difference between the two genera of the bacteria (Table 6).

The plots analysis showed the variability across MICs of flunixin, paracetamol, and diclofenac with respect to Gram staining characteristic of bacteria (Figure 1). The supplementary and variable plot shows projection characteristics of the bacteria and MIC values. The first dimension explained the 41% variability and the second dimension explained the 27% variability. The MIC variables (flunixin, paracetamol, and diclofenac) mainly contributed in the first dimension. However, the second dimension was contributed by the Gram staining characteristic of bacteria. The MVA results indicated that less or no association existed between the MICs of flunixin, paracetamol and diclofenac with respect to Gram staining characteristic of the bacteria.

Genus	Fluxinin				Paracetamol			Diclofenac		
	N	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error
Klebsiella	33	10.23	3.73	0.64	10.24	0.00	0.00	4.21	1.24	0.22
Escherichia	101	9.68	4.10	0.41	10.18	0.50	0.05	5.15	1.06	0.10
Enterococcus	24	9.98	5.023	1.03	10.24	0.00	0.00	4.32	1.29	0.26
Hafnia	17	8.92	4.59	1.12	10.24	0.00	0.00	3.31[a]	1.43	0.34
Paenibacillus	17	.511[a]	1.078	0.26	10.24	0.00	0.00	4.85	1.08	0.26
Proteus	15	10.24	3.24	0.84	10.24	0.00	0.00	2.56[a]	0.00	0.00
Pseudomonas	15	9.88	4.61	1.19	6.48[a]	2.34	0.61	2.24[a]	0.74	0.21
Salmonella	24	9.03	5.16	1.06	9.38	1.94	0.39	2.34[a]	0.81	0.16
Staphylococcus	83	2.77[ab]	4.48	0.49	9.99	1.10	0.12	2.62[a]	1.40	0.15
Streptococcus	22	6.50[b]	5.53	1.18	10.01	1.09	0.23	3.61[a]	1.57	0.38
Total	351	7.433	5.42	0.29	9.93	1.21	0.065	3.79	1.63	0.089

Table 5: Inferential analysis of antimicrobial activity of different NSAIDs on major bacterial genera (≥15 isolates) in the study. The mean MIC value in same superscript column wise for each NSAIDs doesn't differ significantly between the bacterial genera.

Bacteria (no. of isolates)	Minimum inhibitory concentration of drugs in mg/mL																	
	Pheniramine maleate				Meloxicam					Aspirin								
	3.2	6.4	12.8	>12.8	0.08	0.32	0.64	1.28	>5.12	≤0.01	0.01	0.04	0.08	0.32	0.64	1.28	2.56	5.12
Enterococcus (24)	24	24	0	0	0	0	0	0	0	0	0	0	0	1	1	10	10	2
Escherichia (101)	76	1	92	8	0	0	1	24	1	0	0	0	1	1	0	84	13	1
Hafnia (17)	17	0	0	17	0	0	0	0	0	0	0	0	0	0	1	12	4	0
Klebsiella (33)	30	0	5	28	0	0	1	2	0	0	0	0	0	0	0	7	26	0
Paenibacillus (17)	0	5	12	0	2	1	0	14	0	4	0	0	0	4	8	1	0	0
Proteus (15)	15	0	2	13	0	0	0	0	0	0	0	0	0	0	0	8	6	1
Pseudomonas (15)	11	1	14	0	0	0	3	1	9	0	0	0	0	0	0	4	2	0
Salmonella (24)	17	6	18	0	0	2	1	4	0	0	0	0	0	0	0	2	22	0
Staphylococcus (83)	82	1	82	0	0	0	0	1	12	0	0	1	0	1	2	55	11	1
Streptococcus (22)	12	1	21	0	0	0	1	9	0	0	1	0	0	0	0	7	13	1
P value	0.01*				0.01*					0.01*								

Table 6: Frequency distribution of minimum inhibitory concentration (MIC) of NSAIDs and antihistamine for strains of major genera with ≥15 isolates tested.

*P < 0.01 indicates significance at 99 % confidence level.

Discussion

The quest for antibiotic alternatives initiated a lot of research and the repurposing of already approved drugs for their antimicrobial activity is an emerging area of research [7,8]. Though several studies have been conducted for the evaluation of the antimicrobial potential of NSAIDs and antihistaminics, most of the studies

Figure 1: Principal component analysis of different NSAIDs' MIC values with Gram staining characteristics of the bacteria.

were limited to a few reference strains. The novelty of the present study seems to be its magnanimity being one of the largest studies reported which reveals the comparative spectrum of antimicrobial activity of the test drugs. The present study on 499 microbial strains of 117 species indicated the antimicrobial potential of NSAIDs, often used as an adjunct with antibiotics. The study revealed that 92.79%, 44.09%, 54.91% and 30.26% bacterial strains were sensitive to 2.56 mg/mL aspirin, 0.32% flunixin, 0.256% diclofenac and 0.128% meloxicam, respectively. Pheniramine maleate and paracetamol could inhibit many of the microbial strains tested at ≤ 12.8 mg/mL concentration. However, tolfenamic acid showed no antimicrobial activity even at a 12.8 mg/mL concentration. In earlier studies too, tolfenamic acid is shown to inhibit only a strain of *S. aureus* (MIC = 5 mg/mL) but was ineffective against *C. albicans, E. coli* and *P. aeruginosa* strains even at 10 mg/mL concentration [11]. A study on antimicrobial activity of pheniramine maleate reported inhibition of growth of *S. aureus* and *S. epidermidis* at > 20 mg/mL level [18]. However, in the present study 81.91% of strains were inhibited by pheniramine maleate at ≤ 12.8 mg/mL including all strains of *S. aureus* and *S. epidermidis*. The difference might be attributed to the different bacterial strains used.

Aspirin inhibited most of the microbial strains in the study at ≤ 5.12 mg/mL (0.512%). In earlier studies, 100% of strains are shown to be susceptible at a 12.8 mg/mL concentration of aspirin [12] but paracetamol was either almost ineffective or mildly effective as antimicrobial [12,17]. The observations of this study are in concurrence to earlier observations and proving no therapeutically useful potential of paracetamol even in very high (toxic) dosages [12,19].

Flunixin meglumine in animals may be given at a maximum dose of 1 - 1.5 mg/kg body weight [20] but it inhibited the growth of 44.09% bacterial strains ≤ 3.2 g/L concentration in the study while at 11 mg/Kg body weight flunixin meglumine may turn lethal within 12 h [21] that means it can't be used for its antimicrobial activity in non-toxic dosages.

Diclofenac inhibited the growth of 54.91% bacterial isolates tested at 2.56 mg/mL concentration; *Staphylococcus* species were mostly sensitive but all *P. pantothenticus* and *Enterococcus* spp. strains (*E. durans* 2, *E. faecalis* 8, *E. faecium* 9, *E. malodoratus* 1 and *E. solitaries* 4) could be inhibited only at ≥ 10.24 mg/mL of diclofenac sodium. In earlier studies too, diclofenac could inhibit *E. faecalis* strains at ≥ 50 mg/mL [22]. In the present study MIC of diclofenac sodium for *S. aureus* and *C. albicans* were 1.28 - 2.56 mg/mL and 1.28 mg/mL, respectively. In an earlier study on three reference strains each of *S. aureus* and *C. albicans* MIC of diclofenac has been reported 0.7 - 1.18 mg/mL and 0.02 - 0.09 mg/mL, respectively [23]. Another study reported MIC of diclofenac sodium equal to 10 mg/mL for a reference strain of *S. aureus* but at the same concentration strains of *C. albicans, E. coli* and *P. aeruginosa* remained unaffected [11]. The wide variation in MIC of diclofenac reported in different studies might be due to variation in types of strains and a few numbers of strains used in different studies. The present study revealed the cause of variation lucidly using a sizeable number of strains. Due to the lethal nephrotoxicity of diclofenac to vultures (scavengers of dead animals) with LD_{50} of 0.1 - 0.2 mg/Kg [24], its use in animals is prohibited. Even in human being a dose equivalent to 2.5g may induce lethal kidney failure [25,26]. Thus, the antibacterial activity of diclofenac sodium at a concentration of 10.24 mg/mL observed in the study might be of academic interest only and may not be of any therapeutic value.

Meloxicam inhibited 30.26% bacterial strains tested at 1.28 mg/mL concentration. The total human dose for an adult human is just 7.5 mg/day and higher dosages lead to serious toxicity [27] i.e.,

in therapeutic dosages, similar to other NSAIDs in the study, it is also of no use as antimicrobial. Although at 7.81 µg/ml concentration meloxicam is reported to inhibit biofilm formation by *P. aeruginosa* [28], in the present study none of the 9 strains of *P. aeruginosa* could be inhibited to grow at < 6.4 mg/mL and only one strain of *P. stutzeri* was susceptible to 0.2 mg/mL concentration of meloxicam. The MICs of meloxicam for *S. aureus* and *C. albicans* were 1.28 - > 1.28 mg/mL and > 1.28 mg/mL, respectively. The observations in the study are in concurrence to earlier studies on MICs of meloxicam on reference strains of *S. aureus* (0.44 - 1.23 mg/mL) and *C. albicans* (1.23 - 2.46 mg/mL) [23].

The difference in susceptibility of G+ve and G-ve bacteria and strains of different genera and species observed for aspirin, flunixin and diclofenac has also been reported earlier and might be due to different cell wall structure [11,12,23].

Although it is not lucid how aspirin and other NSAIDs can kill or restrains growth of bacteria, they are known to modulate the antimicrobial action of other antimicrobials. It is hypothesized that aspirin and other antipyretics induces changes in phenotypic resistance of bacteria by up- or down-regulating outer membrane proteins or efflux pumps, antibiotic targets, by inducing antibiotic degrading enzyme activity change in the surface hydrophobicity of bacteria to influence their biofilm production, interaction with the transport and release mechanism of antibiotics by killers cells of the host and inturn modify antimicrobial susceptibility of bacteria to antibiotics [12,29,30]. Antipyretics are also shown to alter the frequency of mutations thus affecting the emergence of antimicrobial drug-resistance [31,32]. Thus to understand the antimicrobial mechanism of antipyretics more studies are required for their more judicious mechanism based use as antimicrobials.

Although all NSAIDs and pheniramine maleate tested in the present study could not inhibit most of the microbial strains in therapeutically achievable systemic concentrations of the drugs within biological safety limits, the study achieved its goal to reveal the spectrum of antimicrobial effect of different drugs and scope for their use as antimicrobials in topical preparations to reduce antibiotic use. Besides, the susceptibility pattern of certain bacteria to specific NSAIDs may be useful in their differentiation from related genera and species provided more elaborate studies on more field isolates of bacteria are conducted. For example, all *P. mirabilis* strains were resistant while all *P. pantothenticus* strains were sensitive to flunixin, similarly, all *P. pantothenticus* strains were susceptible but most of the staphylococci were resistant to meloxicam.

Conclusion

Repurposing of drugs especially as an antimicrobial is an emerging area of research to mitigate antimicrobial drug resistance and develop antibiotic alternatives. This study on the evaluation of the antimicrobial potential of medicines commonly used as an adjunct to antibiotics (aspirin, paracetamol, flunixin meglumine, tolfenamic acid and diclofenac sodium and pheniramine maleate) on 499 strains including 475 field isolates and 24 reference strains belonging to 117 species of 36 genera concluded that the tested drugs possess antimicrobial activity but not in therapeutically achievable non-toxic concentrations. However, the broad spectrum antimicrobial activity of some of the molecules may be utilized in the development of a topical antimicrobial formulation to reduce topical antibiotic use.

Recommendations

Though not in systemically achievable non-toxic concentrations, NSAIDs posses broad spectrum antimicrobial activity which can be easily formulated into topically useful antimicrobials as some of the highly toxic antibiotics are used as powders, gels and ointments in clinical practice. Therefore, further studies may be conducted *in-vivo* for development of alternative antimicrobial preparations to reduce antibiotic use.

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