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RESEARCH ARTICLE

Evaluation of Antibacterial Potential of Raw Turmeric, Nano-Turmeric, and NSAIDs against Multiple Drug Resistant *Staphylococcus aureus* and *E. coli* Isolated from Animal Wounds

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A B S T R A C T

Wound healing gets difficult due to antibiotic resistance and requires prolong therapy. Current study was planned to check antibacterial potential of raw turmeric, nano-turmeric, and Non-steroidal anti-inflammatory drugs against multiple drug resistant (MDR) *Staphylococcus aureus* and *E. coli* isolated from animal wounds. Wound samples (n=150) were collected from different animals. Isolation and confirmation of *S. aureus* and *E. coli* was done by microbiological techniques and biochemical tests. Confirmed isolates were subjected to number of antibiotics for MDR *S. aureus* and *E. coli*. Drug modulation of MDR isolates were done by NT, RT, and NSAIDs in combination with antibiotics using well diffusion method. The data were analyzed by non-probability testing at 5% probability using SPSS statistical computer program. Study found 20.7 and 28.7% of MDR *E. coli* and *S. aureus* from animal wounds, respectively. MDR *S. aureus* were found 27.40, 33.30, and 100% sensitive while MDR *E. coli* were found 12.50, 62.50, and 75.00% against oxytetracycline, chloramphenicol, and trimethoprim-sulphamethoxozole, respectively. Nano curcumin showed higher antibacterial activity against MDR *S. aureus* and *E. coli* in comparison with raw curcumin. NSAIDs in combination with antibiotic also showed synergistic effect in inhibition of bacterial growth. Among the assumed risk factors external parasites, body condition and antibiotic use showed significant association. The study found higher prevalence of MDR *S. aureus* and *E. coli* from wounds with significant association of assumed risk factors, along with promising antibacterial effects of nano curcumin, raw curcumin, and NSAIDs in combination with antibiotics.

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INTRODUCTION

Microbial invasion of wound can delay or stop the repairing process and lead to life-threatening complications commonly present in patients. Repairing of wound infection is a basic tool in maintaining the integrity of tissue and for performing of functions. Presence of microbial flora over wound is a major factor in delayed wound healing (Mangoni *et al.*, 2016). The most common bacterial pathogens isolated from wound sites are *Escherichia coli*, *Staphylococcus aureus*, *S. pyogenes*, *Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp, *Enterobacter* spp and anaerobic spp such *Clostridium* (Upreti *et al.*, 2018). The increased rate of wound infections are caused by multi drug resistant bacteria predominately with *S. aureus* and *E. coli* which impose major health problems all over the world due to increase resistance to different classes of antibiotics (Worthington and Melander, 2013). Acquisition of drug resistance by these pathogenic strains has posed serious challenges for the remedy and management of wound infections around the world. The treatment of wound infections is being

more challenging due to antibiotic resistance and involvement of polymicrobial flora. Antimicrobial resistance (AMR) is creating a serious problem in all clinical settings and AMR has become the biggest public health threat globally (Steed *et al.*, 2014). Antibiotics are becoming ineffective day by day due to their extensive use with special context to non-judicial use. To combat AMR issue, new research experiments should be done to find the alternate ways for the better treatment options against these multi drug resistant bacterial pathogens. Non-steroidal anti-inflammatory drugs (NSAID's) such as carprofen, vedaprofen, celecoxib, bromfenac, aspirin and ibuprofen have shown antibacterial activity. Aspirin (anti-inflammatory agent) has been shown to inhibit the growth of *Klebsiella pneumoniae* and *Helicobacter pylori* and increase the antibacterial sensitivity of *Helicobacter pylori*. NSAID's are safe to use with their proven effects against bacteria. They have been reported synergistic effect with antibiotics against bacteria.

In addition, many herbs act as antibacterial as well as antioxidant (Bakr *et al*., 2020; Elghobashy *et al*., 2020). Turmeric, *Curcuma longa* (*C. longa)*, is among such herbs that is rich in vitamin C, E, and other biological active compounds that have therapeutically proven antimicrobial effect. Curcumin is obtained from *Curcuma longa* rhizome belonging to *Zinfiberaceae* family. Many studies have shown that turmeric and its derivatives can be used in treatment of various diseases such as autoimmune diseases, neurodegenerative disorders, cardiovascular diseases, metabolic diseases as well as healing of acute or chronic wounds. It has anti-inflammatory, antiviral, antiangiogenic, antifungal, antibacterial, anticancer and antioxidant properties. However, curcumin is less water soluble due to which it has short half-life and low bioavailability. Such characteristics limit its therapeutic efficacy that may be recovered by its modified form of nano turmeric. Nanoparticles of curcumin are prepared to increase bioavailability, controlled drug release and superior biocompatibility due to small particle size which increases the surface area (Hussain *et al.*, 2017).

Now a days, very few antibiotics are available to treat MDR *S. aureus* and *E. coli*. There is an immediate need for new antibiotics that can be used to treat resistant bacterial infections. Unfortunately, this aspect faces many challenges, as development of new antibiotics takes many years. One such approach is the use of non-antibiotics such as NSAIDs, and curcumin or nanocurcumin to re-sensitize the resistant bacteria to antibiotics. These non-antibiotics may act through mechanisms other than conventional antibiotics to enhance antibiotic activity or reduce antibiotic resistance. (Chockattu *et al*., 2018). Therefore, current study was planned to check the antibacterial potential of non-antibiotics such as NSAIDs, raw curcumin and nano curcumin against MDR *S. aureus* and *E. coli* isolated from wounds of different animal species.

MATERIALS AND METHODS

Sample collection: A total of n=150 swab samples were collected from wounds of different animals (n=22, Cat; n=25, Dog; n=25, Buffalo; n=36, Cattle; n=23, Calf; n=19, Buck) presented at outdoor clinic of Faculty of Veterinary Science, University of Agriculture, Faisalabad. The swab samples were dipped in sterilized phosphate buffer saline before sample collection. Collected samples were transported to microbiological laboratory of Department of Clinical Medicine, University of Agriculture, Faisalabad by maintaining the cold chain (4ºC). The inclusion criterion for sample collection was to include all animals with wounds on their skin presented to veterinary teaching hospital.

Isolation and identification of MDR *E. coli* and *S. aureus:* For better isolation of *Staphylococcus aureus* and *Escherichia coli*, the cotton swab samples were stored in nutrient broth at 37°C for 24 hours. Then, samples were swabbed on their selective media such as Mannitol Salt agar for *S. aureus* and MacConkey agar for *E. coli*. Microbiological and biochemical tests were performed to confirm *Staphylococcus aureus* and *E. coli*. Biochemically characterized isolates were put to various antibiotics belonging to different classes using the disk diffusion test. The isolates were swabbed on Mueller Hinton Agar (MHA) by adjusting with 0.5 McFarland turbidity standard and antibiotic disks were aseptically placed on MHA with the help of disk dispenser at equal distances and kept at 37ºC for 24 hours. The zones of inhibitions were measured in millimeters with the help of Vernier Calipers and compared with standard zones provided by (CLSI, 2016). The isolates showing resistance to ≥2 classes of antibiotics were considered as multiple drug resistant (MDR) *S. aureus* and *E. coli* (Upreti *et al.*, 2018).

Antibiogram of MDR *S. aureus* and *E. coli:* The MDR isolates of *E. coli* and *S. aureus* were put to various antibiotics susceptibility using oxytetracycline (30µg), oxacillin (1µg), chloramphenicol (30µg), vancomycin (30µg), amoxicillin (10µg), ampicillin (10µg), ciprofloxac (5µg), and cefoxitin (30µg) using the disk diffusion test (Fig 3a) according to guidelines of CLSI (2016). The freshly grown (24hr) bacterial cultures adjusted at 1.5×10^8 CFU/ml were swabbed on adjusted Muller Hinton agar (MHA) and incubated at 37ºC for 24 hours. The zone of inhibitions were measured and compared with standard zones provided in Clinical Laboratory and Standard Institute to declare resistant, sensitive and intermediate strains (CLSI, 2016).

In-vitro* drug modulation against MDR *S. aureus* and *E. coli

Nano-turmeric and suspension preparations: For this, grinded curcumin was milled in ball mill for 6 hours (top-down approach). Characterization of these nanoparticles was done using scanning electron microscopy (SEM) (Krausz *et al.*, 2015). 10mg, 1mg and 0.1 mg of curcumin (raw and nano) were dissolved in 1mL of Hydroxypropyl methylcellulose (HPMC) and then sonication was done and later vortex the solution for 5 minutes for homo-genous suspension (Gosangari and Dyakonov, 2013).

Antibacterial efficacy of nano turmeric, raw turmeric, NSAID's, and antibiotics alone and in combination: Antibacterial activity was checked by well diffusion method as shown in Fig. 3b, 3c & 3d, and MHA plates

were prepared and inoculum adjusted at 1.5×10^8 CFU/ml were swabbed on MHA agar plates. Wells were made with the help of sterile steel borer at equal distances. Firstly, different concentrations of turmeric, nano turmeric, NSAIDs, and antibiotic were prepared and dispensed into wells alone and incubated at 37ºC for 24 hours. Then zones of inhibition were measured using Vernier Caliper. Secondly, selected antibiotic (30 µg and 60 µg) in combination with NSAID (125µg and 250µg) at different concentrations were poured into wells and incubated at 37ºC for 24 hours and again zones of inhibition were measured and compared with each other.

Statistical analysis: Prevalence was determined using formula described by (Thrusfield, 2018).

$$\text{Prevalence } (\%) = \frac{\text{No. of infected Animal (n)}}{\text{Total no. of sampled Animals (N)}} \times 100$$

The descriptive statistics was applied for estimation of antibacterial assays, while risk factor analysis was analyzed by chi-square. However, independent t-test and one way ANOVA were used to find significance among different groups at 5% probability using SPSS version 22 statisttical computer program.

RESULTS

Prevalence of MDR *S. aureus* and *E. coli* from animal wounds: The present study overall found higher percentage 28.7% (43/150) of MDR *S. aureus* as compared to *E. coli* 20.7% (31/150) from the wounds of different animals. However, the prevalence of MDR *S. aureus* and *E. coli* were found non-significant (P> *0.05*) among the different animal species with higher percentage 44% (11/25) and 32% (8/25) of MDR *S. aureus* and *E. coli*, respectively was found in dog specie. The prevalence of MDR *S. aureus* were found in other species as calf 39.1% (9/23), cattle 25% (9/36), buffalo 24% (6/25), buck 21.1% (4/19), and cat 18.2% (4/22) while lower percentage of MDR *E. coli* was found in all species as mentioned in Table 1.

***In-vitro* therapeutic efficacy of various antibiotics against MDR *S. aureus* and *E. coli*:** The present study revealed that ciprofloxacin, trimethoprim-sulphamethoxazole, and amikacin showed 100% efficacy against MDR *S. aureus* while lower efficacy of ciprofloxacin (66.70%) and trimethoprim-sulphamethoxazole (62.50%) with equal percentage of amikacin (100%) was noted against MDR *E. coli*. Chloramphenicol and oxacillin showed higher efficacy (75% and 66.09%) against MDR *E. coli* as compared to MDR *S. aureus* (33.30% and 16.60%). However, MDR *S. aureus* and *E. coli* isolates showed 100% resistance to amoxicillin, with 100% and 80% resistance to ampicillin respectively. However, cefoxitin and oxytetracycline showed higher resistance 83.33% and 63.60% respectively against MDR *S. aureus* as compared to *E. coli* (71.42% and 50% respectively) while vancomycin showed 100% resistance against MDR *E. coli* with lower percentage against MDR *S. aureus* 67.80% (Table 2).

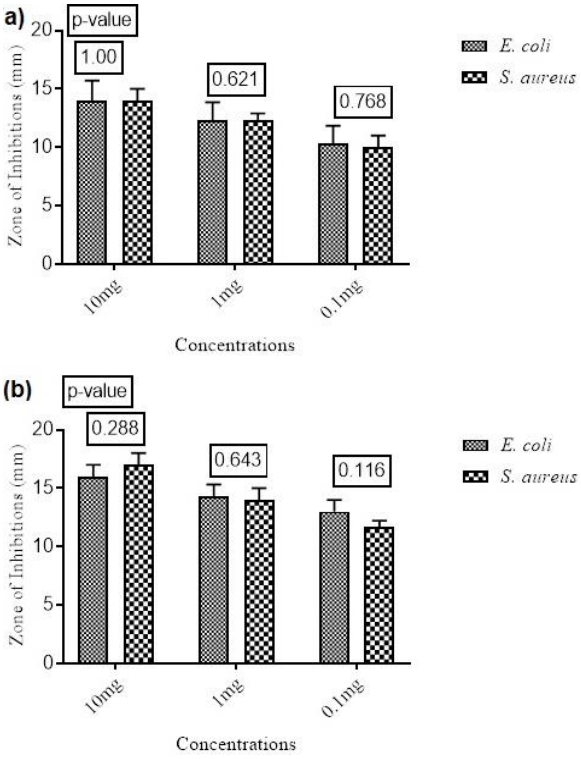

Fig. 1: Response of different turmeric preparations against MDR *E. coli* and *S. aureus*. a) Raw Turmeric b) Nano Turmeric.

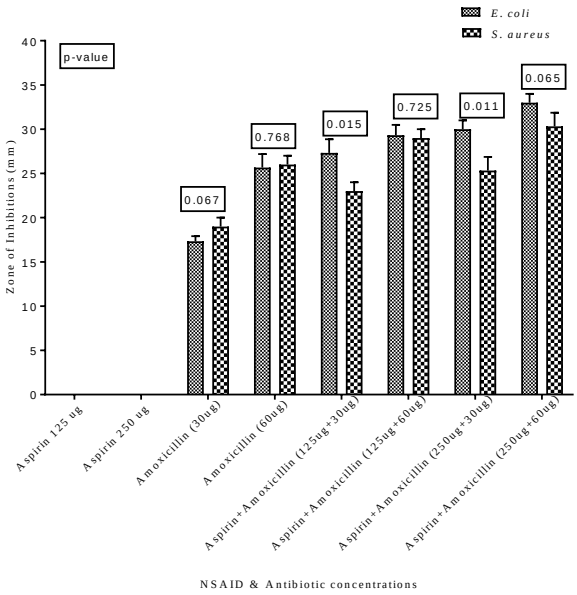

Fig. 2: Response of NSAID and antibiotic alone and in combination against MDR *S. aureus* and *E. coli.*

Efficacy of raw turmeric and nano-turmeric at different concentrations against MDR *S. aureus* and *E. coli*: The therapeutic efficacy of raw turmeric and nano turmeric at different concentrations in terms of zones of inhibition (ZOI) were observed against MDR *S. aureus* and *E. coli*. Nano-turmeric showed higher ZOIs at all concentrations against MDR *S. aureus* and *E. coli* (Fig. 1a & 1b). The present study revealed MDR *S. aureus* and *E. coli* showing almost same response with non-significant difference (P>0.05) in terms of zone of inhibitions (ZOI) at all concentrations of raw turmeric except at 0.1mg

concentration where MDR *E. coli* showed slightly greater ZOI as compared to MDR *S. aureus* while in case of nano turmeric the MDR *E. coli* showed slightly higher response with non-significant difference (P>0.05) in terms of ZOI at all concentrations except at 10mg concentration where less response was observed as compared to MDR *S. aureus*. MDR *S. aureus* and *E. coli* showed significant difference (P<0.05) within each treatment both in case of raw turmeric and nano turmeric except MDR *E. coli* that showed non-significant difference (P>0.05) in case of raw turmeric (Fig. 1a & 1b).

Efficacy of NSAID and antibiotic alone and in combination against MDR *S. aureus* and *E. coli*: The response of NSAID (aspirin) and antibiotic (amoxicillin) alone and in combination at different concentrations in terms of zone of inhibitions (ZOI) against MDR *S. aureus* and *E. coli* isolates were directly proportional to concentration used. The MDR *S. aureus* and *E. coli* showed significant (P<0.05) higher ZOI at all concentrations against NSAID+antibiotic in combination as compared to NSAID and antibiotic alone. However, antibiotic alone showed higher ZOI at all concentrations in comparison with NSAID alone that showed no ZOI against MDR *S. aureus* and *E. coli* (Fig. 2). The higher antibiotic concentration (60ug) showed higher zone of inhibition as compared to lower antibiotic concentration (30ug) both in case of MDR *S. aureus* and *E. coli*. Similar pattern was observed in case of combination of NSAID+antibiotic. The MDR *S. aureus* showed less response with significant difference (P<0.05) at NSAID 125ug+antibiotic 30ug and NSAID 250ug+antibiotic 30ug concentrations while non-significant difference (P>0.05) at NSAID 125ug+antibiotic 60ug and NSAID 250ug+antibiotic 60ug concentrations as compared to MDR *E. coli* in relation to ZOI at all combinations of NSAID and antibiotic (Fig. 2).

Risk factors analysis: The assumed determinants studied for the prevalence of MDR *S. aureus* and *E. coli* from wounds of different animal species showed significant association (P<0.05) related to presence of external parasites and type of treatment approach while all other factors such as specie, sample site, sex, body condition, any other infection, vaccination, and previous antibiotic use showed non-significant association (P>0.05) in case of MDR *S. aureus* while in case of MDR *E. coli*, presence of external parasites, body condition, and previous antibiotic use showed significant association (P<0.05) for acquiring the *E. coli* while all other showed non-significant association. Among the non-significant risk factors, dog's species, parasitic wounds and bite wounds, female sex, presence of any other infection and absence of infection, vaccinated and non-vaccinated animals showed higher percentage of MDR *S. aureus* and *E. coli*, respectively, than their all-other respective categories. However, overall surgical wound site, presence of external parasites, vaccinated animals and self-treatment approach levels presented significant association (P<0.05) in acquiring both MDR *S. aureus* and *E. coli* while all other levels showed non-significant association (Table 5).

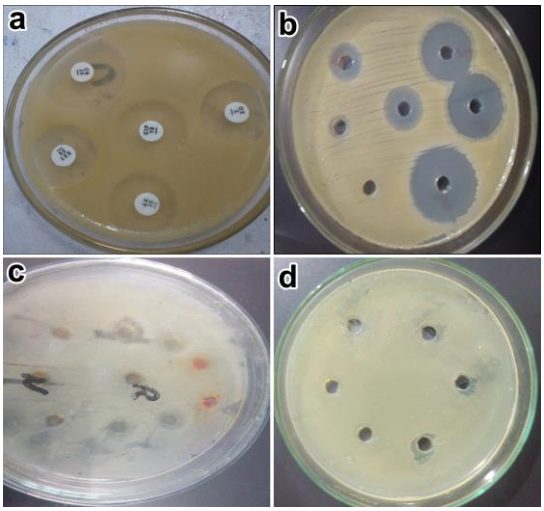

Fig. 3: Zone of inhibitions of different preparations against MDR *S. aureus* and *E. coli* a) antibiotics alone b) NSAID in combination with antibiotic c) nano-turmeric d) raw turmeric.

Table 1: Prevalence of MDR *S. aureus* and *E. coli* isolated from wounds of different animals

Sample sources	MDR *Staphylococcus aureus*					MDR *E. coli*				
	Total	Positive	Percentage (%)	C.I	P-*value*	Total	Positive	Percentage (%)	C.I (95%)	P-*value*
Cat	22	4	18.2	0.0731-0.3851		22	3	13.6	0.0475-0.3334	22qq246712www56
Dog	25	11	44.0	0.2667-0.6293		25	8	32.0	0.1573-0.5355	
Cattle	36	9	25.0	0.1375-0.4107		36	7	19.4	0.0975-0.3502	
Buffalo	25	6	24.0	0.1150-0.1150		25	3	12.0	0.0417-0.2996	
Calf	23	9	39.1	0.2216-0.5921		23	7	30.4	0.156-0.5086	
Buck	19	4	21.1	0.0851-0.4333	0.275	19	3	15.8	0.0552-0.3757	
Total	150	43	28.7	-	-	150	31	20.7		-

P<0.05 indicate significant difference, CI=confidence interval.

Table 2: *In-vitro* efficacy of various antibiotics against MDR *S. aureus* and *E.coli* isolated from wounds of different animals

Antibiotics	Resistant %			Intermediate %			Sensitive %		
	S. aureus	*E. coli*	*p-value*	*S. aureus*	*E. coli*	*p-value*	*S. aureus*	*E. coli*	*p-value*
Cefoxitin	83.33	71.42	0.044	0.000	0.000	N/A	16.67	28.58	0.044
Oxacillin	66.70	33.91	0.000	16.60	0.000	0.000	16.70	66.09	0.000
Vancomycin	67.80	100.0	0.000	32.20	0.000	0.000	0.000	0.000	N/A
Ampicillin	100.0	80.00	0.000	0.000	0.000	N/A	0.000	20.00	0.000
Chloarmphenicol	0.000	25.00	0.000	66.70	0.000	0.000	33.30	75.00	0.000
Amoxicillin	100.0	100.0	N/A	0.000	0.000	N/A	0.000	0.000	N/A
Ciprofloxacin	0.000	33.30	0.000	0.000	0.000	N/A	100.0	66.70	0.000
Oxytetracycline	63.60	50.00	0.046	9.000	37.50	0.000	27.40	12.50	0.012
Trimethoprim-Sulphmethoxazole	0.000	25.00	0.000	0.000	12.50	0.000	100.0	62.50	0.000
Amikacin	0.000	0.000	N/A	0.000	0.000	N/A	100.0	100.0	N/A

P<0.05 indicate significant difference.

Table 3: Risk factors' association with acquisition of MDR *Staphylococcus aureus* and *E. coli* isolated from wounds of different animals

Variable	Levels	Total	MDR *Staphylococcus aureus*						MDR *E. coli*						*E. coli* and *S. aureus*
			Positive	(%)	Negative	(%)	C.I	*P-value*	Positive	(%)	Negative	(%)	C.I	*P-value*	*p*-value
Species	Cat	22	4	18.2	18	81.8	0.0731-0.3851	0.275	3	13.6	19	86.4	0.0475-0.3334	0.367	0.680
	Dog	25	11	44.0	14	56.0	0.2667-0.6293		8	32.0	17	68.0	0.1573-0.5355		0.382
	Cattle	36	9	25.0	27	75.0	0.1375-0.4107		7	19.4	29	80.6	0.0975-0.3502		0.571
	Buffalo	25	6	24.0	19	76.0	0.1150-0.1150		3	12.0	22	88.0	0.0417-0.2996		0.269
	Calf	23	9	39.1	14	60.9	0.2216-0.5921		7	30.4	16	69.6	0.156-0.5086		0.536
	Buck	19	4	21.1	15	78.9	0.0851-0.4333		3	15.8	16	84.2	0.0552-0.3757		0.676
Site of Sample	Dehorn wounds	30	11	36.67	19	63.33	0.2188-0.5449	0.274	9	30.00	21	70.00	0.1666-0.4788		0.584
	Bite wounds	24	5	20.83	19	79.17	0.0924-0.4047		8	33.33	16	66.67	0.1797-0.5329	0.073	0.330
	Parasitic wounds	18	8	44.44	10	55.56	0.2456-0.6628		5	27.78	13	72.22	0.125-0.5087		0.298
	Ear infection	22	4	18.18	18	81.82	0.0731-0.3851		2	9.09	20	90.91	0.0253-0.2781		0.380
	Surgery	56	15	26.78	41	73.22	0.1696-0.396		7	12.5	49	87.5	0.0619-0.2363		0.057
Sex	Male	80	22	27.50	58	72.50	0.1892-0.3814	0.279	15	18.75	65	81.25	0.1171-0.2866	0.535	0.189
	Female	70	25	35.71	45	64.29	0.2550-0.4741		16	22.85	54	77.15	0.1459-0.3395		0.095
External parasites	Yes	55	30	54.54	25	45.46	0.4232-0.7541	0.000	18	32.72	37	67.28	0.2182-0.2182	0.025	0.021
	No	95	13	13.68	82	86.32	0.0817-0.2201		16	16.80	79	83.20	0.1064-0.2562		0.545
Body Condition	Normal	79	19	24.05	60	75.95	0.1597-0.3453	0.187	10	12.65	69	87.35	0.0702-0.2176	0.004	0.064
	Weak	71	24	33.80	47	66.20	0.2388-0.4538		23	32.39	48	67.61	0.2265-0.4393		0.858
Any other infection	Yes	90	28	31.11	62	68.89	0.2248-0.4128	0.417	17	18.88	73	81.12	0.1214-0.2818	0.510	0.058
	No	60	15	25.00	45	75.00	0.1578-0.3723		14	23.33	46	76.67	0.1444-0.3543		0.831
Vaccination	Yes	85	26	30.58	59	69.42	0.2181-0.4105	0.552	15	17.64	70	82.36	0.11-0.271	0.296	0.049
	No	65	17	26.15	48	73.85	0.1702-0.3795		16	24.61	49	75.39	0.1576-0.3631		0.840
Previous use of antibiotic	Yes	130	28	21.53	102	78.47	0.1534-0.2937	0.794	18	13.84	112	86.16	0.0894-0.2083	0.000	0.104
	No	20	15	60.0	5	40.0	0.5313-0.8881		13	65.00	7	35.0	0.4329-0.8188		0.490
Treatment approach	Self	45	20	44.44	25	55.56	0.3093-0.5882	0.005	11	24.44	34	75.56	0.1423-0.3867	0.636	0.046
	Vet visit	105	23	21.90	82	78.10	0.1506-0.3072		22	21.00	83	79.00	0.1426-0.2969		0.866

DISCUSSION

Now, multidrug resistance is a worldwide issue, the current study found an overall 28.7% prevalence of MDR *S. aureus* from different animal species that is lower than reported by (Yadav *et al.*, 2018) who reported 40% prevalence of MDR *S. aureus*. Current study found 20.7% prevalence of MDR *E. coli* that is much higher than the study conducted by (Upreti *et al.*, 2018) who found 8.6% prevalence of MDR *E. coli* from wound infections at territory care hospital of Nepal with the most predominant bacteria was found to be MDR *S. aureus* (56.9%). Another study conducted by (Nolff *et al.*, 2016) found 48% MDR bacteria, predominately *E. coli* and staphylococcus species during open wound management. The variation may be due to type of sample, geographical area, wound site, type of wound and specie etc.

The current study revealed that MDR *S. aureus* and *E. coli* isolates showed 100% resistance to amoxicillin, with 100 and 80% resistance to ampicillin respectively. However, cefoxitin and oxytetracycline showed higher resistance 83.33 and 63.60% respectively against MDR *S. aureus* as compared to *E. coli* (71.42 and 50% respectively) while vancomycin showed 100% resistance against MDR *E. coli* with lower percentage against MDR *S. aureus* 67.80%. A study conducted by (Tadesse *et al.*, 2018) found *S. aureus* isolates were 100% resistance to ampicillin, 68.4% to oxacillin and cefoxitin, 57% to tetracycline. Another study conducted by (Hasan *et al.*, 2016) found 37.93% *S. aureus* isolates from burn wound infections were resistant to vancomycin. Another study conducted by (Alharbi *et al.*, 2019) found that more than 50% of *E. coli* isolates were resistant to ampicillin, tetracycline, cefazolin, ciprofloxacin and moxifloxacin.

The efficacy of nano-curcumin and raw curcumin against MDR *S. aureus* and *E. coli* in current study showed higher antibacterial activity against gram positive bacteria as compared to gram negative bacteria which is in line with

findings of previous study (Zorofchian Moghadamtousi *et al.*, 2014). The difference in antibacterial activity may be due to difference in cell membrane structures. Gram positive have an outer peptidoglycan layer while Gram negative have phospholipid layer, both of which undergo different types of mechanisms when interact with curcumin (Basniwal *et al.*, 2011). Another study conducted by Tyagi *et al.* (2015) demonstrates that curcumin is equally effective against gram positive and gram negative bacteria. Furthermore, present study found nano curcumin showed higher antimicrobial efficacy as compared to curcumin against both pathogens which is in line with the study reported by Shome *et al.* (2016). The enhanced antibacterial activity of nanocurcumin is because of its higher solubility and dispensability in aqueous phase. The mechanism involved in antibacterial activity is accumulation of reactive oxygen species (ROS) that destroy the permeability and confirmation of membrane leading to destruction of bacterial cell. Similarly, another study conducted by Shome *et al.* (2020) found higher antibacterial activity of nano curcumin against *S. aureus* and *E. coli* as compared to curcumin.

The study found no antibacterial activity of NSAIDs alone against both MDR *S. aureus* and *E. coli* which contradicts with the findings of Chan *et al.* (2017). Moreover, present study found higher antimicrobial activity of antibiotics in combination with non-steroidal anti-inflammatory drugs (NSAIDs) that is in line with findings of Ahmed *et al.* (2017). The present study found higher antibacterial activity of NSAID and antibiotic in combination against gram negative bacteria in contrast to gram positive bacteria that is contraindicated with study conducted by Chan *et al.* (2017). Chan *et al.* (2017) also found that antibiotics in combination with NSAIDs exhibit higher antibacterial activity and reduces the minimum inhibitory concentration (MIC). Many other studies reported about synergistic effects of antibiotics with NSAIDs and revealed that they are equally effective against gram positive and gram-negative bacteria. Several studies showed that NSAIDs have antibacterial activity, and acts through different mechanisms but the exact mechanism is not known. Some studies have proposed NSAIDs inhibits the synthesis of DNA of bacteria, impairing membrane activity, altering genes which encode transport, down regulation of efflux pumps, reduced quorum sensing-controlled motility leading death of bacterial cell (Chockattu *et al*., 2018).

Conclusions: The study concluded that higher prevalence of multiple drug resistant *S. aureus* and *E. coli* were found from wound infections of different animal species. The study found higher sensitivity of ciprofloxacin, trimethoprim-sulphamethoxazole and amikacin against MDR *S. aureus* and *E. coli* with higher resistance to ampicillin, amoxicillin, oxytetracycline and vancomycin. Non-steroidal anti-inflammatory drugs (NSAIDs) in combination with antibiotics showed higher antibacterial potential as compared to their alone effects. Nano curcumin exhibited higher antibacterial activity as compared to raw curcumin. The study found promising antibacterial potential of NSAIDs, raw curcumin and nano curcumin against highly pathogenic MDR *S. aureus* and *E. coli*.

Authors contribution: IS, AA, AM and AIA conceived and designed the study. IS, MIS, AAB, ZAB, MFAK, MAN and AI executed the experiment and analyzed the sera and tissue samples. MS analyzed the data.

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