

Original Research Article

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Study of Antimicrobial Sensitivity Pattern of Bovine Mastitis using Various Tea Extracts

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ABSTRACT

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In the present study 105 numbers of microbial isolates were isolated from 96 milk samples and were processed as per routine method of microbial isolation. Various types of tea extracts like black tea and green tea were used in combination with antibiotic discs obtained from HiMedia, Mumbai. The synergistic effects of antibiotics and tea extracts against resistant bovine pathogens lead to new choices for development of combination therapy during chronic mastitis. In the present study methanol was used as solvent for green and black tea samples in various concentrations. It is revealed that the tea extracts in combination with chloramphenicol, tetracycline, levofloxacin and gentamicin enhanced the zone of inhibition in comparison to individual therapy. In order to establish the above finding a more elaborative study on mastitogenic microbial isolates the antimicrobial synergistic testing is needed.

Introduction

The incidence of clinical and subclinical mastitis in cross bred cows is a common problem for coastal and remote areas of Odisha in summer season. Bovine mastitis is an inflammatory condition of mammary gland

most often caused by bacterial intramammary infection. The principal pathogens causing mastitis are pathogenic *Staphylococcus* spp. and *Streptococcus* spp. (Boonyayatraet *al.*, 2014). It adversely affects milk production and causes widespread economic losses. The subclinical mastitis has been 15-40 times more

prevalent than the clinical mastitis (Sudhan and Sharma, 2010). And controlling subclinical mastitis can substantially reduce 75% of economic losses in milk production (Constable *et al.*, 2017).

Clinical and subclinical cases of mastitis are routinely treated with antimicrobial therapy both intramammary and parenterally. The use of antimicrobials over long period has triggered the development of multidrug resistant strains, which has resulted in the use of increasing dose of antimicrobials, causing the danger of increasing amount of drug residues in the milk, a potential biohazard (Dhanabalan *et al.*, 2008). In some MDR cases, the use of combination of the antibiotic drugs with different bioactive compounds is the possible option for the treatment (Bag and Chattopadhyay, 2015). The combination therapy of antibiotics and herbs or beverages can be used to improve the antimicrobial spectrum, to prevent the emergence of the resistant mutants, to minimize toxicity and to obtain synergistic antimicrobial activity (Pankey and Ashcraft, 2005). Some studies (Braga *et al.*, 2005, Dickson *et al.*, 2006 and Gibbons *et al.*, 2003) have reported that plant extracts can increase the in-vitro activity of antibiotics against MDR strains of *Staphylococcus aureus* and other pathogens.

Tea extract from the leaves of plant *Camellia sinensis* has been shown to have wide range of antioxidant, anti-inflammatory, anti-carcinogenic and antibacterial activity against many pathogens (Hamilton-miller, 1995). This antimicrobial activity of the tea extract is due to the presence of active ingredient catechin mainly epigallocatechingallate, EGCG (Song and Seong, 2007).

In the present study, antibiogram study of field isolates and evaluation of antimicrobial effects of tea (*Camellia sinensis*) extracts on bovine Mastitogenic isolates was conducted.

Materials and Methods

Sample collection

In this study a total of 96 milk samples were obtained from TVCC of C.V.Sc. & A.H. Bhubaneswar, A.D.R.I. Phulnakhara and other organized dairy farms. The milk samples were collected aseptically from the affected quarter and found positive for mastitis through Modified Californian Mastitis Test.

Bacterial isolation and identification

The isolation of the bacteria was done by growth on selective media. The selective media used were Mannitol Salt Agar for Staphylococci, Edwards' Modified Blood Agar for Streptococci (Buxton and Fraser, 1977), *Pseudomonas* Isolation Agar for *Pseudomonas* spp. (Figure 1). The test isolates were then identified by various biochemical and sugar fermentation tests. For the isolation and identification of Enterobacteriaceae the method outlined by Edwards and Ewing (1972) was followed (Figure 2 and 3). The biochemical tests performed to identify the isolates were Indole, MR, VP, Citrate utilization test, H₂S production test (Cruickshank *et al.*, 1980). And hemolysis, coagulase test (Merchant and Packer, 1967) and motility test were also performed.

Preparation of antibiotic and tea discs

The detail procedure of Esimone *et al.*, (2003) was followed with slight modification. According to the procedure circular filter paper discs (6mm diameter) were prepared with the aid of an office paper perforator. The discs were placed in a petri dish and sterilized in an autoclave. Solutions of the methanolic tea extract of different concentrations (5, 10, 15, 25, 30, 35, 40, 45, 50 mg/ml) and a suitable antibiotic were made in different test tubes. The paper discs were then aseptically

transferred into the tubes containing the drugs' solutions and allowed to absorb the solution for about 15 seconds. The discs were then aseptically transferred to empty sterile tubes and allowed to air dry while the mouth of the test tube is still plugged with cotton wool.

Each of the test tubes containing the dried discs was well labelled with the strength of the solution of the drug in which the paper discs were dipped. It had been established that the 6mm disc has a capacity of absorbing 10 µl of drug solution at saturation.

Antibacterial susceptibility test

The antibacterial activity of various antibiotics against the mastitogenic isolates obtained were assayed by disk diffusion method according to Bauer *et al.*, (1996) and zone of inhibition is recorded. For the test Mueller Hinton Agar Media (HiMedia, Mumbai) was used.

The antibiotics discs used were Tetracycline (30 mcg), Ampicillin (10 mcg), Neomycin (30 mcg), Erythromycin (15 mcg), Penicillin (10 units), Ciprofloxacin (5 mcg), Gentamicin (10 mcg) and Doxycycline (30 mcg). The antibiotic discs were obtained from HiMedia, Mumbai.

In order to know the combined effects of the antibiotics along with methanolic tea extracts, the above prepared discs were used (Figure 4).

Results and Discussion

In this study, a total of 105 bacterial isolates were recovered from 96 milk samples. The predominant isolates recovered were *Staphylococcus aureus* and *Escherichia coli* followed by *Streptococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. It was revealed

that 85% of Coliform spp. Isolates were highly sensitive to Gentamicin. For *Streptococcus* spp. tetracycline was found to be 75% sensitive like was for *Staphylococcus* spp. Ciprofloxacin (75%) for *Proteus* spp. Tetracycline (70%), for *Corynebacterium* spp. both the antibiotics Ampicillin (86.5%) and Ciprofloxacin (85%) respectively (Table 1).

The crude methanolic extract of green tea and black tea when mixed with Chloramphenicol showed good results at the concentrations of 25-50 mg/ml but it gave poor results at other lower concentrations.

The synergistic activity of crude methanolic extracts of green tea and black tea in combination with Levofloxacin, Tetracycline and Gentamicin showed best activity against *Staphylococcus aureus* at all concentrations (Table 2). The synergistic combination showed better results as compared to the green tea and black tea alone.

This study has shown that crude extract of the leaves of *Camellia sinensis* exhibits potentials of synergy in combination with some antibiotics against pathogenic bacteria often presenting problems of drug resistance.

Such synergistic attributes of crude extracts of *Camellia sinensis* leaves and antibiotics demonstrates that this plant possesses antibiotic resistance modifying compounds.

It is concluded that tea extract did not impair the antimicrobial properties of antibiotics; rather, it enhanced its activity in an additive manner which could be beneficial in the treatment of staphylococcal infections. The synergistic effect from the association of antibiotic with tea extracts against resistant bacteria leads to new choices for the treatment of mastitis.

Table.1 Sensitivity pattern of bacterial isolates that was isolated from mastitic cows to different antibiotics

Antibiotics	Dose	Coliform spp.	Strepto. spp.	Staph. spp.	Proteus spp.	<i>Corynebacterium</i> spp.
Tetracycline(T)	30 mcg	52.5%	75%	71.8%	70%	85%
Ampicillin(A)	10 mcg	3%	69%	79%	0%	86.5%
Neomycin(N)	30 mcg	12%	13.5%	24.6%	11.5%	12.5%
Erythromycin(E)	15 mcg	9%	81%	34%	49%	60.5%
Penicillin G(P)	10 units	3%	54.4%	72%	0%	80%
Ciprofloxacin(CF)	5 mcg	73%	70%	75%	25%	85%
Gentamicin(G)	10 mcg	85%	60%	65%	15%	75%
Doxycycline(DO)	30 mcg	12%	10%	13%	29%	11%

Table.2 Synergistic Antibacterial activity of crude methanolic extract of green tea and black tea samples with Chloramphenicol, Tetracycline, Levofloxacin and Gentamicin antibiotics

Sl. No.	Conc. (mg/ml)	Zone of inhibition (mm) of green and black tea							
		Chloramphenicol		Tetracycline		Levofloxacin		Gentamicin	
		Green tea	Black tea	Green tea	Black tea	Green tea	Black tea	Green tea	Black tea
1	5	-	12	16	15	17	18	14	15
2	10	-	-	18	18	17	18	16	16
3	15	-	-	17	16	13	12	14	12
4	20	-	-	19	17	18	17	15	15
5	25	14	13	16	18	18	19	16	14
6	30	13	12	17	15	16	15	16	15
7	35	17	15	18	18	17	18	17	16
8	40	17	15	16	16	19	19	17	15
9	45	15	14	16	17	19	19	18	16
10	50	18	17	19	18	19	18	19	18

Fig.1 *Pseudomonas* sp. colonies on *Pseudomonas* Isolation Agar (PIA)



Fig.2 *E. coli* colonies on MacConkey Lactose Agar (MLA)



Fig.3 *E. coli* colonies grown on EMB Agar



Fig.4 Zone of inhibition against *Staphylococcus aureus* using combination of tea extract at different concentration with antibiotics



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