

ANTIMICROBIAL ACTIVITIES OF COW DUNG EXTRACTS AGAINST HUMAN PATHOGENS

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ABSTRACT

Objective: For control of microbial infections and diseases, various synthetic drugs and chemical formulations are currently in use. But due to the problem of microbial drug resistance, new alternative synthetic drugs have been explored. Similarly, antimicrobial activities of so many natural products have also been explored.

Methods: In this various study extracts of cow dung possessed antimicrobial property against human pathogens like *Klebsiella pneumonia* and *Escherichia coli*.

Results: The Indian cow dung extracted possessed superior antimicrobial activity than other cow dung types and showed antimicrobial property against all the test microorganisms. Since cow dung and buffalo dung are abundant in nature, which make the process cost effective with processing ease and thus are a promising solution for a variety of health problems in the near future.

Conclusion: The medicinal properties of these cow dung and buffalo dung can be exploited to formulate drugs for several diseases caused by antibiotic resistant pathogenic microorganisms.

Keywords: Cow dung, *Klebsiella pneumonia*, *Escherichia coli*, Antimicrobial activity

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INTRODUCTION

In India, cattle's rearing is a tradition in the country and intimately limited to the agricultural economy. Different products obtained from cow milk, ghee, curd, urine, and dung are used widely in a number of ayurvedic formulations. Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. The addition of cow dung increases the mineral status of soil, enhances the resistance of plant against pests and diseases; stimulate plant growth and other beneficial activities such as sulphur oxidation and phosphorus solubilization [1].

The Hindu Vedas say that the cow is holy and should be worshiped. In India, cows are very important animal resources and are highly useful in agriculture and dairy industry [2]. Panchagavya is a term used to describe five major substances, obtained from cow, which include cow's urine, milk, ghee, curd and dung. All the five products possess medicinal properties against many disorders. This kind of treatment is called Panchagavya therapy or cowpaths [3]. Cowpathy is an old system of medicine mentioned in ancient Indian literature (Ayurveda) as *Panchagavya Chikitsa*. The ayurvedic medicines of animal origin are mainly prepared from *Panchagavya* which boost up the body immune system and makes the body refractory to various diseases [4]. Although some Indian literature mentioned the medicinal property of cow excretion, only a few were proved. Several useful properties of cow urine got confirmed by researchers patent also. But there is no report available on antimicrobial activity of cow dung.

Cow dung is basically the rejects of herbivorous matter which is acted upon by symbiotic bacteria residing within the animal's rumen. The resultant faecal matter is rich in minerals. Cow dung is the undigested residue of plant matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. Cow dung is comprised of organic matter including fibrous material that passed through the cow's digestive system, among other liquid digesta that has been left after the fermentation, absorption and filtration, then acidified, then absorbed again. The chemical composition mostly carbon, nitrogen, hydrogen, oxygen, phosphorus, etc. with salts, cells sloughed off as the digesta went through the digestive tract, some urea, mucus, as well as cellulose, lignin and hemicellulose.

The addition of cow dung increases the mineral status of soil, enhances the resistance of plant against pests and diseases; stimulate plant growth and other beneficial activities such as sulphur oxidation and phosphorus solubilization. Normally, Composition of cow dung is about 80% water and supports a matrix of undigested plant material that is rich in nutrients, micro-organisms, and their byproducts. Cow dung microflora contains an abundant number of bacilli, lactobacilli and cocci and some identified and unidentified fungi and yeasts [5].

Escherichia coli, is a Gram-negative, facultative anaerobic and non-sporulating bacteria. *Escherichia coli* use mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide [6]. It is a common kind of bacteria that lives in the intestines of animals and humans and most are harmless. Eating unwashed greens such as spinach, lettuce or green onions or undercooked beef can cause the infection [7]. The spores are heat, chemical and pH resistant, the extracts were passed through a membrane filter (Millipore corp; 47 mm diameter; 0.2 µm pore size). Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *Escherichia coli* vary widely. Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms [8].

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines [9]. *Klebsiella pneumoniae* tends to affect people with underlying diseases, such as alcoholism, diabetes and chronic lung disease. Multiple-resistant *Klebsiella pneumoniae* have been killed *in vivo* via intra-peritoneal, intravenous or intranasal administration of phages in laboratory tests. While this treatment has been available for some time, a greater danger of bacterial resistance exists to phages than to antibiotics [10].

With this view, this study focused on the antimicrobial activities of dung extracts of Indian and imported cows and buffalos against human pathogens.

MATERIALS AND METHODS

Cow dung collection

Different cow dung (Indian cow, Jersey, *Holstein*) and buffalo dung were collected in and around Dharmapuri District.

Culture collection

The two-gram negative pathogenic strains namely *Escherichia coli* and *Klebsiella pneumonia* samples were collected from Gopi hospital; Salem Were collected for this study.

Powdered cow dung

- 1000g of cow dung from Indian cow (Dharmapuri) was collected and shadow dried for 5 d. The moisture content of the cow dung was lower than the other types. The dried cow dung was powdered. The powdered material had a net weight of 290g.
- 1000g of cow dung from *Jersey* was collected and shadow dried for 5 d. The moisture content was high when compared to cow dung from Indian cow. The dried cow dung was then powdered. The powdered material had a net weight of 250g.
- 1000g of cow dung from *Holstein* was taken and shadow dried for 5 d. The moisture content was high when compared to cow dung from *Sindhu*. The dried cow dung was then powdered. The powdered material had a net weight of 220g.
- 1000g of buffalo dung was taken and shadow dried for 5 d. The moisture content was high when compared to cow dung from *Jersey*. The dried buffalo dung was then powdered. The powdered material had a net weight of 190g.

Preparation of cow dung extracts

100 ml of acetone and ethanol was added in 10 g of powdered different cow dungs (Indian cow, Jersey, Holstein and buffalo dung) in a conical flask and it was kept in a rotary shaker for 3 d. The extract was then filtered using Whatman No 1 filter paper and stored in a vial for future use.

Preparation of the disc containing cow dung extract

The empty discs were impregnated with 50µl (2 mg/disc) of acetone extracts of cow dung from Indian cow, Jersey, Holstein and buffalo dung separately and dried in the oven. Similarly, the empty discs were impregnated with 50µl (2 mg/disc) of ethanol extracts of cow dung from Indian cow, Jersey, Holstein and buffalo dung separately and dried in the oven. This process was repeated until the disc was completely saturated with the extract. The disc was then used to study the antimicrobial activity of cow dung extracts against human pathogens [11].

Antibiotic sensitivity test

Kirby-Bauer method also known as disc diffusion antibiotic sensitivity testing is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. It is based on the observation that the degree of inhibition of bacterial growth on agar medium surrounding an antimicrobial compound containing disc correlates with susceptibility to the agent. The zone of inhibition determines whether the organism is sensitive, resistant or intermediate to a particular antibiotic or the antimicrobial compound.

Four to five similar colonies of identified organism from pure culture plates were transferred into the nutrient broth and incubated at 37 °C for 24 h. To determine the antimicrobial sensitivity, the inoculum was spread on the entire surface of the Mueller-Hinton agar plates with the sterile cotton swab. The commercially available antibiotic discs and one disc containing the antimicrobial compound were gently pressed onto the microbe carpeted plate at a distance 15 mm away from the edge and 24 mm apart from each other and incubated at 37 °C overnight (18-24 h). The diameter of the zone of bacterial growth inhibition around each disc was measured and the susceptibility or resistance to the agent in each disc was determined according to the standardized table provided by the Hi-media Laboratories, Mumbai. Antibiotics used were Ampicillin, Amikacin, Chloramphenicol, Gentamycin, Ofloxacin, Vancomycin, Methicillin and Penicillin-G (5).

Phytochemical screening of cow dung extract

The ethanol and acetone extract of *cow dung* were used for phytochemical screening. [12, 13]

Test for flavonoids

Lead acetate test

To 0.5 ml of the extract, a few drops of lead acetate solution were added. The yellow color precipitate was formed in the presence of flavonoids.

Test for glycosides

A small amount of the cow dung extract was dissolved in 1 ml of water, and then aqueous 10% Sodium hydroxide solution was added. Formation of yellow color indicated the presence of glycosides.

Test for steroids

Salkowski test

To 2 ml of extract, added 2 ml of chloroform and 2 ml of concentrated H₂SO₄ and shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence, in the presence of steroids.

Test for tannins

To 5 ml of the extract, 1 ml of 10% lead acetate solution was added. Formation of yellow precipitate showed the presence of tannins.

Test for phenols

A small quantity of the extract was dissolved in 0.5 ml of 20% Sulphuric acid solution. Followed by addition of a few drops of 2% Sodium hydroxide solution, it turned blue in the presence of phenols.

RESULTS AND DISCUSSION

Antimicrobial activity

Antimicrobial activity of cow dung from Indian cow

Indian cow dung had possessed superior antimicrobial activity than cow dung. All the test microorganisms were sensitive to the Indian cow dung. Ethanol extract of the Indian cow dung had shown antimicrobial activity against the entire test organism, while acetone extract had shown antimicrobial activity against *Klebsiella pneumonia* and *Escherichia coli* only. The test microorganisms were resistant to cow dung extract from Jersey. Only the *Klebsiella pneumonia* was sensitive to acetone extract of cow dung. Other extract had no antimicrobial activity against test microorganisms. Cow dung extract from Holstein had shown antimicrobial activity against *Klebsiella pneumonia* only. The other two test microorganisms were resistant to cow dung extracts. Both acetone and methanol extract had shown antimicrobial activity against *Klebsiella pneumonia*. Cow dung extract from buffalo dung had shown partial antimicrobial activity against test microorganisms. The *Klebsiella pneumonia* was sensitive to acetone extract of buffalo dung, but the same microorganisms were resistant to ethanol extract of the buffalo dung. The *Escherichia coli* were resistant to ethanol and acetone extract. This shows that the buffalo dung had partial antimicrobial activity (table 1).

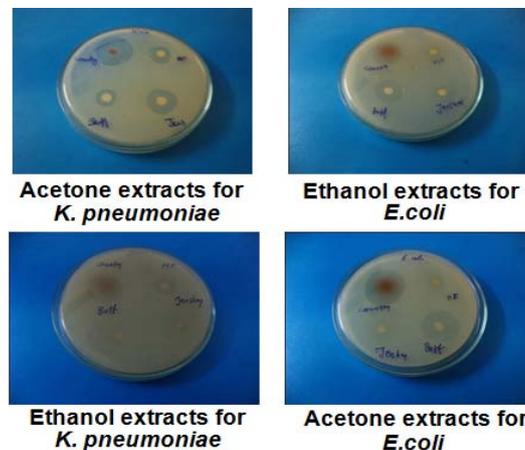


Fig. 1: Antimicrobial sensitivity test

Table 1: Antimicrobial activities of acetone and ethanol extracts of various cows and buffalo dung

S. No.	Test organisms	Antibiotics	Zone of inhibition(mm)	Inhibitory pattern	Acetone	Inhibitory pattern	Ethanol	Inhibitory pattern
1.	<i>E. coli</i>	Ampicillin	20	S	CAE(I) 20 mm	S	CEE(I) 16 mm	S
		Amikacin	24	S	CAE(J) No zone	R	CEE(J) No Zone	R
		loramphenicol	22	S	CAE(H) No Zone	R	CEE(H) No Zone	R
2.	<i>Klebsiella pneumonia</i>	Gentamicin	No zone	R	BAE 16 mm	S	BEE 18 mm	S
		Ofloxacin	No zone	R	CAE(I) 22 mm	S	CEE(I) 18 mm	S
		Vancomycin	20	S	CAE(J) 16 mm	S	CEE(J) No zone	R
					CAE(H) 20 mm	S	CEE(H) 18 mm	S
					BAE 20 mm	S	BAE No zone	R

Table 2: Phytochemical analysis of acetone and ethanol extract of different cow dung

S. No.	Phytochemical	Extract							
		Acetone				Ethanol			
		I	J	H	B	I	J	H	B
1.	Flavonoids	+	+	+	+	+	+	+	+
2.	Glycosides	+	+	+	+	+	+	+	+
3.	Steroids	+	+	+	+	+	+	+	+
4.	Tannins	+	+	+	+	+	+	+	+
5.	Phenols	+	-	-	-	+	+	-	+

**Phytochemical analysis of acetone extraction****Fig. 2: Phytochemical analysis of ethanol extraction**

DISCUSSION

In Indian Vedas, the cow is considered the most valuable and religious animal of Hindus. In India, cows are a very important animal and useful in agriculture and dairy industry. The cow dung has been used as organic fertilizer and in the production of biogas. The evaporated extract of cow dung is called "Dalang" or "Dalam" in northeast Nigeria and in some part of Northern Cameroun and has been used as soup condiment and in the treatment of infections. Indian cow, jersey, Holstein and buffalo a different healthy cows Cow dung was collected in the early morning from cattle breed of dharmapuri. Cow dung was also put for shed dried. 1000g of different cow dung was collected and shadow dried for 5 d. The dried cow dung was powdered. The powdered material 100 ml of acetone and ethanol was added in 10 g of powdered different cow dungs (Indian cow, Jersey, Holstein and buffalo dung) in a conical flask and it was kept in rotary shaker for 3 d. The extract was then filtered using What man No 1 filter paper and stored in vial for future use the cow dung extraction procedure was followed by [14] Phytochemical analysis was performed by each cow dung extract present the flavonoids, Glycosides, tannins, saponins and phenols this result was similar to [15] which reports these phytochemical compound are present the cow urine. The antimicrobial activities of disc diffusion technique at different cow dung extract against *K.*

pneumonia and *E. coli*. Indian cow dung extracts were activity against the both pathogens in this result are similar to [16] which report that the cow dung extract was inhibition zone of *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

CONCLUSION

From the experiment conducted it was concluded that the various extracts of cow dung possessed partial antimicrobial property against human pathogens. The cow dung from various cow had antimicrobial property against *klebsiella pneumonia*. Besides the Indian cow dung extracts possess superior antimicrobial activity than other cow dung and that shown antimicrobial property against all the test microorganisms. Since cow dung and buffalo dung are abundant in nature, cost effective and easy to be processed, they are a promising solution for a variety of health problems in the near future. The medicinal properties of these cow dung and buffalo dung can be exploited to formulate drugs for several diseases caused by antibiotic resistant pathogenic microorganisms.

CONFLICT OF INTERESTS

Declare none

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Research Article

**Isolation and characterization of bacteria from cow
dung of desi cow breed on different
morpho-biochemical parameters in Dehradun,
Uttarakhand, India.**

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ABSTRACT

The present study includes the collection, isolation and characterization of microorganisms from the cow dung of local varieties from different places of Dehradun, Uttarakhand, India. Four different strains of bacteria B1, B3, B4, B5 were isolated in which three are gram positive (cocci form) and one is gram-negative (bacillus form). Only one strain shows (B4) the formation of endospore. The enzymatic activity of four isolates revealed that strains B3, B5 and both control 1 (*E. coli*) and control 2 (*B. cereus*) showed amylase activity whereas none of the strains showed protease and lipase activity. To test the susceptibility of isolated strains against chemotherapeutic agents, eight antimicrobial drugs were used to treat the susceptibility patterns of isolated bacteria. Among four isolates (B1, B3, B4, B5) strain B3 and control 2 (*B. cereus*) shows resistance to penicillin and rest of the strains were sensitive to all these antibiotics and also shows antagonistic activity against different human pathogens. The strains B1 and B3 shows moderate inhibition zone against *Listeria monocytogenes* ATCC 657, *Klebsiella pneumonia* MTCC5615 and *Bacillus pumilis* MTCC 1607 whereas strain B4 and B5 shows maximum zone of inhibition against *Listeria monocytogenes* ATCC657 and *Bacillus pumilis* MTCC 1607. Therefore, intensive efforts must be initiated to identify and preserve all the indigenous breeds of cows for comparative chemical, microbiological and immunological analysis of milk, urine and dung with special reference to their agricultural, medicinal and nutritional significance.

KEY WORDS: antimicrobial activity, cow dung, hydrolytic activity, antagonistic effect.

INTRODUCTION

Cattle rearing in India has been a tradition and intimately limited to agricultural economy. Different products obtained from cow milk, ghee, curd, urine, and dung are used widely in number of Ayurvedic formulations. Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. The addition of cow dung increases the mineral status of soil, enhances resistance of plant against pests and diseases; stimulate plant growth¹ and other beneficial activities such as sulphur oxidation and phosphorous solubilization. Normally, Composition of cow dung is about 80% water and supports a matrix of undigested plant material that is

rich in nutrients, micro-organisms, and their byproducts. Cow dung micro flora contains abundant number of bacilli, lactobacilli and cocci and some identified and unidentified fungi and yeasts². According to Ware *et al.* (1988), lower part of the gut of the cow contains various microorganisms including *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *B. subtilis*, *Enterococcus diacetylactis*, *Bifido bacterium* and yeasts (commonly *Saccharomyces cerevisiae*) having probiotic activity³. Normally aged cow dung gets invaded with several soil contaminants such as bacteria, fungi, *Trichoderma* and *Actinomyces*². There are several evidences to show that fresh cow dung and cow urine are antifungal and antiseptic in

nature⁴, which might be due to secretion of antimicrobial metabolites by cow dung micro flora. Our endeavor through this study is to isolate and characterized the bacteria from cow dung of desi cow breed on different morphological and biochemical basis and study their usefulness with the preliminary biological screening of microbes.

MATERIAL AND METHODS

Material required:

Different samples of cow dung were collected from different regions of Dehradun district, aseptically in sterile poly bags and transported to Microbiology laboratory of the Department of Life Sciences for the evaluation of microbial analysis.

Preparation of cow dung suspension:

Cow dung suspensions were prepared by serial dilution method. The collected and labelled, 1gm of cow dung samples were mixed in 10 ml sterilized phosphate buffer and vigorously shaken in vortex for 2 minutes for proper mixing of sample. Before plating, all the samples were incubated at 37⁰C for 30-40 minute in an incubator for activation of microorganism. After incubation dilutions of each sample were prepared by using standard dilution method with the help of sterilized pipette. In this method, Phosphate blanks were prepared, each contain 9 ml of sterilized phosphate buffer. The labeled tubes were placed in test tube stand then 1ml of activated standard solution was transferred aseptically in test tube number 1, and further 1ml of sample was transferred to number 2 and same procedure was repeated for each dilution.

Isolation and Purification of microorganisms:

The different bacterial cultures were purified by using streak plate method on Nutrient agar medium. Using sterilized inoculating loop, slightly picked up the colony from the spread plate dragged the loop over the surface of another plate in a zigzag motion. Sterilized the loop over the flame, turned the plate to 90⁰ and dragged the loop over the area streaked before in similar manner. Again sterilized the loop over the flame and the same process was repeated again, all the plates were incubated for 24 hours. The heaviest growth was seen in the first sector, and the isolated colonies were in the third sector. This method was repeated several times until purified colonies were obtained. The purified bacterial cultures were maintained over Nutrient agar slant.

Characterization and identification of microorganisms:

After the pure culturing method, the isolated colonies of microorganisms were observed for colony

morphology determination; colour, shape, size, surface, edges, margins and elevation. These cultures were identified by different staining such as Gram's staining, endospore staining etc.

Antibiotic susceptibility assay:

The most common method for antibiotic susceptibility test is Kirby Bauer method or Disc diffusion method. In this test bacterial isolate is inoculated uniformly into the surface of an agar plate. A filter disc impregnated with a standard amount of an antibiotic is applied to the surface of the plate and the antibiotic is allowed to diffuse into the adjacent medium. The result is a gradient of antibiotic surrounding the disc. Following incubation, a bacterial lawn appears on the plate. Zones of inhibition of bacterial growth may be present around the antibiotic disc. The size of the zone of inhibition is depend on the diffusion rate of the antibiotics, the degree of sensitivity of the micro-organisms and the growth rate of bacterium. Discs with very small zones or no zones of inhibition means that the bacteria is not susceptible to the antibiotic. Large zones indicate the levels of susceptibility: Susceptible (S), Intermediate (I), or Resistance (R).

Hydrolytic enzyme activities:

Biochemical tests are mainly done to identify bacteria capable of producing various exoenzymes which explore their properties of hydrolyzing waste material. The biochemical tests were done basically to identify the secretion of three exoenzymes viz . protease, amylase and lipase. Agar plates were prepared containing protein, starch and lipid for testing protease, amylase and lipase activity, respectively. If the inoculated bacterium secretes the respective exoenzymes, a clear zone of hydrolysis is observed around the inoculums. The relative protease, amylase and lipase activities were calculated by the given formula:

$$\text{Relative activity} = \frac{\text{Zone of hydrolysis}}{\text{Colony size}}$$

Antibacterial assay of isolates from cow dung against human pathogens:

All isolates were screened for antibacterial activity against 8 test organisms using the disc diffusion method.

Test organisms included five Gram-positive bacteria (*Staphylococcus epidermis*, *Bacillus pumilis*, *Bacillus licheniformis*, *Listeria monocytogens*, *Streptococcus mutans*) and three gram negative bacteria (*Klebsiella pneumoniae*, *Salmonella typhimurium* and *Pseudomonas aerogenosa*). The plates were

incubated at 30°C for 24 hours. The inhibitory spectrum of the antibacterial agents against gram-negative and gram-positive bacteria was determined.

RESULT AND DISCUSSION

In present study, different samples of cow dung were collected from different localities of Dehradun which were subjected for morphological and biochemical characterization. These isolated bacterial strains were further evaluated for antibiotic resistance and antagonistic test against several bacteria which causes different diseases in human. Finding of the present study were presented and discussed as follows:

Morphological and biochemical characteristics of isolated strains:

Microorganisms produce colonies with characteristics which could be seen by naked eyes that are called as cultural characteristics. The cultural characteristics were observed on Nutrient Agar Medium plates after incubation. These morphological characteristics were observed in different forms such as colony form, colony elevation, surface of the colony and colony colour. The collected samples of cow dung were enumerated for their microbial load of total bacteria. The maximum number of bacterial population was exhibited in dilution 10^{-4} which ranged from 60.5×10^4 to 175×10^4 cfu/ml and minimum concentration was exhibited in dilution 10^{-6} which ranged from 23.5×10^6 to 80.5×10^6 . The morphological examinations of the isolates were determined by standard procedure of basic stain, gram stain and endospore stain⁵. Out of four strains, three strains B1, B3 and B5 were gram positive, cocci form and rest of the strain B4 is gram negative, bacillus form (table 1,2). Among these isolated strains, only one strain B4 shows endospore formation. Similar type of work was performed by Teo and Teoh (2011)⁶. Cavaletti *et al.* (2006) also reported two isolates K2 and K4, both were gram-positive micro-organisms, capable of forming endospore⁷.

All the biochemical reaction that proceeds both outside and inside the cell were precisely controlled by some governing factors, the enzymes. These are mainly hydrolytic enzymes that degrade by the addition of water, high molecular weight substances (like polysaccharides, lipids and proteins) into smaller component (e.g. glucose that can enter into the cell and later assimilated cow dung in Kampar Malaysia⁶). Strains isolated from cow dung were able to produce protease, esterase and esterase lipase. Protease is an enzyme that catalyses proteolysis which breaks down proteins to its respective amino acids. In addition, they have a variety of applications

mainly in the detergent and food industries. Microbial proteases account for approximately 40% of the total worldwide enzyme sales⁸. Esterase and esterase lipase belong to hydrolyses enzyme which splits esters into acids and alcohols in a chemical reaction of hydrolysis process involving addition of water molecules⁹. In present study, the isolated four strains B1, B3, B4, and B5 from the cow dung have been evaluated for enzymatic activity. No strains shows protease and lipase activity. Strains B3, B5 and both control 1 (*E. coli*) and control 2 (*B. cereus*) shows amylase activity (Table 3). Amylases from several *Bacillus* spp. i.e. *B. cereus*, *B. megaterium*, *B. subtilis* and *B. stearothermophilus* are available commercially. Preliminary studies of Singh and Hayashi in 1995¹⁰ shown that all these *B. subtilis* strains were thermo tolerant (up to 60 °C), which may be useful in producing commercial enzymes. The fact that *B. subtilis* strains are also producers of cellulose is of interest from the biotechnological point of view and in relation to the decomposition of agricultural residues remaining in the field after the crop is harvested. Therefore studies can be conducted to elucidate the mechanism underlying biocontrol and growth stimulation by *Bacillus* sp. According to the studies conducted by Pandey *et al.*, (2008) revealed that *Bacillus* spp. known to produce α -amylases which have wide application in industrial processes, especially in starch industry¹¹. Besides these studies sulphuroxidizing- *Pseudomonas* sp. PRK786, cellulase producing bacterial strains were isolated and characterized biochemically and molecular basis^{12,13}.

Antibiotic susceptibility of isolated strain:

Antibiotic susceptibility test (AST) is usually carried out to determine which antibiotic will be most successfully in treating a bacterial infection *in vivo*. Testing of antibiotic sensitivity is often done by Kirby-Bauer method. Among four isolates (B1, B3, B4, B5), the B1 strain of sample is sensitive to all antibiotics i.e. penicillin, amoxicillin, ofloxacin, ciprofloxacin, chloramphenicol, erythromycin, streptomycin and tetracycline, whereas B3 strain is resistance to penicillin and streptomycin. Rests are sensitive. B4 strain shows no resistance against these antibiotics. Furthermore B5 strain was sensitive to all antibiotics. Control 1 (*E. coli*) is sensitive to all these antibiotics as compared to other strains, whereas Control 2 (*Bacilli*) is resistance to penicillin and rest are sensitive to all these antibiotics (Table-4). Antibiotic resistance may occur due to natural processes such as transformation, transduction and conjugation, or due to human mediated activity such as antibiotics mediated activity such as antibiotics abuse, particularly in farming and agricultural

industry¹⁴. There are several evidences to show that fresh cow dung and cow urine are antifungal and antiseptic in nature⁴, which might be due to secretion of antimicrobial metabolites by cow dung micro flora.

Antagonistic activity of isolated strains against the pathogenic bacteria:

Four strains isolated from cow dung along with two controls show antimicrobial activity against different human pathogens in which some of the strains shows either maximum inhibition zone or no zone (table5). Moderate inhibition zone is shown by B1 against *Listeria monocytogenes* ATCC 657 and *Klebsiella pneumoniae* ATCC 109 and rest of the pathogenic strains does not shown any zone. Strain B3 shows moderate zone of inhibition against *Listeria monocytogenes* ATCC 657, *Bacillus pumilis* MTCC 1607 and *Klebsiella pneumoniae* MTCC 432 strains and rest of the strains does not show any zone. Strong inhibition is shown by B4 against *Staphylococcus epidermis* whereas strain B5 shows maximum zone of inhibition against *Listeria monocytogenes* ATCC 657), *Pseudomonas aerogenosa* ATCC 424 and *Salmonella typhimurium* MTCC 1255 and rest of the strain does not shows any zone of inhibition. Studies conducted by Shrivastava *et al.* (2014) evaluated

antibacterial and antifungal properties of cow dung extract in distilled water, ethanol and n- hexane against *Candida, E.*

coli, Pseudomonas and *Staphylococcus aureus* and found it highly effective against these microbes. The study revealed that cow dung extract possess antimicrobial properties, which can be used to fight against certain pathogenic diseases and other ailments^{15,16, 17}. Besides these, the antibacterial activities some of the isolates exhibited nematicidal activity and probiotic activities^{18,19}.

CONCLUSION

The present study shows that isolated bacterial strains can be used to prevent diseases caused by pathogenic strains. Thus, Cow dung serves as a purifier of all wastes in the nature, is a rich source of microflora which can be used as probiotics, live microbial food supplements modifying the intestinal microbiota. Therefore, the intensive efforts must be initiated to identify and preserve all the indigenous breeds of cows for comparative chemical, microbiological and immunological analysis of milk, urine and dung with special reference to their agricultural, medicinal and nutritional significance.

Table 1
Microbial count from the cow dung samples

S.No.	Dilutions	Method used	Total bacteria count		
			Sample 1	Sample 2	Sample 3
1.	10 ⁻⁴	Serial dilution method	192.5x10 ⁻²	175.0x10 ⁻²	60.5x10 ⁻²
2.	10 ⁻⁵	Serial dilution method	141.5x10 ⁻³	95.5x10 ⁻³	33.5x10 ⁻³
3.	10 ⁻⁶	Serial dilution method	80.5x10 ⁻⁴	50.5x10 ⁻⁴	23.0x10 ⁻⁴
4.	10 ⁻⁷	Serial dilution method	24.0x10 ⁻⁵	20.0x10 ⁻⁵	15.0x10 ⁻⁵

Table 2
Morphological characteristics of isolates from cow dung

Characteristics	ISOLATES			
	B1	B3	B4	B5
Form of colony	Circular	Circular	Circular	Circular
Translucency and opacity	Opaque	Opaque	Opaque	Opaque
Elevation of colony	Convex	Flat	Convex	Convex
Surface of colony	Smooth	Smooth	Smooth	Smooth
Pigmentation	Creamy white	Yellow	Pink	White
Cell shape	Coccus	Coccus	Bacillus	Coccus
Gram stain reaction	+	+	-	+
Spore stain	No	No	Yes	No

Table 3
Enzymatic assay of four isolates and two controls

Enzymes	Protease	Amylase	Lipase
Isolates			
B1	-	-	-
B3	-	+++	-
B4	-	-	-
B5	-	+++	-
Control 1 (<i>E. coli</i>)	-	+++	-
Control 2 (<i>Bacillus cereus</i>)	-	++	-

*Degree of inhibition: + = moderate clear zone (1-9mm); ++ = strong clear zone (10-19mm); +++ = very strong zone (20 or more); - = no inhibition zone.

Table 4.
Antibiotic sensitivity of isolated bacteria from cow dung

Isolates	Zone of inhibition in (mm)					
	B1	B3	B4	B5	Control 1 (<i>E. coli</i>)	Control 2 (<i>Bacillus cereus</i>)
Antibiotics						
Penicillin	15.0	Nil	Nil	0.13	11.0	Nil
Amoxicillin	25.0	23.0	23.0	22.0	15.0	13.0
Ofloxacin	35.0	30.0	26.0	27.0	19.0	23.0
Ciprofloxacin	38.0	38.0	31.0	33.0	28.0	28.0
Chloramphenicol	24.0	23.0	22.0	21.0	22.0	25.0
Streptomycin	27.0	26.0	19.0	18.0	13.0	12.0
Tetracycline	23.0	22.0	10.0	0.5	10.0	10.0
Erythromycin	14.0	14.0	12.0	11.0	16.0	12.0

Table 5
Antagonistic effect of isolates on pathogenic bacteria

Isolate	B1	B3	B4	B5	<i>Bacillus cereus</i>	<i>E.coli</i>
Bacteria						
<i>Listeria monocytogenes</i> ATCC657	+	+	-	+	-	+
<i>Streptococcus mutans</i> ATCC890	-	-	-	-	-	-
<i>Pseudomonas aerogenosa</i> ATCC424	-	-	-	+	+	-
<i>Bacillus pumilis</i> MTCC1607	-	+	-	-	-	-
<i>Bacillus licheniformis</i> ATCC1483	-	-	-	-	-	-
<i>Staphylococcus epidermis</i> MTCC432	-	-	++	-	-	-
<i>Klebsiella pneumoniae</i> MTCC5615	-	+	-	-	-	+
<i>Salmonella typhimurium</i> MTCC1255	-	-	-	++	-	+

*Degree of inhibition: += moderate inhibition zone; ++ = strong inhibition zone; +++ = very strong inhibition zone; - = no inhibition zone.

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