Study of antimicrobial activity of cow dung against *Escherichia coli* and *Staphylococcus* species

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IN

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By

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CERTIFICATE-I

This is to certify that the thesis entitled **"Study of antimicrobial activity of cow dung against *Escherichia coli* and *Staphylococcus* species"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Veterinary Microbiology)** to the Odisha University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Bukka Rahul** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"Study of antimicrobial activity of cow dung against *Escherichia coli* and *Staphylococcus* species"** submitted by **Bukka Rahul** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Veterinary Microbiology)** has been approved/ disapproved by the students' advisory committee and the external examiner.

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Bhubaneswar **(Bukka Rahul)**

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LIST OF ABBREVIATIONS

°C	:	Degree Centigrade
μ	:	Micrometer
μg	:	Microgram
μl	:	microlitre
ABST	:	Antibiotic Sensitivity Testing
BHI	:	Brain Heart Infusion Broth
CFU	:	Colony Forming Unit
Conc.	:	Concentration
EMB	:	Eosine Methylene Blue Agar
et al.	:	Co-workers
g/L	:	Gram/Litre
ISO	:	International Organization for Standardization
lit	:	Litre
MHA	:	Mueller Hinton Agar
ml	:	milliliter
mm	:	millimeter
MRSA	:	Methylene Resistant *Staphylococcus aureus*
MTCC	:	Microbial Type Culture Collection and Gene Bank
PBS	:	Phosphate Buffer Saline
sp.	:	Species

ABSTRACT

Now a days due to increase in antimicrobial resistance, there is a quest for alternative to synthetic drugs. As cow dung is a natural product with reported antimicrobial activity. In the present study 30 cow dung samples were collected from desi cattle and different breeds present in and around Bhubaneswar, Odisha were tested against the *Escherichia coli* and *Staphylococcus species* isolates from milk. Antibiotic sensitivity test was conducted using commercially available antibiotic disks, it was observed that the test organisms are showing resistance towards antibiotics. In order to combat antimicrobial resistance a new alternate natural product like cow dung can be explored against *Escherichia coli* and *Staphylococcus species* infections in animals. Methanol, Ethanol and Aqueous extracts of cow dung were prepared as per the routine procedure and its antimicrobial properties were assessed as per Disk Diffusion method. Disks prepared from cow dung showed the Zone of inhibition in petri plates. Results obtained for antimicrobial sensitivity of different cow dung extracts procured by using solvents like Methanol, Ethanol, Aqueous against *E. coli* were noted in the (Table 4.3) and against *Staphylococcus species* were noted in the (Table 4.4). Out of 30 samples it was found that Methanol extracts of 24(80%) cow dung samples are showing high sensitivity towards the test organism *E. coli* and around 21(70%) are showing towards *Staphylococcus species* when compared to their Ethanol and Aqueous extracts. They are even found to exhibit a high or an almost equal sensitivity pattern against test organism when compared with the zone of inhibition pattern of commercially available antibiotics noted in (Table 4.2). These extracts are even showing high antimicrobial activity when compared with some other antibiotic because they are resistant to test organism based on zone of inhibition noted in (Table 4.2). Methanol extracts of desi cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross. Methanol extracts of desi cattle (21.6±1.226) was statistically ($p<0.05$) higher as compared to the HF (13.67±), Jersey (10.67±0.667) and HF cross (7.67±0.882) against *E. coli*. Methanol extracts of desi cattle (20.87±0.975) was significant ($p<0.05$) difference when compared to HF (15.83±0.477), Jersey (14.0±0.00) and HF cross (10.83±1.138) against *Staphylococcus sp*ecies . The phytochemical analysis conducted for the samples showed no precipitation changes and no colour changes. This emphasizes that phytochemical substances plays no role in contributing towards the antimicrobial activity of cow dung extracts.

INTRODUCTION

In dairy industry, there are many infectious diseases affecting the production animals, out of which mastitis was the most devastating one that causes significant losses at all stages of production .The treatment for mastitis was also to expensive and in turn this indiscriminate antibiotics usage leads to negative consequences for this disease like decreased milk production, increased antibiotic resistance to bacteria .It was difficult to diagnose this disease and its control because it requires frequent bacteriological analysis of milk ,antibiotic therapy and slaughtering of infected animals .Most commonly isolated bacteria are *Escherichia coli and staphylococcus spp.* (Costa, 2019).

Over the last three decades, large numbers of antibiotics have been synthesized, leading to increased bacterial resistance. Bacteria gains resistance to antibiotics by several methods like chromosomal changes or genetic material exchange via transposons and plasmids. Almost all older antibiotics were now resistant to *E. coli* and *Staphylococci*, as well as members of the Enterobacteriaceae family. Many mechanisms such as antibiotic control programs, improved hygiene and the developing the agent's alternative to antibiotics with improved antimicrobial activity must be carried out (Neu, 2012).

Antibiotic resistance has been evolving since their discovery, and it is being exacerbated because of increased usage and even misuse of antimicrobials agents leading to serious public health related problems. Isolated bacterial strains were streaked at the center of Baird-Parker agar plates and incubated at 37 for 24 hrs. *Bacillus cereus* (MTCC6728), *Bacillus subtilis* (MTCC441), *Staphylococcus* (MTCC7443), *Vibrio cholera* (MTCC 3904), *Salmonella typhi* (MTCC3216) and *Escherichia coli* were used as test organisms (Rana, 2016).

Escherichia coli is a bacteria that can be found both in humans and animal guts as well as in water, soil, air and vegetation. Antimicrobial resistance in *Escherichia coli* has been reported all over the world& it has become a growing concern in both developed and developing countries. Due to this antibiotic resistance, it is becoming more difficult to treat infections. Among 146 milk samples collected 14.2% was found with *Escherichia coli* in them. On Antibiotic sensitivity test it is found that *Escherichia coli* isolates are resistant to erythromycin [89.4%], amoxicillin [86%], tetracycline [86%], nitrofurantoin on the other hand has higher sensitivity [96.4%], while norfloxacin has very low sensitivity [9.4%] (Abera2, 2011).

In clinical setting, antibiotic resistance is a major issue. Due to indiscriminate use of antibiotics, no proper disposal of wastage & due to transfer of antibiotic resistant genetic material in community there is an emergence & spread of antibiotic resistance (Choudhury, 2012).

From the ancient time, it was believed that antimicrobial properties are present in natural products. Cow urine concentrate and dung were found to have antibacterial activity against a variety of bacteria, including *B. subtilis, P. aeruginosa, Staphylococcus aureus, E. coli* and others .It's an antiseptic of some sort .As a result ,it is customary in India to wash and sprinkle cow dung solution on the floors of homes(Satyaprakash, 2018).

Cattle raising was a long - standing tradition in India, and it is intimately linked to the agricultural economy. Ayurvedic formulations frequently include products made from cow milk, ghee, curd, urine, and dung. Cow dung is primarily composed of water, with a mixture of partially digested and vegetative material that is undigested and rich in microorganisms. In the micro flora of cow dung bacillus, lactobacillus and cocci, as well as other fungi and yeasts are abound (Rajeshwari, 2016).

The five products of the cow, known as "Panchgavya" are a valuable gift to our society, and include milk, curd, ghee, urine, and dung are used to test the antibacterial and antifungal properties of cow dung extract using solvents like distilled water, ethanol, and n-hexane against *Candida, Escherichia coli, Pseudomonas,* and *Staphylococcus sp.*, a study was conducted, and it was found to be highly effective against these microbes. Cow dung extract has antimicrobial properties, according to this study (Sushmita Shrivastava, 2014).

Cow dung has wide antimicrobial properties and to contain a diverse range of microorganism. Several studies were conducted to determine the antimicrobial traits of dung samples, including pathogenic ones, as well to evaluate the samples microbial diversity. A total of eight freshly collected cow dung samples were used in this experiment. All of the samples had high bacteria and fungi concentrations, averaging

108 and 107 cfu/g, respectively. Bacterial species such as *Staphylococcus sp., E. coli, Bacillus sp.* were the most common pathogenic found in all samples (Munshi1, 2018).

On *Staphylococcus sp., E. coli and P. aeruginosa,* isolated from clinical specimens, the methanol extracts of the plants grown on soil supplemented with anaerobically digested cow dung slurry showed marked antimicrobial activities. All the plants that were grown on digested cow dung slurry shows higher mean zones of inhibition values on all microorganisms that is more than that of the plants grown with undigested dung (Yongabi, 2007).

The main objective is to investigate the antimicrobial properties of cow dung extracts as well as photochemical screening to see if they can be used to kill pathogens causing infections. Antimicrobial properties of natural products are also being researched. Desi cow dung chloroform extracts possess antimicrobial activity against *Escherichia coli* and *Klebsiella pneumonia* bacteria. Phyto-chemical analysis is done on flavenoids, glycosides, steroids, tannins and phenol substances. The disc diffusion method was used to test antimicrobial sensitivity (Nayak, 2019).

Outline of the research work:

In order to evaluate the antimicrobial property, the following parameters will be earned in the present study.

1. Cow dung extract will be prepared from Desi cattle, Holstein, Buffaloes present in and around Bhubaneswar, Odisha.
2. A cow dung disc containing cow dung extract will be prepared in the Dept. of Veterinary Microbiology laboratory, College of veterinary science & animal husbandry, OUAT.
3. Antimicrobial tests will be carried out against both *Escherichia coli* and *Staphylococcus* species.
4. A comparative analysis will be made between cow dung antimicrobial activity with standard antimicrobial discs.
5. An attempt will be made to study the phytochemical analysis of Aqueous, Methanol and Ethanol extract of different cow dung obtained from various breeds of cattle and Buffaloes.
6. All the data will be subjected to statistical analysis.

REVIEW OF LITERATURE

2.1 Cow dung -microbial analysis and antimicrobial activity

Muhammad & Amusa, 2003 said that the cow dung possess some properties to inhibit disease causing microorganisms, for example antifungal agents that inhibits the growth of coprophilous fungi, antibacterial agents that has been successfully been extracted from cow dung revealing that cow dung has antimicrobial property. From the above findings, in present study an attempt was done to find that cow dung has some antimicrobial properties against microbes.

Saswant *et al.* (2007) reported that Cow dung contains abundant microorganisms with many species of bacteria, protozoa and yeast such as *Citrobacter koseri, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae,., Morgarella morganii, Pasteurella spp., Providencia alkaligenes, P. stuartii, Pseudomonas sp., Nocardia, Mucor* and *Rhizopus sp*. have been isolated from cow dung.

Geetha *et al.* (2008) noticed that Slurry was created when cow dung was combined with cow urine. *Aspergillus, Pseudomonas sp, Sarcina, Nocardia, MucorSp, RhizopusSp, Aspergillus, E. coli* and *Penicillium sp* are among the bacteria, fungi*, and Actinomycetes* found in cow dung slurry.

Yongabi *et al.* (2009) reported that In general, all the extracts obtained from plants showed activity against the test organisms, indicating that they have antimicrobial potential as previously reported. The results of this study show that when these plants growing on the soil fertilized with cow dung showing increased antibacterial activity revealing that cow dung ha some antimicrobial activity.

Randhawa *et al.* (2011) found that many species of bacteria and fungi namely *Actinomyces, Streptococcus spp., Staphylococcus spp., Sarcina, Escherichia coli, Nocardia, Mucor spp., Rhizopus, Aspergillus spp.* and *Pencillium* microbes are present in cow dung slurry. From this it was proved that cow dung is a surplus source of microflora and is not even too expensive.

Raja *et al.* (2011), Experimented with various cow dung extracts. In some places, clothes are washed or rinsed with a mixture of cow dung ash and water before being used. This belief was still held by Hindus, who believe that if microbes are present, the cow dung ash mixture will kill them. These customs confirm that our forefathers believed in the antiseptic properties of cow dung. The extractives acted as antimicrobials against pathogenic microbes, including fungi, found in the human body. In particular, when compared to the other extractives, the methanol extractive of cow dung had the best antimicrobial action, acting on almost all of the microbes tested.

Obuekwe *et al.* (2014) observed that microbes like *Staphylococcus spp., Bacillus spp., Escherichia coli, Nocardia, Micrococcus spp., Neisseria spp.,* are isolated from the cattle dung and its dung contaminated with soil settlements of cattle revealing the microbial composition of cow dung.

Kartikey Kumar Gupta *et al.* (2016) said that the objective of the study was to evaluate the ability of cow dung microflora to produce antimicrobial metabolites. 24 isolates were tested for antimicrobial potential in this study, and 2 of them showing broad spectrum antimicrobial activity against all of the organisms. Till then, only some investigations have shown that the dung has medicinal properties. Antimicrobial activity has been found in 51 microbes. Teo & Teoh (2011) found that one cow dung isolate had antimicrobial activity against *E. coli*. Isolates from *Escherichia coli* and *Citrobacter sp.* were found to be promising antimicrobials in this study.

Manyi-Loh *et al.* (2016) noticed that there are different practices carried in livestock according to the geographical area and it varies even from one person to another, which causes changes in the output excreta or manure voided out by the animal. Likewise the microbial composition varies from one animal to another animal. Salmonella spp., Escherichia coli , protozoa like Giardia lamblia are abundant while the others are not commonly found.

Sharma and Singh *et al.* (2016) observed that on microbial analysis of the cow dung samples procured for studies, they enumerated the bacterial load and others. The maximum bacterial load found ranges from 60×10^4 -180×10^4 cfu/ml and the minimal load was around 23×10^6-75×10^6. This reveals that a lot of microflora is present in cow dung.

Atnafel *et al*. (2017) reported that Due to its rich microflora, cow dung can be investigated as a source of possible antibacterial substances. Against all of the bacteria examined, all of the cow dung samples showed considerable antibacterial activity. *E. coli, Klebsiella spp., Staphylococcus sp., Vibrio sp., Pseudomonas sp.* and *Bacillus* species were found to efficiently limit the growth of all bacteria examined.

Annanda Das *et al.* (2018) emphasized that the cow dung is used as an antiseptic. Hence washing the floor of houses and sprinkling of cow dung solution is an Indian custom. Medicinal plants like ginger, garlic grown on cow dung slurry showed increased antibacterial activity against *S. aureus, E. coli, P. aeroginosa* and higher antifungal activity against *Aspergillus niger.* Products obtained from animals and plants have antimicrobial activities due to the presence of a variety of active principles or secondary metabolites or phytochemicals (in case of plants) like lectins, alkaloids, tannins, terpenoids, flavenoids, proteins & polypeptides, quinones, coumarins, essential oils polyphenols & phenolic compounds, enzymes, lysozymes, phagocytic cells etc..

Raja *et al.* (2021) said that there are five products obtained from cow called as “Panchagavya” that include the directly obtained products like cow milk, dung , urine and the other secondary products like ghee, curd derived from the primary products. In therapeutic and medicinal purposes cow dung and urine are used as they are believed to possess antimicrobial activity.

2.2 Antibiotic resistance profile of different microorganisms

Kirby and Craig *et al.* (1981) noticed that there was usage of antimicrobial agents from the beginning of synthesis of Pencillin. Antibiotic resistance has risen dramatically, rendering many antibiotics ineffective. Antibiotics must be closely monitored due to the emergence of immunocompromised cases, increased Antibiotic Resistance and new strains of disease-causing agents.

Mandell *et al.* (1995) evaluated that *Staphylococcus aureus* resistance to methicillin was first described in 1960, shortly after the drug was introduced into clinical practice in hospitals (Jevons, 1960). Since then methicillin–resistant *Staphylococcus aureus* common (MRSA) has gradually spread and began to cause problems. In the 1970s, there were a lot of serious nosocomial infections all over the world (Locksley and colleagues, 1982). MRSA had become widespread by the mid-1990s.Because the strains are so common it became a major issue. Multiple resistance to tetracycline, aminoglycosides, macrolides, lincosamides and other antibiotics were used for alternative and it was spreading.by forming new communities.

Sato *et al.* (2005) conducted Antibiotic Sensitivity Test for many samples including urine, milk, skin swabs in which almost all *Escherichia coli* & *Staphylococcus* are present in their microflora and after incubating plates at 37^0C for 24-36hrs, it was found that isolates are moderately sensitive to antibiotics like Azithromycin, Ampicillin, Amoxyclav and are resistant to ketoconazole, cephalexin, cefotaxime, cefpodoxime and highly sensitive to Enrofloxacin, Gentamicicn and Streptomycin.

Srinivasan *et al.* (2007) evaluated the antimicrobial susceptibility for *E. coli* isolates using 10 different antimicrobial agents. An isolate was termed as multidrug resistant when it shows resistance to more than 2 or more different antimicrobial agents. *E. coli* is termed as multidrug resistant and in this case, *E. coli* were resistant to ampicillin (98.4%), streptomycin (40.3%) and tetracycline(24.8%).

Sumathi *et al.* (2008) evaluated that, from 60 clinical mastitis milk samples a total of 75 bacterial species were isolated. Among 60 samples, 21 (35%) yielded pure cultures, including 6 gram-positive and 15 gram-negative organisms, while the remaining 39 (65%) yielded mixed cultures. 49 (65.3%) of the 75 isolates were gram-positive, while the remaining 26 (34.67%) were gram negative. *Staphylococcus aureus* (24%) and *Escherichia coli* (20%) were the most common bacteria found, followed by *Staphylococcus epidermidis* (16%), *Streptococcus sp.* (16%), *Klebsiella sp.* (10.67%) and *Bacillus sp.* (10.67%). On performing Antibiotic Sensitivity Test, it revealed that gentamicin was the most effective drug (90 %), followed by enrofloxacin (88%), ciprofloxacin (85%), chloramphenicol (75%), tetracycline (60%), colistin (57%), neomycin (50%), nitrofurantoin (50 %), furazolidone (50%), cephalexin (30%) to the isolated bacterial species.

Bergman *et al.* (2011) reported that his studies evaluated that E. coli isolates were showing resistance to minimum of single group of antimicrobials used in the farms. Bivariate analysis revealed positive significant correlations between ampicillin and amoxicillin, amoxicillin and tetracycline, and gentamicin and amikacin resistance profiles, as well as negative significant correlations between tetracycline and ceftazidime resistance patterns, ampicillin and ceftazidime resistance patterns, and amoxicillin and ceftazidime resistance patterns. The antimicrobial resistance pattern of *E. coli* isolates correlates with the antimicrobial used in the respective farms, implying that antimicrobial overuse or misuse may be linked to resistance development. However, in two *E. coli* isolates from two different sources, phenotypic azithromycin resistance was discovered.

Teo and Teoh *et al.* (2011) while conducting his studies in Nigeria isolated many bacterial species and evaluated their Antibiogram profile using cow dung extracts procured by using different solvents. They found that Indian cow dung has high degree of antimicrobial activity against many isolates including *Staphylococcus spp*., *Klebsiella* and others.

Thaker *et al.* (2012) reported the antibiogram profile of *E. coli* in 100 raw milk samples by inoculating the enriched samples at 38^0C for 24 hrs for cultural and morphological characteristics the plates were inoculated on Mac Conkey Agar at 37^0 C for 24 hrs. The presumptive *E. coli* shows high resistance against Ampicillin. On EMB agar it was found that they are resistance towards Streptomycin (58%), Amoxyclav (12%) and Oxytetracycline(48%). Then resistance was observed towards Co-trimoxazole (14%) and Chloramphenicol (5%) respectively.

Aminu *et al.* (2015) reported the Antimicrobial susceptibility of *E. coli* revealed that antimicrobial resistance was increasing even among commensal bacteria and has become a growing problem. Reasons behind this are indiscriminate use of antibiotics in humans and in animal feeds as growth promoters. Antimicrobial resistance in food animals was a problem that needs to be addressed. The higher level of *E. coli* susceptibility to Ciprofloxacin (89.3%) observed was consistent with reports of *E. coli* sensitivity of 80 percent and 80.1 percent in food animals, respectively. This could indicate that this antibiotic was still effective in the treatment of bacterial infections.

Munsi *et al.* (2015) evaluated the *Staphylococcus aureus* antibiogram profile by disc diffusion method and the reults obtained in terms of zone of inhibition were 33 mm for oxytetracycline, 25 mm for Amoxyclav, 36 mm for Ciprofloxacin, 29 mm for Cefotaxime, 32 mm for Ceftriaxone, 31 mm for Azithromycin, and 26 mm for Erythromycin; in case of *E. coli*, were 5 mm for Oxytetracycline, 9 mm for Amoxicillin, 22 mm for Ciprofloxacin, 30 mm for Cefotaxime, 31 mm for Ceftriaxone, 15 mm for Azithromycin, and 0 mm for Erythromycin.

Batabyal *et al.* (2018) noticed that the Resistance to antibiotics such as ampicillin (86.11%), amoxicillin-clavulanic acid (63.89%), cefotaxime (100%), ceftazidime (66.67%), tetracycline (72.22%), and gentamicin (61.11%) was previously reported by *E. coli* pathogens in their study. In their study, they found that ampicillin, cefotaxime, ceftazidime and cefuroxime (all 100%) and tetracycline (93.54%) were highly resistant to the *E. coli* strains isolated from cattle, but imipenem (100%) was highly sensitive.

Munshil *et al.* (2018) in studies conducted antibacterial analysis against 11 test organisms including Gram-positive (*Staphylococcus sp., Bacillus subtilis, B. cereus, S. epidermis, Micrococcus luteus, Enterococcus faecalis* and *Bacillus sphaericus*) and Gram-negatives (*Salmomella, Escherichia coli, Pseudomonas, Proteus vulgaris*).after incubating plates for 24hrs most of them are found to shoe sensitivity towards Gentamicin(65%) while others are moderately sensitive to Enrofloxacin and cephalosporin group of drugs.

Kaviraj *et al.* (2019) emphasized that the cow dung is a good source of probiotic used for many uses. He conducted Antibiotic Susceptibility Test for the isolates obtained from cow dung. Out of which *E. coli* was showing sensitivity towards antibiotics namely streptomycin, ampicillin, norfloxacin and showed resistance towards tetracycline, penicillin G and amphotericin B.

Ogunkeyede Akinyemi *et al.* (2020) isolated Gram positive bacteria like *Staphylococcus* and Gram negative bacteria like *klebsiella, Alcaligens, Edwardsiella and Acinetobacter* in his studies. Among them some of the bacteria shows antibiotic resistance which causes severe public health issues and found the cow dung biodigester that has been used in agricultural purpose to minize this this pathogenic effects.

Waksita *et al.* (2020) reported that The bacterium Staphylococcus aureus was a major cause of mastitis in West Java, particularly in the Bandung Regency. This bacterium frequently causes both clinical and subclinical mastitis infections. *Staphylococcus aureus* was a threat to increasing the quality and quantity of cow's milk in dairy farming. The difficulty in handling and preventing mastitis infection poses a threat. Ampicillin, Oxytetracycline, Sulfametoxazole-Trimethoprim, Ciprofloxacine and Chloramphenicol are five antibiotics for the treatment of mastitis that have become resistant, according to the findings of this study. Sulfametoxazole - Trimethoprim, Ciprofloxacin, and Chlorampenicol antibiotics had the lowest percentage of resistance (3.12%), while Ampicilin had the highest (100%)

2.3 Effect of cow dung extracts on microorganisms

Akhter *et al.* (2006) stated that when cow dung suspension is combined with *Azardiracta indica* at 2.5% concentration, they inhibit the 100% conidial germination. When treated with *Rauvolfia serpentina*, *Azardiracta indica*, *Datura metel and Swietiena macrophylla* extracts at concentrations of 1.0 to 1.5 %, conidial germination inhibition was not observed. When treated with *Rauvolfia serpentina* extract, the lowest conidial germination was inhibited, with 30 percent conidial germination inhibition counted only at 2.5 % concentration after 24 hours of incubation. The remaining plant extracts had a moderate to poor inhibitory effect on *B. sorokiniana* conidial germination. In this study, they observed that the increasing concentrations of plant extracts causes increase in inhibition of conidia germination rate.

Waziri *et al.* (2012) emphasized that the cow dung extract antimicrobial activities at different concentration rates against different bacteria like *Staphylococcus sp., B. subtilis* and *E. coli* by considering the zone of inhibition values exhibited by the bacteria. When compared with different concentrations of 0.3 mg/ml, 0.45 mg/ml, and 0.9 mg/ml, the 2.5 mg/ml concentration showed the most antibacterial activity. When compared to the other bacteria tested, *Staphylococcus sp.* (11 mm, 12 mm, and 14 mm in diameter) had the highest antibacterial activity. In this studies *Staphylococcus sp.* and *Cyanobacteria* are the most susceptible organisms because among four concentrations significant zones are observed at three concentrations when tested, but none of the bacteria tested showed any activity at 0.3 mg/ml. Many pathogens found in manure, according to Pachepsky *et al*. (2010) *Salmonella sp.,* *Campylobacter jejuni., Listeriamonocytogenes, Yersinia enterocolitica, Escherichia coli* and some protozoan species.

Omojowo *et al.* (2013) noticed that out of one hundred and thirty-two (132) bacteria were isolated from 1,000 samples of cow dung. *Escherichia coli* 66 (50%) was the most common, followed by *Staphylococcus aureus* 12 (9.1%), and the rest are other microbes. Non-fermentative typical strain O157:H7 *Escherichia coli* is found in twelve (12) of the (66) *E. coli* isolates from cow dung, accounting for 9.1 % isolates from cow dung. All isolates tested positive for Tetracycline, Ampicillin (85.6 %), Amoxicillin (83.3 %), Gentamicin (47.6%), Chloramphenicol (66 %), Erythromycin (44.4 %), and Nalidixic acid (44.4%) (18.3%). Multiple antibiotic drug resistance profiles in entericbacteria from human and animal sources have been reported.

Sushmitha Shrivastava *et al.* (2014) observed that as antimicrobial resistance is becoming a major threat now a days, so in order to avoid this, they conducted experiments regarding antifungal and antibacterial properties of cow dung extracts obtained by using solvents like distil water, acetone, ethanol, n-hexane and chloroform against *Staphylococcus sp, E. coli* and *Salmonella sp.* the results procured are highly effective against them. This proves that cow dung extracts has some antimicrobial properties that help to combat some infections.

Rajeshwari *et al.* (2016) said that the cow dung has high antimicrobial activity. It acts against all the test microorganisms in this study. Ethanol extract shows antimicrobial activity against all test organisms while acetone extracts shows only against *Klebsiella* and *E. coli* only. Jersey cow dung extract does not act against all test organisms. During this study it was concluded that, the ethanol extracts possess high antimicrobial activity against *E. coli* and *Klebsiella*. *E. coli* is resistant to both acetone and ethanol extracts of jersey cow dung extracts.

Atnafel *et al.* (2017) reported that extracts from cow dung had shown antimicrobial activity against some bacteria. Extracts obtained by using solvents like acetone and methanol had shown high antimicrobial activity. The acetone extract of buffalo dung exhibited high antimicrobial activity against *Klebsiella*, but the same organism is resistant to ethanol extract of the buffalo dung. The *Escherichia coli* were resistant to cow dung extracts obtained by using solvents like acetone and ethanol.

Nayak *et al.* (2019) reported that as many microorganisms became resistant to antibiotics and synthetic antimicrobial compounds, known natural alternatives can be used to treat a variety of diseases caused by these microorganisms. The antimicrobial activities of different cow dung extracts were then investigated using the disc diffusion technique against *Escherichia coli* and *K. pneumonia*. Pathogens like *Escherichia coli* and *K. pneumonia* were both active against the extracts from desi cow dung. The inhibition zone of microorganisms was visible in Indian cow dung extracts such as Jersey, Holstein, buffaloes, and others. Desi cow dung had antimicrobial properties against *Klebsiella pneumoniae and Escherichia coli.* In his studies he even evaluated the phytochemical analysis of various cow dung chloroform extracts.

Huygens *et al.* (2021) noticed that the extracts obtained from cow dung by using solvents like distilled water, acetone and n-hexane are showing high antibacterial and antifungal properties against *Candida, E. coli, Pseudomonas Staphylococcus aureus.* These investigations revealed that cow dung extract is likely to exhibit antimicrobial activity in order to fight against pathogenic diseases. Along with this antibacterial property it also has some nematicidal activity and also exhibit some probiotic activities.

Manishimwe *et al.* (2021) evaluated the antimicrobial activity in ethanol extract of Indian cow dung on test organisms in his studies. Acetone extract is also showing antimicrobial activity against both *E. coli* and *Staphylococcus*, but same organisms are resistant to cow dung extract from jersey.

MATERIALS AND METHODS

3.1 Sample collection

30 freshly voided dung samples were obtained from TVCC and different dairy farms near and around Bhubaneswar, stored in a sterile aseptic polythene bag and used in the laboratory for conducting research work.

3.2 Media used for culture

Standard protocols are used while preparing all the media used in the lab for this studies. The chemical reagents were dissolved by boiling, pH adjusted and sterilized by using autoclave at 15 lbs pressure at 121 ° C for a 15 mins. For some of the ingredients, sterilization by boiling water was done. The sterility of the prepared media was checked by incubating at 37° C for 24 hours. Generally media used for the study are prepared fresh and as per the necessities and if anything prepared is remaining or extra then it is to be stored at 4^0C in refrigerating after proper wrapping by aluminium foil.

Selective agar: The broth culture were streaked onto MacConkey lactose agar plate and Nutrient agar plate then incubated at 37°c for 24 hours. Characteristic growth of specific colonies can be transferred into EMB agar and MSA agar for further observation.

Some organisms were picked up in pure form in nutrient agar slant. Identification can be done by colony morphology, colour, size of colony, pH and nature of colony and different biochemical test like indole, catalase, oxidase, MR-VP, citrate and nitrate test.

All the antibiotic disks, media and agar used in these studies were obtained from Hi-media laboratory, Mumbai.

3.3 Microbial analysis

Standard broth like Brain heart infusion broth (BHI) was used for inoculating the collected samples and incubated at 37°c for 24 hrs. Different bacteria were isolated using the selective media like Eosine Methylene Blue agar (EMB), MacConkey lactose agar (MLA) and Mannitol Salt agar (MSA). Then based on colony morphology (size, colour and nature), pigment production and by various biochemical tests the organisms were identified.

3.4 Preparation of cow dung extracts (Methanol, Ethanol and Aqueous extracts)

Collected cow dung samples were dried with the help of hot air oven for 36-48hrs, then the dried powdered dung samples devoid of moisture were obtained.10gms of powdered dung was taken in three different conical flasks each separately and then 100ml of Methanol, Ethanol and distilled water were added in those conical flasks separately. Then those flasks were kept in rotary shaker for 3 days or shaken manually with the help of hands at a time interval of 15 minutes continuously for 3 days. After 3 days the extract was filtered into a conical flask using whatman no.1 filter paper (Rajeshwari, 2016).

3.5 Preparation of disks containing cow dung extracts

The empty disk was filled with 30µl of the Methanol, Ethanol and Aqueous extract of cow dung from desi cow and dried in oven. If the disk was not completely saturated with extract, then that process was repeatedly done until the disks get saturated with extracts (Rajeshwari, 2016).

3.6 Antibiotic sensitivity test

Disc diffusion antibiotic sensitivity test also known as Kirby-Bauer method is used to study antibiotic sensitivity of different bacteria using antibiotic disks saturated with a particular concentration. Zone of inhibition is the parameter used to evaluate the susceptibility of a particular bacteria that correlates with the degree of inhibition of growth of a particular bacteria on agar surrounding the antimicrobial ingredient containing disk. Whether organism is showing high or moderate sensitivity and resistance to a particular antibiotic disk was determined by the zone of inhibition.

After sample collection, it was inoculated into BHI broth at 37^0 C for 24hrs. Three to four similar colonies were taken from the pure bacterial culture slant of identified organism and then inoculate into the nutrient broth and incubated at 37`c for 24 hours. Initially 100 µl culture was spread with the spreader evenly on all over plate of the MHA for testing the antimicrobial activity of cow dung extract. Then saturated disk containing different extracts of cow dung and the other commercially available antibiotic discs were gently pressed on to the microbe inoculated Muller-Hinton agar plate at the scientific distance from each other in different plates correspondingly. Then Hi-Media scale was used to measure the zone of inhibition around the antibiotic used and around the cow dung extract impregnated disks.

3.7 In-vitro evaluation of antimicrobial susceptibility pattern of cow dung

In order to evaluate the antimicrobial activity, a method known as modified agar gel diffusion method was done on MHA. Liquid cultures inoculated with bacteria prepared using normal saline with the turbidity equal to that of the 0.5 ml McFarland standard such as *Escherichia coli* and *Staphylococcus sp.* were introduced on to the MHA plates, and they were laid on Muller-Hinton agar (MHA). Then empty disks impregnated with cow dung extract were placed on above inoculated MHA plates. The plates were incubated at 37^0C for 24hrs and later zone of inhibition was measured. Zone of inhibition was calculated in mm using Hi-Media scale.

3.8 Phytochemical screening of cow dung extract

The cow dung extracts obtained were used for phytochemical screening. Collect all the required materials like test-tubes, auto pipettes, sterile tip-box and racks to the working area.

3.8.1 Test for flavonoids

Lead acetate test

0.5 ml quantity of the cow dung extract was taken with the help of auto pipette in the test-tube. Then lead acetate solution was added drop by drop slowly to the cow dung extract. The yellow colour precipitate formed indicates that flavonoids are present in the cow dung.

3.8.2 Test for steroids

Salkowski test

2 ml of cow dung extract was collected using the auto pipette in the glass test-tube, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 were added to it and shaken

well. If the chloroform layer turns red and acid layer exhibits greenish yellow fluorescence, it indicates that steroids are present in the cow dung**.**

3.8.3 Test for tannins

5 ml of the cow dung extract was taken with the help of auto pipette in glass test-tube, then mixed with 1 ml of 10% lead acetate solution. If yellow precipitate is formed, it indicates the presence of tannins in the cow dung.

3.8.4 Test for phenols

Some quantity of the cow dung extract was taken in the glass test-tube. Then 0.5 ml of 20% sulphuric acid solution was used to dissolve the extract. Following it few drops of 2% Sodium hydroxide solution was added, if it turns blue it indicates that the cow dung contains phenols**.**

3.8.5 Test for glycosides

Some quantity of the cow dung extract was taken in the glass test-tube and then dissolved in 1 ml of water, later aqueous 10% Sodium hydroxide solution was added to the mixture of cow dung extract and water. The yellow color precipitation formed indicates the presence of glycosides in the cow dung**.**

RESULTS

Table 4.1.1: Sources of cow dung samples collection

Name of the institution/dairy farm from which cow dung sample is collected	Number of cow dung samples collected	Percentage of dung samples collected from different institute /dairy farm
Teaching Veterinary Clinical Complex, C.V.Sc., Bhubaneswar, OUAT	12	40%
Instructional Livestock Farm, OUAT, Bhubaneswar	6	20%
Ranjan Dairy Farm, Siripur, Bhubaneswar	3	10%
Veterinary Microbiology Department, O.V.C, Bhubaneswar	9	30%
Total	30	100

Table 4.1.2: Sources of cow dung samples collection (Breed wise)

Breed	Desi cattle	Holstein Friesian	Jersey	HF cross	Total
No. of Samples	15	6	3	6	30
% out of total samples	50%	20%	10%	20%	100

Table 4.2: ABST of *E. coli* and *Staphylococcus sp.* with commercially available antibiotics

Name of the Antibiotic	Zone of inhibition for *E. coli* culture in mm	Zone of inhibition for *Staphylococcus spp.* culture in mm
Amoxyclav (AMC^{30})	12mm	14mm
Ampicillin (AMP^{2})	2mm	4mm
Amikacin (AK^{30})	20mm	14mm
Doxycycline (DO^{30})	22mm	20mm
Enrofloxacin (EX^{10})	26mm	28mm
Gentamycin (GEN^{30})	22mm	23mm
Oxy tetracycline (O^{30})	4mm	6mm
Streptomycin (S^{25})	19mm	18mm
Vancomycin (Va^{30})	6mm	4mm

From the antibiogram studies conducted against *Escherichia coli* and *Staphylococcus sp.* isolated from mastitis milk samples, the results obtained were like *E. coli* (Gram-negative) and *Staphylococcus sp.* (Gram-positive) are highly sensitive to the antibiotics like Amikacin (AK^{30}), Doxycycline (DO^{30}), Enrofloxacin (EX^{10}), Gentamycin(GEN^{30}), Streptomycin(S^{25}) and are showing resistance towards antibiotics like Ampicillin (AMP^{2}), Amoxyclav (AMC^{30}), Oxytetracycline (O^{30}) and Vancomycin (Va^{30}).

Table 4.3: Mean ± standard error values of zone of inhibitions against *E. coli*

	Desi cattle	HF	Jersey	HF cross
Ethanol	5.6±0.559	5.5±1.118	3.0±0.557	5.5±0.847
Aqueous	2.53±0.559	1.5±0.719	2.67±0.333	3.0±1.155
Methanol	21.6 ± 1.226^{bcd}	13.67 ± 0.558^{ad}	10.67 ± 0.667^{a}	7.67 ± 0.882^{ab}
Antibiotics	15.93 ± 0.825^{bd}	11.83 ± 1.721^{ad}	16.67 ± 3.667^{d}	6.0 ± 0.966^{abc}

Values having different superscript differ significantly ($p<0.05$)

Ethanol extracts of the desi cattle dung (5.6±0.559) had shown high zone of inhibition followed by Holstein Friesian (5.5±1.118), HF cross (5.5±0.847) and Jersey (3.0±0.557).There was no significant difference found in between different breeds of cattle among ethanol extracts.

Aqueous extracts of HF cross cattle dung (3.0±1.155) dung had shown high zone of inhibition followed by desi cattle (2.53±0.559), Jersey (2.67±0.333) and HF (1.5±0.719).There was no significant difference found in between different breeds of cattle among aqueous extracts.

Methanol extracts of desi cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross. Methanol extracts of desi cattle (21.6±1.226) was statistically ($p<0.05$) higher as compared to the HF (13.67±), Jersey (10.67±0.667) and HF cross (7.67±0.882).Methanol extracts of HF has significant ($p<0.05$) difference when compared to desi cattle and HF cross. Methanol extracts of Jersey cattle has significant ($p<0.05$) difference when compared to desi cattle. Methanol extracts of HF cross has significant ($p<0.05$) difference when compared to desi cattle and HF.

In case of antibiotics Jersey (16.67±3.667) cattle dung has higher zone of inhibition followed by desi cattle (15.93±0.825), HF (11.83±1.721), HF cross (6.0±0.966). Desi cattle dung has significant ($p<0.05$) difference as compared to HF and HF cross. HF cattle dung has significant difference as compared to desi cattle and HF cross. Jersey cattle dung has significant ($p<0.05$) difference as compared to HF cross. HF cross cattle dung has significant ($p<0.05$) difference as compared to desi cattle, HF and Jersey cattle dung.

Results obtained for antimicrobial sensitivity of different cow dung extracts procured by using solvents like Methanol, Ethanol, Aqueous and antibiotics against *E. coli* were noted in the (Table 4.3). Out of 30 samples it was found that Methanol extracts of 24 (80%) cow dung samples in which it accounts for 13 samples of desi cattle, 5 samples of HF, 2 samples of jersey and 4 samples of HF cross were showing high sensitivity towards the test organism compared to the Ethanol and Aqueous extracts. They were found to exhibit a high or an almost all equal sensitivity pattern against test organism when compared with the zone of inhibition pattern of commercially available antibiotics noted in (Table 4.2). These extracts were even showing high antimicrobial activity when compared with some other antibiotic because they are resistant to test organism based on zone of inhibition noted in (Table 4.2).

Table 4.4: Mean ± standard error values of zone of inhibitions against *Staphylococcus sp*.

	Desi cattle	**HF**	**Jersey**	**HF cross**
Ethanol	5.2 ±.0763	6.33±1.706	9.0±0.577	4.67±1.145
Aqueous	3.73±.483	4.0±1.183	5.0±0.577	4.0±1.211
Methanol	$20.87±0.975^{bcd}$	$15.83±0.477^{ad}$	$14.0±0.00^{a}$	$10.83±1.138^{ab}$
Antibiotics	17.93±1.560	13.83±1.851	14.33±2.33	14.5±2.50

Values having different superscript differ significantly ($p<0.05$)

Ethanol extracts of the Jersey cattle dung (9.0±0.577) has shown high zone of inhibition followed by Holstein Friesian (6.3±1.706), desi cattle (5.2±0.763) and HF cross (4.67±1.145). There was no significant difference found in between different breeds of cattle among ethanol extracts.

Aqueous extracts of the Jersey cattle dung (5.0±0.577) has shown high zone of inhibition followed by Holstein Friesian (4.0±1.183), HF cross (4.0±1.211) and desi cattle (3.73±0.483). There was no significant difference found in between different breeds of cattle among aqueous extracts.

Methanol extracts of desi cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross. Methanol extracts of desi cattle (20.87±0.975) has significant ($p<0.05$) difference when compared to HF (15.83±0.477), Jersey (14.0±0.00) and HF cross (10.83±1.138). Methanol extracts of HF has significant ($p<0.05$) difference when compared to desi cattle and HF cross. Methanol extracts of Jersey cattle has significant ($p<0.05$) difference when compared to desi cattle. Methanol extracts of HF cross has significant ($p<0.05$) difference when compared to desi cattle and HF.

In case of antibiotics desi cattle (17.93±1.560) has shown high zone of inhibition followed by HF cross (14.5±2.50), Jersey (14.33±2.33) and HF (13.83±1.851). There was no significant difference found in between different breeds of cattle among antibiotics.

Results obtained for antimicrobial sensitivity of different cow dung extracts procured by using solvents like Methanol, Ethanol, Aqueous against *Staphylococcus sp.* were noted in the (Table 4.4). Out of 30 samples it was found that Methanol extracts of 21 (70%) cow dung samples in which it accounts for 10 samples of desi cow, 5 samples of HF, 2 samples of jersey and 4 samples of HF cross are showing high sensitivity towards the test organism compared to the Ethanol and Aqueous extracts. They were found to exhibit a high or an almost all equal sensitivity pattern against test organism when compared with the zone of inhibition pattern of commercially available antibiotics noted in (Table 4.2). These extracts showed high antimicrobial activity when compared with some other antibiotic because they are resistant to test organism based on zone of inhibition noted in (Table 4.2).

Fig. 1. Cow dung sample collection

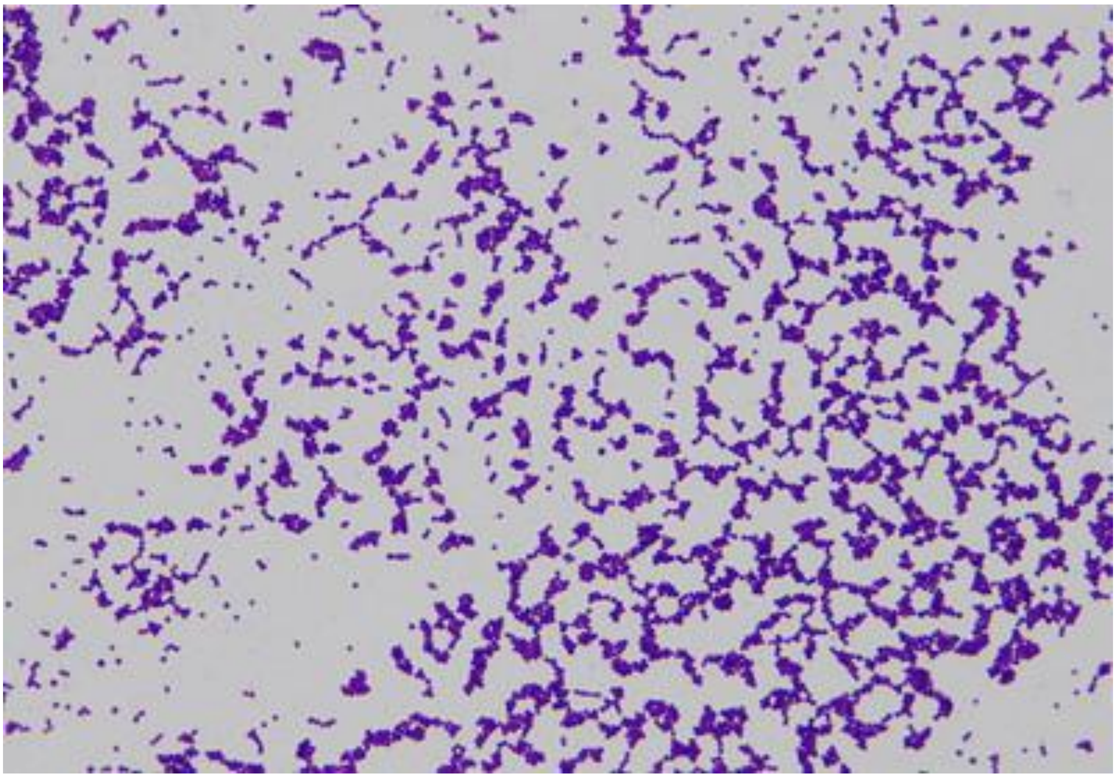


Fig. 2. *Staphylococcus sp.* in Grams staining

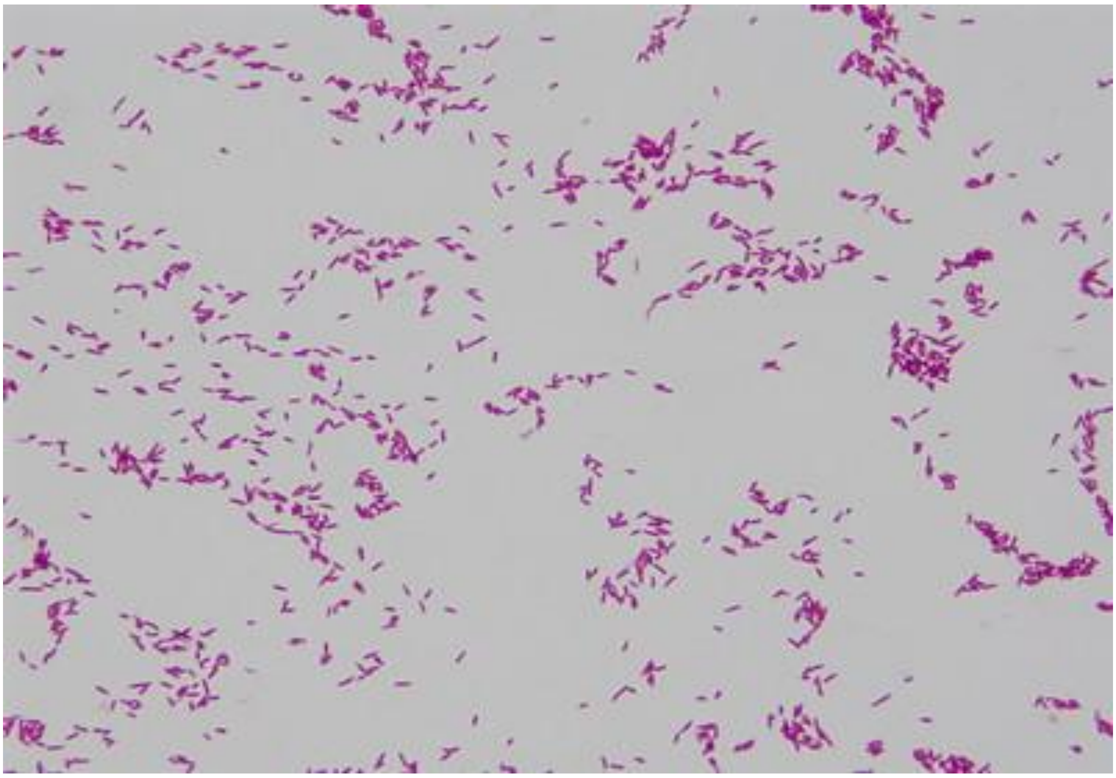


Fig. 3. ***Escherichia coli*** **in Grams staining**

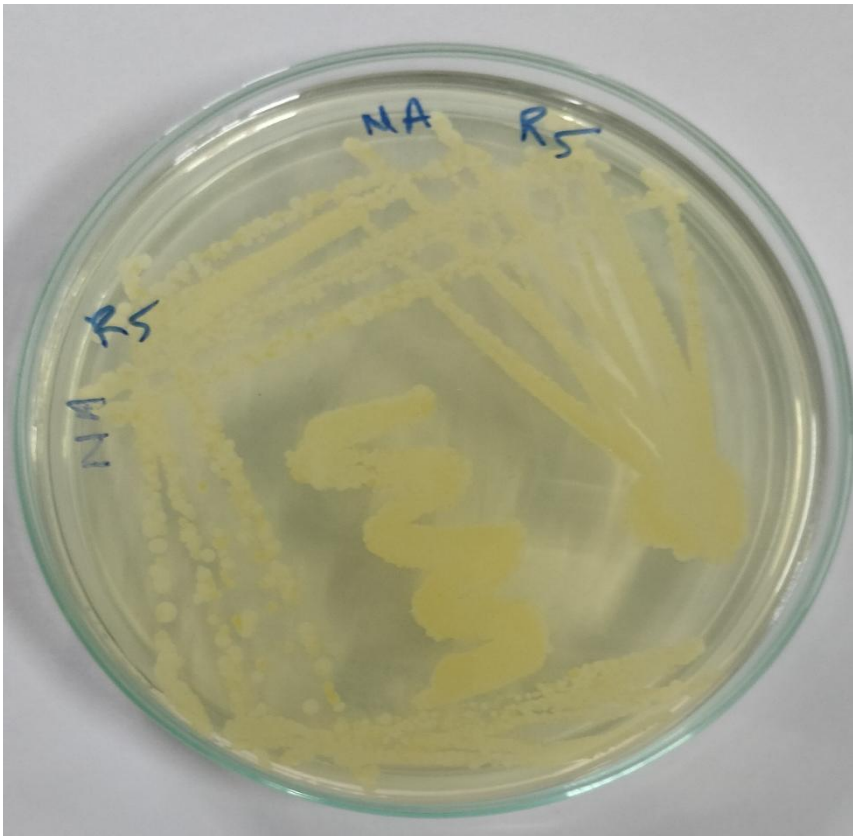


Fig. 4. Streaking of milk sample

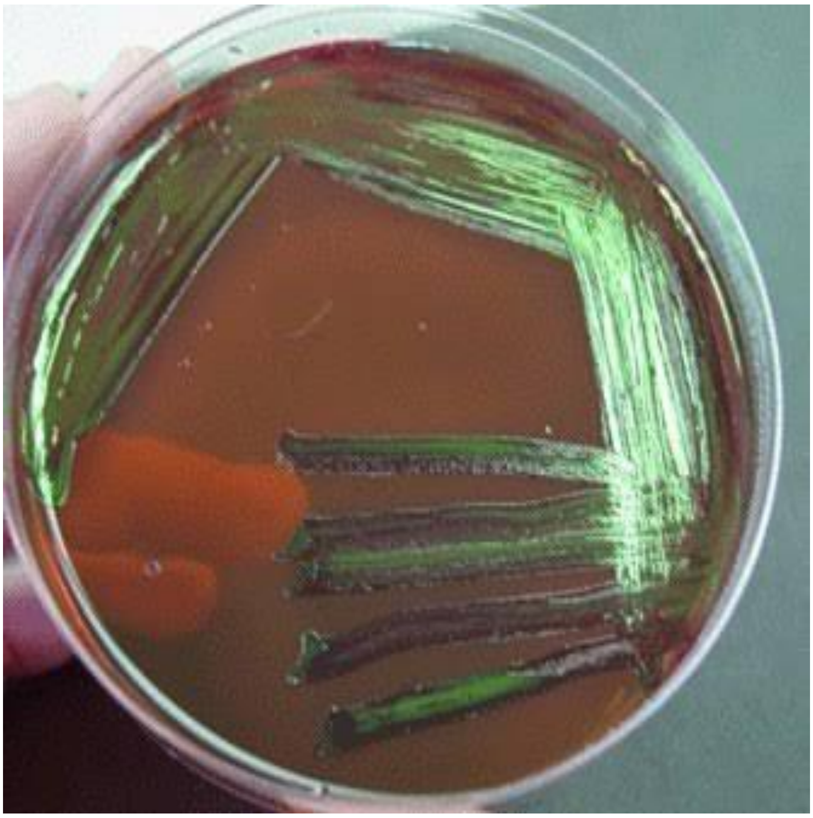


Fig. 5. ***E. coli*** **metallic sheen on EMB agar**

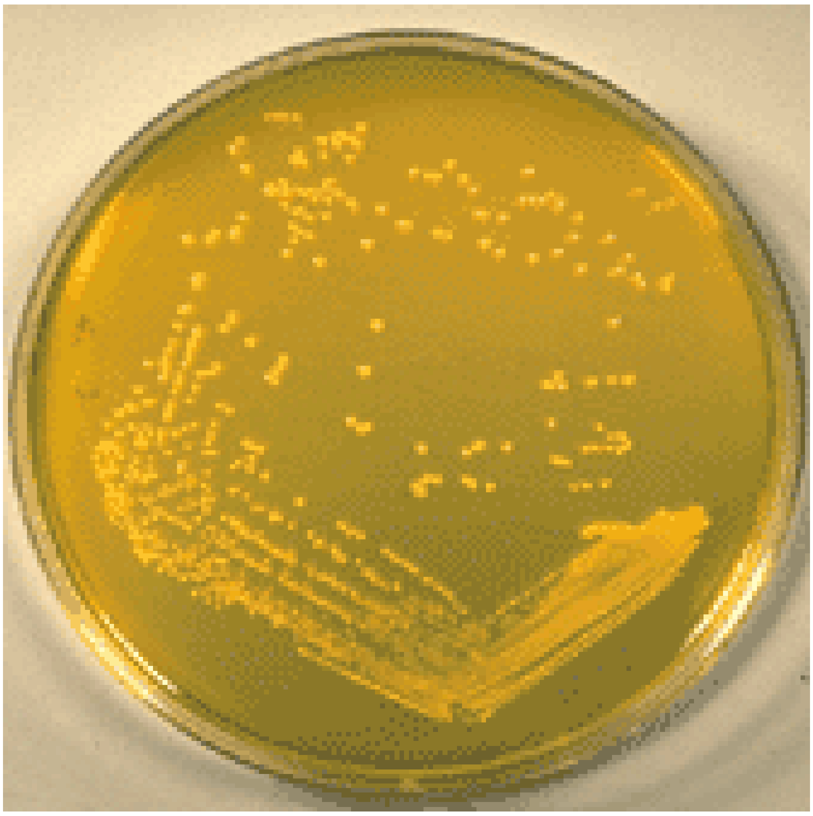


Fig. 6. ***Staphylococcus sp.*** **on MSA**

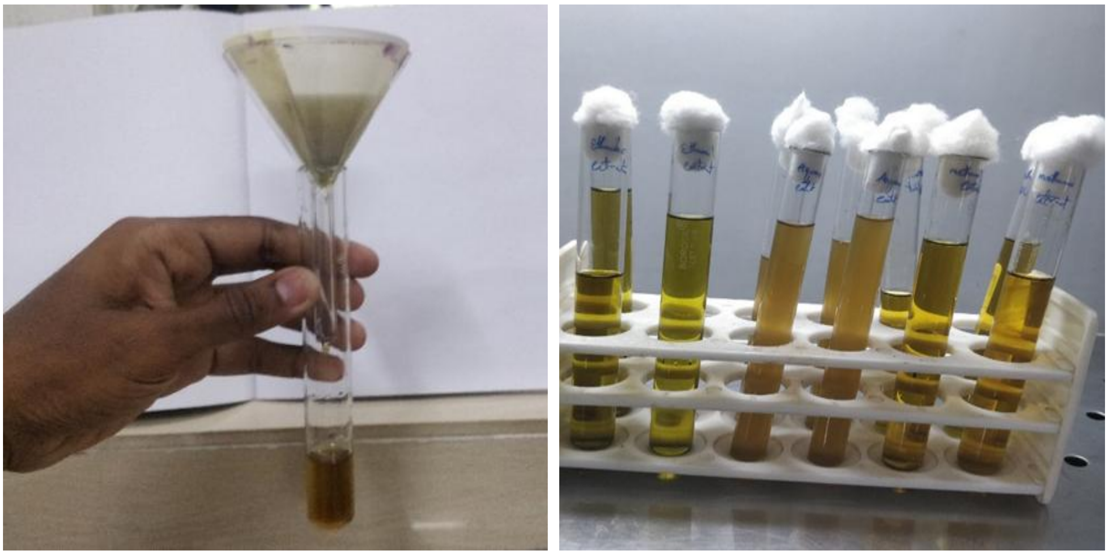


Fig. 7. Filtered cow dung extracts

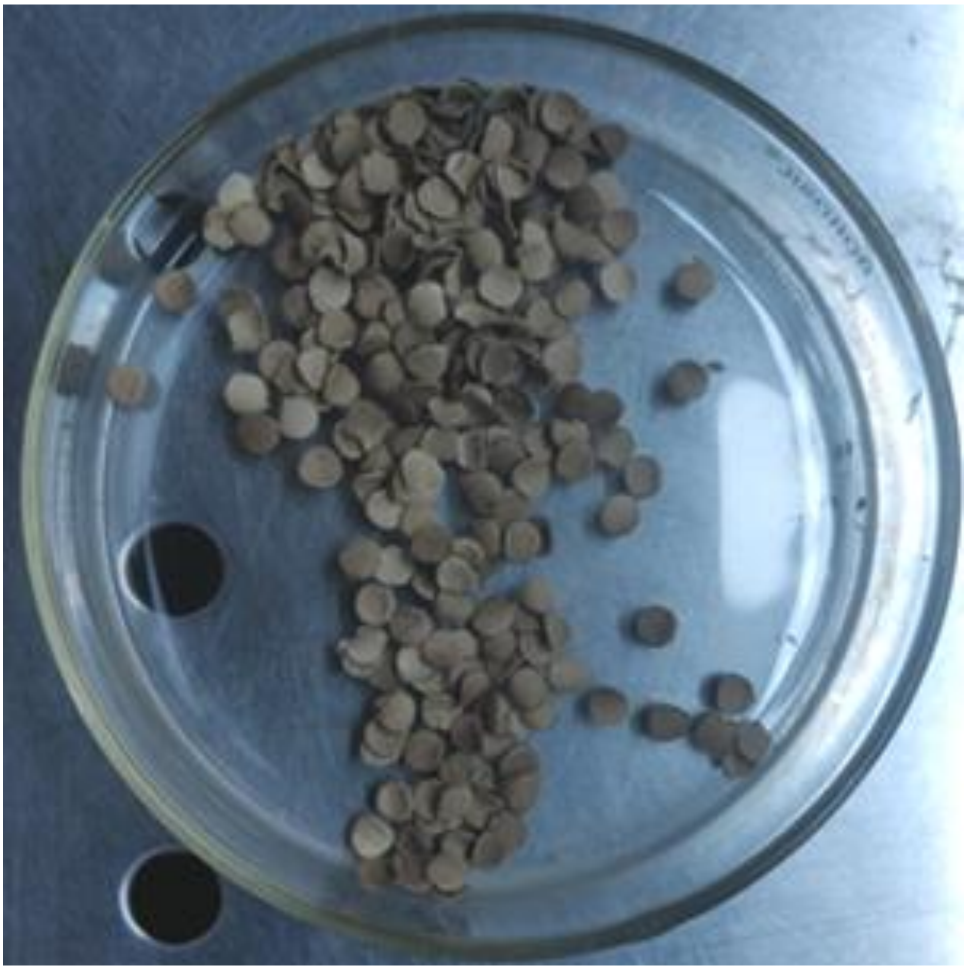
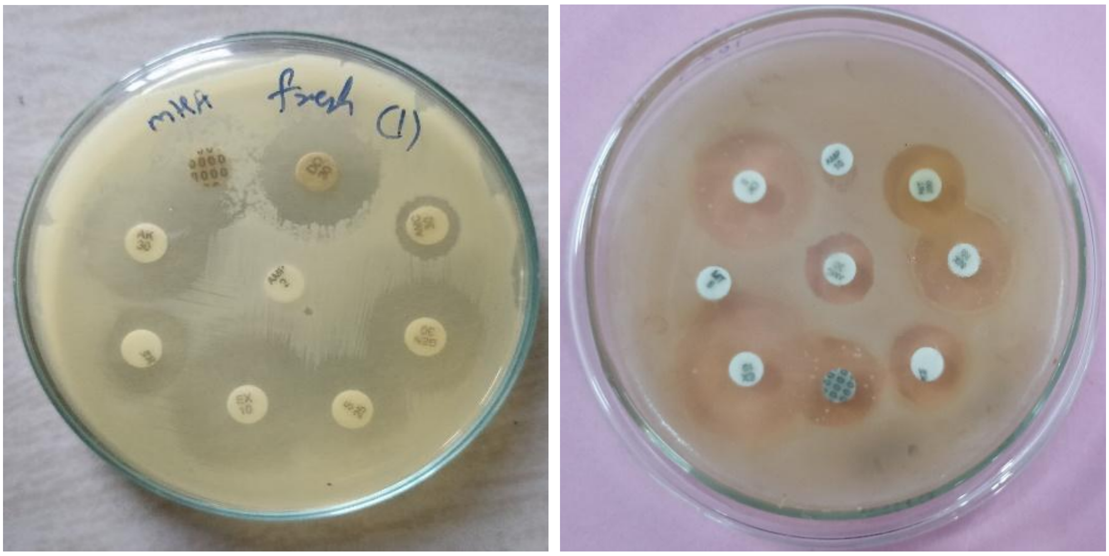


Fig. 8. Disks prepared from cow dung extracts

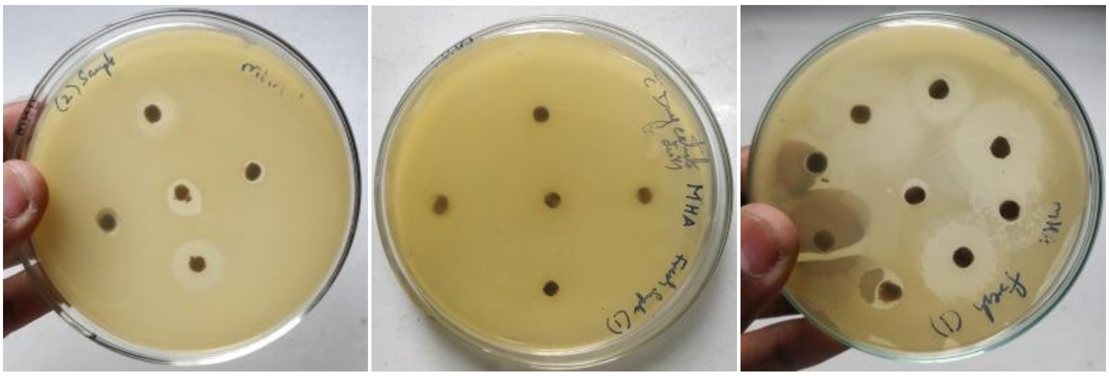


E. coli *Staphylococcus sp*

Fig. 9. ABST of *E. coli* and *Staphylococcus sp.* using antibiotic disks

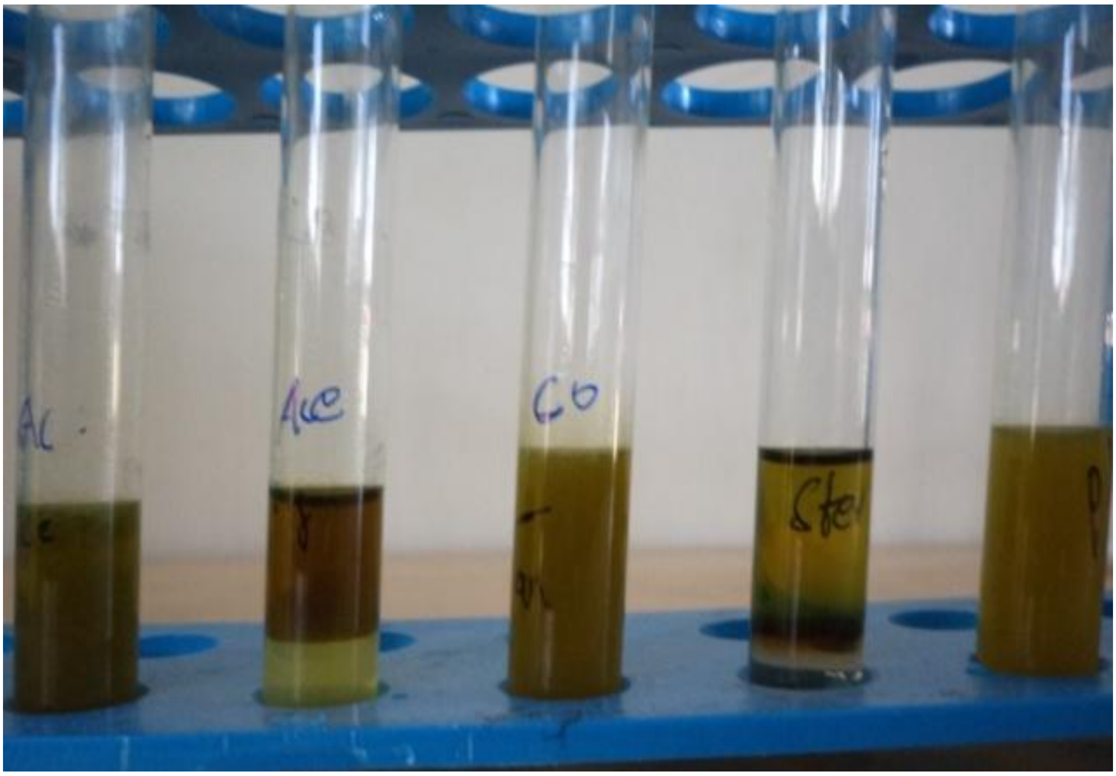
Ethanol extracts **Aqueous extracts** **Methanol extracts**

Fig. 10. ABST of *E. coli* using Ethanol, Aqueous, Methanol cow dung extracts

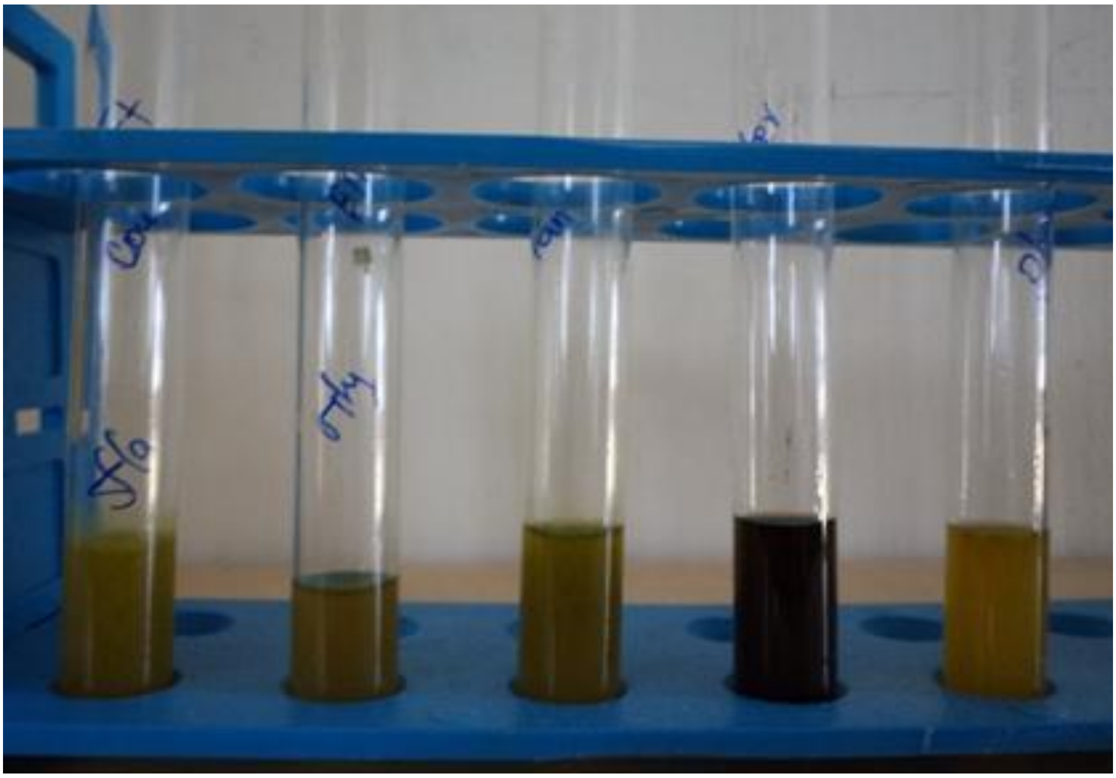


Ethanol extracts **Aqueous extracts** **Methanol extracts**

Fig. 11. ABST of *Staphylococcus sp.* using Ethanol, Aqueous, Methanol cow dung extracts



Test for phenol, steroids, glycosides, tannins in ethanol extracts



Test for phenol, steroids, glycosides, tannins in methanol extracts

Fig. 12. Phytochemical analysis of cow dung extracts

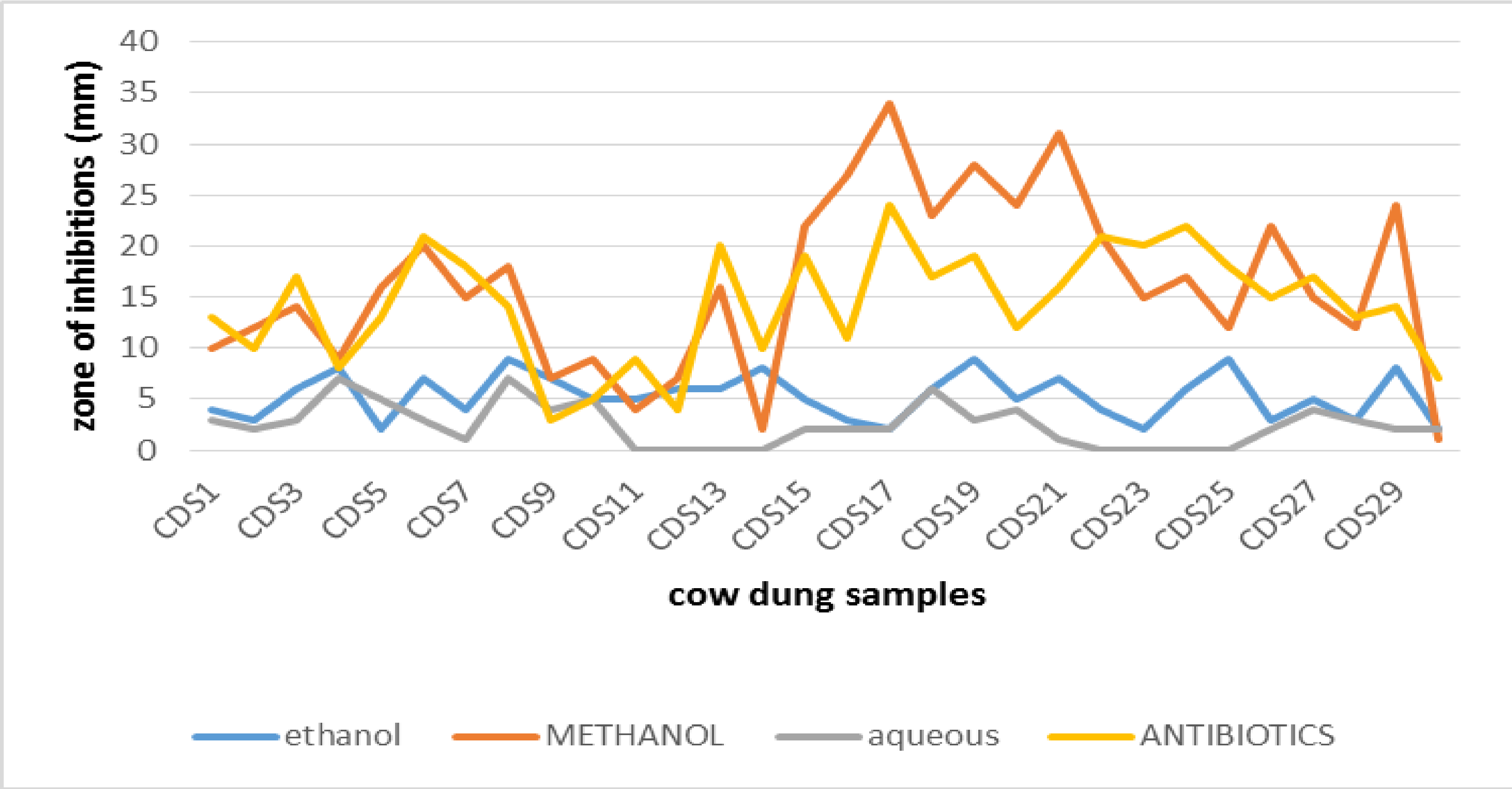


Fig. 13. Comparative analysis of antimicrobial effect of antibiotics and cow dung extracts against *E. coli*

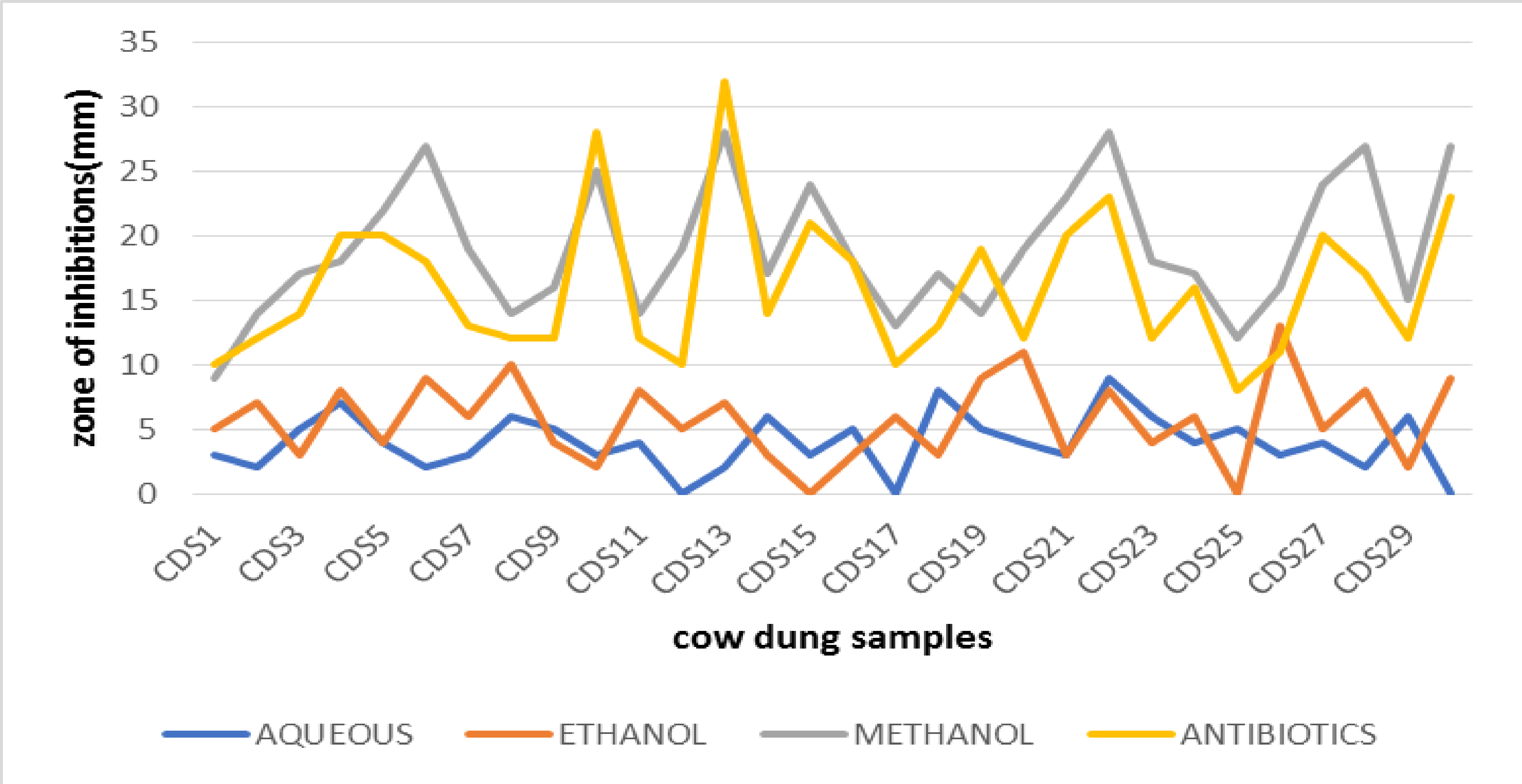


Fig. 14. Comparative analysis of antimicrobial effect of antibiotics and cow dung extracts against *Staphylococcus sp.*

DISCUSSION

Antibiotic resistance became a major issue that was making the microorganisms sensitive and they are becoming more pathogenic by acquiring many antibiotic resistance mechanisms (Borah *et al.,* 2014). As we all know that mastitis was the most common infectious disease in dairy industry causing severe loss in production parameters like decreased milk production. The treatment of mastitis also involves antibiotics that are too costly that even causes a burden to farmer and even during these days all the bacteria are showing resistance towards even higher antibiotics. It was observed that the incidence of mastitis cases in cows is a common problem among cattle breeds present in and around Bhubaneswar, Odisha. *Escherichia coli* and *Staphylococcus sp.* are the most common pathogenic causatives of these mastitis cases in cattle. So it is necessary to find an alternative to antibiotics in treating these infectious diseases.

From the studies done on "**Study of antimicrobial activity of cow dung against *Escherichia coli* and *Staphylococcus* species**" we focused on some of the naturally obtained products like cow dung to be used as an alternative to antibiotics as it was believed to have some antimicrobial activity (Rajeshwari, 2016) and has some antibacterial activity (Bag *et al.*, 2021). *E. coli* and *Staphylococcus sp.* are isolated from the milk samples collected from cattle suffering with mastitis because they are the most common pathogenic causative for mastitis. Even an antimicrobial analysis using commercially available antibiotic disks has also been conducted on pathogenic causative organisms. Antibiotics like Amoxyclav (AMC^{30}), Ampicillin (AMP^{2}), Amikacin (AK^{30}), Doxycycline (DO^{30}), Enrofloxacin (EX^{10}), Gentamycin (GEN^{30}), Oxytetracycline (O^{30}), Vancomycin (Va^{30}), Streptomycin (S^{25}). Then all the plates were incubated at 37^{0} C for 24 hrs then zone of inhibition is recorded. On observation it was found that they were showing resistance to most of the antibiotic (Sato, 2005 and Dhama *et al.*, 2005).

As discussed above 30 freshly collected dung samples were collected aseptically in containers and dried in hot air oven for 36 hrs to get dried powdered cow dung and then it was used in preparation of Ethanol, Methanol and Aqueous extracts of cow dung. Then empty antibiotic discs were impregnated with different extracts and prepared cow dung disks for evaluating their antimicrobial activity on the above isolated microorganisms. MHA plates inoculated with pure cultures of *E. coli* and *Staphylococcus sp.* were used for antibiogram using antibiotic disks prepared from Ethanol, Methanol and Aqueous extracts of cow dung as like done by chloroform extracts (Liu, 2018). It was also believed that phytochemical substances are present in cow dung extracts plays no role in contributing for their antimicrobial activity (Nayak, 2019).

From the results procured it was observed that Methanol extracts of cow dung are exhibiting high microbial activity when compared with other extracts. Out of 30 samples, around 24 (80%) and 21 (70%) of methanol extracts of cow dung were showing more activity when compared with the antibiotics showing resistance towards *E. coli* and *Staphylococcus sp.* based on the zone of inhibition values as mentioned in (Tables 4.2, 4.3, 4.4). For some samples it was even showing similar zone of inhibition. So it was advisable to use cow dung as an alternative to commercially available antibiotics in order to decrease antibiotic resistance and even in treating mastitis cases caused by pathogenic organisms like *E. coli* and *Staphylococcal* infections that even makes the cost of treatment cheaper when compared with expensive antibiotics.

SUMMARY AND CONCLUSION

From the above study it was concluded that the most common pathogenic microorganisms causing the mastitis infection are *Escherichia coli* and *Staphylococcus sp*. Mastitis was the most common disease among dairy cattle leading to decreased milk production and cost of treatment for mastitis includes too expensive antibiotics that in turn affects the economy of farmer leading to financial burden. Due to indiscriminate use of antibiotics, antibiotic resistance also became a booming issue leading to increased antimicrobial resistance of microorganisms towards commercially available antibiotics. So in this study we made an attempt to use some of the naturally obtained products like cow dung possessing some antimicrobial activity as an alternative to antibiotics against *Escherichia coli* and *Staphylococcus sp.* and observed their antimicrobial activity on these test organisms.

In the present study we prepared aqueous extract, methanol extract and ethanol extract from cow dung samples obtained from desi cattle, Jersey, HF and HF cross bred cows. Out of which it was observed that methanol extracts of cow dung are showing almost all equal sensitivity to some antibiotics like Amikacin (AK^{30}), Streptomycin (S^{25}) and more than some of the antibiotics like Ampicillin (AMP^{2}), Amoxyclav (AMC^{30}). For *E. coli*, methanol extracts of desi cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross. Methanol extracts of desi cattle (21.6±1.226) was statistically ($p<0.05$) higher as compared to the HF (13.67±), Jersey (10.67±0.667) and HF cross (7.67±0.882). Methanol extracts of HF was significant ($p<0.05$) difference when compared to desi cattle and HF cross. Methanol extracts of Jersey cattle was significant ($p<0.05$) difference when compared to desi cattle. Methanol extracts of HF cross was significant ($p<0.05$) difference when compared to desi cattle and HF. For *Staphylococcus sp.*, methanol extracts of desi cattle dung has shown high zone of inhibition followed by HF, Jersey and HF cross. Methanol extracts of desi cattle (20.87±0.975) was significant ($p<0.05$) difference when compared to HF (15.83±0.477), Jersey (14.0±0.00) and HF cross (10.83±1.138). Methanol extracts of HF was significant ($p<0.05$) when compared to desi cattle and HF cross. Methanol extracts of Jersey cattle was significant ($p<0.05$) difference when compared to desi cattle. Methanol extracts of HF cross was significant ($p<0.05$) difference when compared to desi cattle and HF.

So finally it was concluded that methanol extracts of cow dung samples are exhibiting high antimicrobial activity against *Escherichia coli* and *Staphylococcus sp.* revealing that cow dung possess antimicrobial activity and can be used to prevent *E. coli* and *Staphylococcus* infections in treating mastitis cases and it is even helping farmer because cow dung being least expensive, abundant in nature and also easy for processing. Phytochemical substances present in the cow dung includes glycosides, tannins, phenols plays no role in contributing towards antimicrobial activity of cow dung extracts.

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