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## Antibacterial activity of ethanolic extract of garlic (*Allium sativum*) against *Escherichia coli* isolated from postpartum metritis affected crossbred cows

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### Abstract

Postpartum metritis and injudicious antibiotic intrauterine therapy are the most common causes of infertility that contribute to economic losses for cattle owners. Moreover, it is crucial to find novel, potent antibacterial agents to solve the problem of antibacterial resistance in cows. Hence, the present study aimed to evaluate the antimicrobial activity of the ethanolic extract of garlic (*Allium sativum*) against *Escherichia coli*. A total of 18 postpartum metritis cows were selected based on their history, clinical signs, vaginal and per-rectal examinations. Under aseptic procedure, *E. coli* isolates were recovered from the uterine samples of metritis cows and subjected for antibacterial sensitivity test. The antibacterial activity of crude, 20%, 10% ethanolic extract of garlic and commercially available antibiotics were tested against *E. coli* using the disc diffusion method. Crude extract of garlic yielded higher antimicrobial activity (19.66±0.42 mm) next to gentamicin (21.22±0.55 mm) against *E. coli* followed by 20%, 10% ethanolic extract. The zone of inhibition formed by 20%, 10% garlic extract was higher and found equivalent to cefuroxime, chloramphenicol, tetracycline and ciprofloxacin. Whereas, ampicillin and sulpha/Trimethoprim showed resistance for *E. coli*. Based on the above results, it was found that ethanolic extract of garlic had *in vitro* antimicrobial activity against *E. coli*, similarly it may also act *in vivo*. In conclusion to combat antibiotic resistance garlic extract can be recommended as alternate choice for treating postpartum metritis in cows.

**Keywords:** *Allium sativum*, antibacterial activity, ethanolic extract, metritis

### 1. Introduction

Several conditions that can lead to infertility in the dairy industry include retained fetal membrane, pyometra, endometritis, and metritis (Deori and Arundhati, 2015) [3]. Metritis is more common in dairy and beef breed cattle and often appears ten days following parturition. The most common symptoms of metritis include an enlarged uterus and a watery, reddish-brown to viscous, off-white, purulent uterine discharge that is frequently accompanied by an unpleasant odour. Metritis is categorized into three grades. Cow with grade 1 metritis show significantly enlarged uterus and purulent uterine discharge but no other clinical signs. Cows with grade 2 metritis exhibit fever along with an enlarged uterus and purulent uterine discharge. Grade 3 metritis cow displays toxæmic signs such as inappetence, cold extremities, depression and/or collapse (Sheldon *et al.*, 2009) [17].

Sheldon and Dobson (2004) [15] reported that during the first 14 days after calving, up to 40% of animals develop metritis; in 10 to 15% of these cases, the infection continues for at least three more weeks, leading to chronic endometritis. A variety of aerobic species were cultured in the early postpartum cows, which includes *Escherichia coli*, *Fusobacterium* spp., *Clostridium* spp., *Bacteroides* spp., and *Trueperella pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus* spp., *Pasteurella multocida*, and others (Dohmen *et al.*, 2000; Sheldon *et al.*, 2008 and Leblanc *et al.*, 2011) [4, 16, 10]. It has been reported that 36 percent of the bacterial isolates from the postpartum uterus were coliforms. According to Smith and Risco, (2002) [18] *E. coli* accounted for 29% of the bacterial isolates recovered from the cows with metritis.

Systemic or local antibiotics, antiseptics and hormones are being used as treatment for postpartum metritis. However, they have their own limitations as well.

According to Hussain and Daniel (1991) [8], these limitations include development of drug resistance, compromised uterine defense, high cost of treatment, milk withdrawal and uncertain recovery rate. Hence, there is need to evaluate alternative and effective antibacterial therapies for uterine diseases like herbs, nutrition, immunomodulators and others (Jadhav and Bhutani, 2005) [9].

Many medicinal plants have been shown to have a role in treating infertility in recent years. Garlic (*Allium sativum*), one of the valuable spice also used as a medicine. Research has demonstrated that garlic has wide range of medicinal benefits, including as lipid-lowering, antiviral, antibacterial, antifungal, anticancer, anti-inflammatory, and antihypertensive (Tesfaye, 2021) [19].

The present study was carried out to evaluate the antibacterial activity of garlic extract against *Escherichia coli* isolates from the uterine discharge of postpartum metritis cows and comparing it with the antibiotics used to treat postpartum metritis.

## 2. Materials and Methods

### 2.1. Preparation of garlic extract

About 250 g of peeled garlic cloves were ground into paste and then filtered through muslin fabric to yield 150 ml of crude extract. To that 150 ml of crude extract, 100 ml of 90% ethanol was added. Distilled water was added to the crude extract to make up the volume to 500 ml. After being allowed to mature for one night at room temperature, the extract was filtered using Whatman filter paper No. 1 and incubated for six hours at 40 °C. The resulting crude extract of garlic was refrigerated at 4 °C until it was used again. 10% and 20% ethanolic extract of garlic were made by diluting the extract with regular saline (Sarkar *et al.*, 2006; Enejiyon *et al.*, 2020) [14, 5].

### 2.2. Isolation of organism

After cleaning the perineum, adhering to the aseptic precautions, the uterine discharge was collected using a sterile AI sheath by aspiration technique using sterile syringe adopting negative pressure technique. All uterine samples

were transferred to sterile containers and transported in ice to the laboratory. A loop full of uterine samples were inoculated in nutrient broth and incubated at 37 °C overnight and observed for turbidity on the next day which indicated growth of organism. The culture from the nutrient broth was streaked on EMB (Eosin Methylene Blue) agar plate (HiMedia, Mumbai) and incubated overnight at 37 °C (Mekibib *et al.*, 2023) [11]. *Escherichia coli* was identified based on the colony morphology (Figure. 1).

### 3. Evaluation of Antibacterial sensitivity by agar disc diffusion method

Pure culture colonies after isolation was inoculated in nutrient broth and incubated overnight at 37 °C. Next day, a sterile cotton swab after being dipped into the culture, was spread gently on the Mueller Hinton Agar (MHA) plates. A sterile filter paper discs were prepared and impregnated with crude, 20% and 10% garlic extract and seeded on MHA plates. Using sterile forceps, the disc was carefully pressed in order to ensure the contact (Gabriel *et al.*, 2022) [7]. To compare the antimicrobial activity of garlic extract with the antibiotics used to treat bovine postpartum metritis, antibiotic discs (HiMedia, Mumbai) were used. The antibiotic diffusion discs contain Chloramphenicol (25 µg), Ampicillin (25 µg), Tetracycline (25 µg), Gentamicin (30 µg), Sulpha/Trimethoprim (25 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg) and Ciprofloxacin (10 µg). The antibiotic discs were placed on MHA plates and incubated at 37 °C overnight. Following incubation, the diameter of zone of inhibition was measured (Mekibib *et al.*, 2023) [11].

Statistical Package for the Social Sciences (SPSS-21) was used for Statistical analysis. 't'-test was used to compare the mean values as a test of significance.

### 4. Results and Discussion

Using agar disc diffusion method, the antibacterial activity of ethanolic extract of garlic was assessed against *Escherichia coli* and compared with commercially available antibiotics. The diameter of zone of inhibition was measured. The results are illustrated in Figure. 2 and Figure. 3.

**Table 1:** Antibacterial activity of different concentrations of ethanolic extract of garlic against *E. coli*

Isolate	Diameter of zone of inhibition (mm)			p-value
	Crude extract	20% garlic extract	10% garlic extract	
<i>Escherichia coli</i>	19.66±0.42	16.77±0.36	13.72±0.51	0.00***

\*\*\*- highly significant

**Table 2:** Antibacterial activity of commercially available antibiotics against *E. coli*

Antibiotics	Diameter of zone of inhibition (mm)
Chloramphenicol	14.05±1.40
Tetracycline	10.77±1.52
Gentamicin	21.22±0.55
Ceftriaxone	16.77±2.02
Cefuroxime	12.11±1.69
Ciprofloxacin	14.05±1.61

p – value: 0.00 (Highly significant)

The results showed that the *E. coli* exhibited different susceptibility for ethanolic extract of garlic at different concentrations of crude (100%), 20% and 10% which was similar to the results obtained by Mohammed *et al.*, (2020) [12]. Among them, crude extract of garlic yielded maximum zone of inhibition of 19.66±0.42 mm followed by 20% and 10% garlic extract whose zone of inhibition was 16.77±0.36

mm and 13.72±0.51 mm (Table. 1) respectively. There is significant difference between various concentrations of garlic extracts.

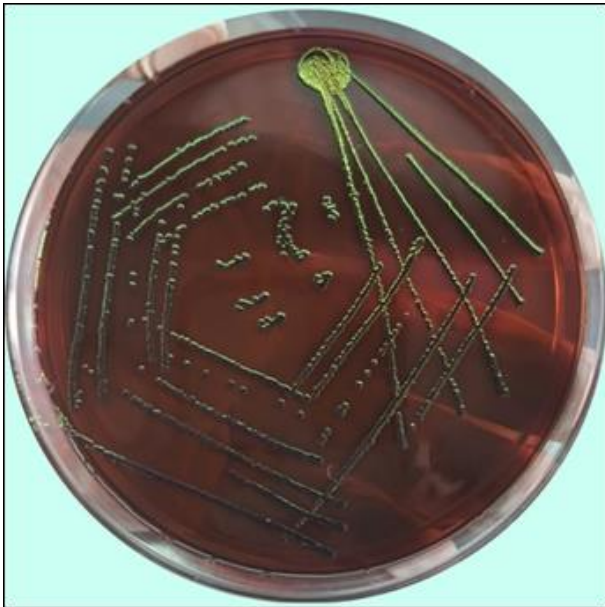
The mean zone of inhibition for *E. coli* by gentamicin was 21.22±0.55 mm which was higher than other antibiotics. The zone of inhibition by other antibiotics like chloramphenicol, tetracycline, ceftriaxone, cefuroxime and ciprofloxacin was 14.05±1.40 mm, 10.77±1.52, 16.77±2.02, 12.11±1.69 and 14.05±1.61 (Table. 2) respectively. *E. coli* was resistant to Ampicillin and Sulpha/Trimethoprim. Additionally, it was found that *E. coli* isolated from individuals showed resistance to some antibiotics and this could be due to repeated previous exposure of these individuals to that antibiotics. Basbas *et al.* (2022) [1] isolated *E. coli* from metritis cows and assessed antibacterial resistance against *E. coli* and concluded that *E. coli* become resistant to oxytetracycline or chlortetracycline in farms where these antibiotics were used to treat metritis, compared to those farms that does not practice this.



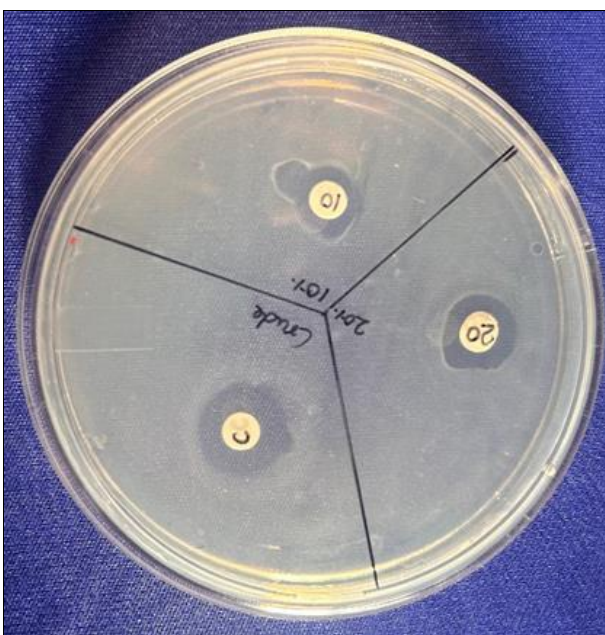
Gentamicin yielded higher zone of inhibition when compared to garlic extract. However, crude garlic extracts showed higher antimicrobial activity next to gentamicin than other antibiotics. The zone of inhibition formed by 20%, 10% garlic extract was moreover equal to cefuroxime, chloramphenicol and tetracycline. There is significant difference between garlic extract and other antibiotics.

The antimicrobial activity of the garlic is due to presence of sulphur containing compound allicin. Allin in the garlic is converted into allicin by the enzyme alliinase when garlic is cut or crushed (Daynea *et al.*, 2014) [2]. According to the study done by Feldberg *et al.*, (1988) [6], allicin partially inhibits the synthesis of bacterial DNA and proteins and totally inhibits the synthesis of RNA.

The findings of the current study is in consistent with the studies carried out by Rahi *et al.*, (2013) [13] and Enejiyon *et al.*, (2020) [5].



**Fig 1:** *E. coli* on Eosin Methylene Blue agar showing Green Metallic Sheen



**Fig 2:** Antimicrobial activity of garlic extract of different antibiotics against *E. coli* In Muller Hinton Agar



**Fig 3:** The zone of inhibition of different antibiotics against *E. coli* in Muller Hinton Agar

## 5. Conclusion

Ethanollic extract of garlic showed higher zone of inhibition next to gentamicin. *In vitro* antimicrobial activity of garlic against *E. coli* was higher when compared to other commercially available antibiotics. Similarly, it may also act *in vivo*. In conclusion, it is suggested that garlic extract can be used as an alternate drug for postpartum metritis treatment in cows to nullify antibiotic resistance.

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