

Original Research Article

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Isolation of *E. coli* from Bovine Mastitis and their Antibiotic Sensitivity Pattern

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ABSTRACT

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A total of 300 milk samples from buffaloes were evaluated for mastitis and incidence of *E. coli*. Overall, 157 milk samples (52.33%) were positive for mastitis and out of these positive isolates, 27 bacterial isolates (17.19 %) were identified as *E. coli*. All the 27 isolates of *E. coli* were tested for *in vitro* sensitivity towards 15 antibacterial drugs. The highest resistance was attributed towards antibiotic Kanamycin followed by Cefotaxime, Trimethoprim, Cefotaxime + Clavulanic acid, Amoxicillin, Cefixime, Tetracycline, Ampicillin + Salbactam, Amikacin, Chloramphenicol, Nitrofurantoin, Gentamicin, Cefoperazone, Ceftriaxone and Ciprofloxacin. Simultaneously resistance to 2 to 9 antibacterial agents, was observed in all 27 (100%) isolates.

Introduction

Mastitis is considered to be the most common cause of injudicious antibiotic use in dairy animals which leads to treatment failure, escalated treatment costs and development of resistance to antimicrobials.

Inappropriate usage of antimicrobials such as wrong dose, drug or duration may contribute to the increase in antimicrobial resistance without improving the outcome of treatment (Williams, 2000).

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature. It is mainly caused by

microorganisms usually gram-negative and gram-positive bacteria, mycoplasmas, yeasts and algae (Zadoks *et al.*, 2011). The multiplicity of the cause and emergence of resistance due to indiscriminate and prolonged use of antibiotics in absence of antibiogram is a major hurdle in the control of mastitis (Jeykumar *et al.*, 2013).

The usage of antibiotics correlates with the emergence and maintenance of antibiotic resistant traits within pathogenic strains. These traits are coded by particular genes that may be carried on the bacterial chromosome, plasmids, transposones or on gene cassettes that are incorporated into integrons (Daka *et al.*, 2012) thus are easily transferred among isolates.

Materials and Methods

A total of 300 milk samples were collected from buffaloes and animals found positive for clinical and subclinical mastitis (SCM) were included in the present study. The cases of clinical mastitis were diagnosed on the basis of physical examination of udder (swelling, redness, pain on palpation, induration) and milk color- (yellow or blood tinged and consistency- watery, flakes, etc.). The subclinical mastitis was diagnosed by California mastitis test (Schalm *et al.*, 1971).

Isolation of bacteria

Milk samples from clinical cases and those showing (++) or more reaction in CMT were cultured bacteriologically to isolate and identify the *E. coli* bacteria. Positive sample was inoculated in BHI and incubated aerobically at 37 °C for 24 hrs and then streaked on nutrient agar media and incubated aerobically at 37 °C 24 hrs to obtain isolated single bacterial colonies.

The differential media and selective media used were MacConkey media and EMB, respectively. The gross cultural characteristics of the colonies were observed and after that Gram staining of cultures was done.

Identification of bacteria

The presumptive identification of bacterial isolates as *E. coli* was accomplished by colonial and bacterial morphology. Further, the biochemical tests were carried out as per procedure described by Cruickshank *et al.*, (1973), Barrow and Feltham (1993), Collee *et al.*, (1996) and Malik (2003).

Both traditional methods and readymade kits (Hi *E. coli* identification kits, Hi media) were used for biochemical characterization of *E. coli* isolates.

Antibiotic sensitivity test

In vitro antibiotic sensitivity test of the *E. coli* isolates towards 15 antibacterial drugs was conducted as per the method of Bauer *et al.*, (1966). Antibiotics used in the present study were: Amikacin, Amoxicillin, Ampicillin, Cefixime, Cefoperazone, Ceftriaxone, Cefotaxime/clavulanic acid, Cefotaxime, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Nitrofurantoin, Tetracycline and Trimethoprim.

A loopful of the growth from slant was inoculated in BHI and incubated at 37 °C for 18-24 hrs. and then the surface of preincubated and sterile Muller Hinton agar (Hi Media) was uniformly smeared with the culture swab and the plate was kept at room temperature for 30 min to allow the inoculums to be adsorbed on the surface and then the antibiotic discs (Hi Media) were placed on the media surface at equal distances and sufficiently separated from each other. The plates were incubated overnight at 37 °C. Diameters of clear zone of inhibition around antibiotic discs were measured in mm. The interpretation of result was made in accordance with the instruction supplied by manufacture.

Results and Discussion

Antimicrobial agents are of great value for devising curative measures against bacterial infections. But antibiotic-resistant bacteria pose a severe challenge to both veterinary and health professions and dairy animal producers because they have a negative impact on therapy. Development of resistance has been attributed to the extensive therapeutic use of antimicrobials (Abo-Shama, 2014).

E. coli is the major pathogen causing environmental mastitis and it is one of the most important coliforms that have received

more attention due to its high incidence relatively to other mastitis pathogens (El-Sayed Lamey, 2013).

A total of 300 milk samples from buffaloes were evaluated for mastitis and incidence of *E. coli*. Overall, 157 milk samples (52.33%) were positive for mastitis. Buffaloes have some characteristics that may contribute to greater risk of mastitis. For example, the udder is more pendulous in comparison with cattle (Moroni *et al.*, 2006). The overall prevalence recorded in the present study was different from the reports of Farooq *et al.*, 2008 who reported only 9.3% prevalence and Bhanot *et al.*, 2012 who reported high (78.1%) prevalence of buffalo mastitis.

Out of 157 buffalo mastitis positive isolates, 27 bacterial isolates (17.19 %) were identified as *E. coli*. The frequency of *E. coli* observed in present study was in close agreement with the data obtained by Khan and Muhammad (2005), Farooq *et al.*, (2008), Hameed *et al.*,

(2008), Ali *et al.*, (2011), Bhanot *et al.*, (2012), El-Sayed Lamey *et al.*, (2013) who observed 18%, 16%, 15.38%, 16.18%, 16.3%, 18.47% frequency of *E. coli*, respectively in buffalo milk samples.

Antimicrobials are routinely used for treatment of dairy animals affected with clinical and subclinical infections. Over the years, antimicrobial susceptibility testing has become under scrutiny because of concerns about antimicrobial resistance, changes in methodology and the relationship between *in vitro* results and on-farm clinical outcomes (Idriss *et al.*, 2014).

All the 27 *E. coli* isolates were tested for *in vitro* sensitivity towards 15 antimicrobial drugs and interpreted according to the manufacturer's (HiMedia) instructions (Plate 1). These 15 antibiotics belonged to the seven groups viz. fluoroquinolones, aminoglycosides, tetracycline, cephalosporins, penicillin, beta lactum and sulphonamide.

Fig.1 Frequency of sensitivity to different antimicrobial agents

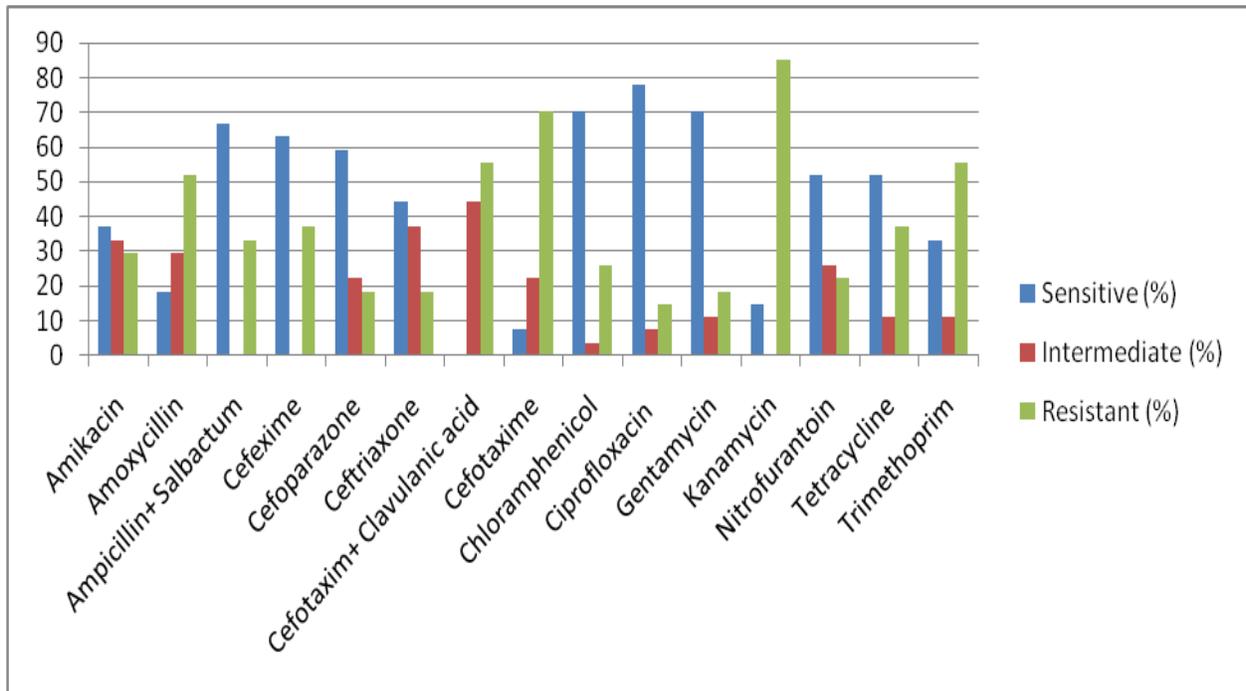


Fig.2 Multiple drug resistance in *E. coli* isolates

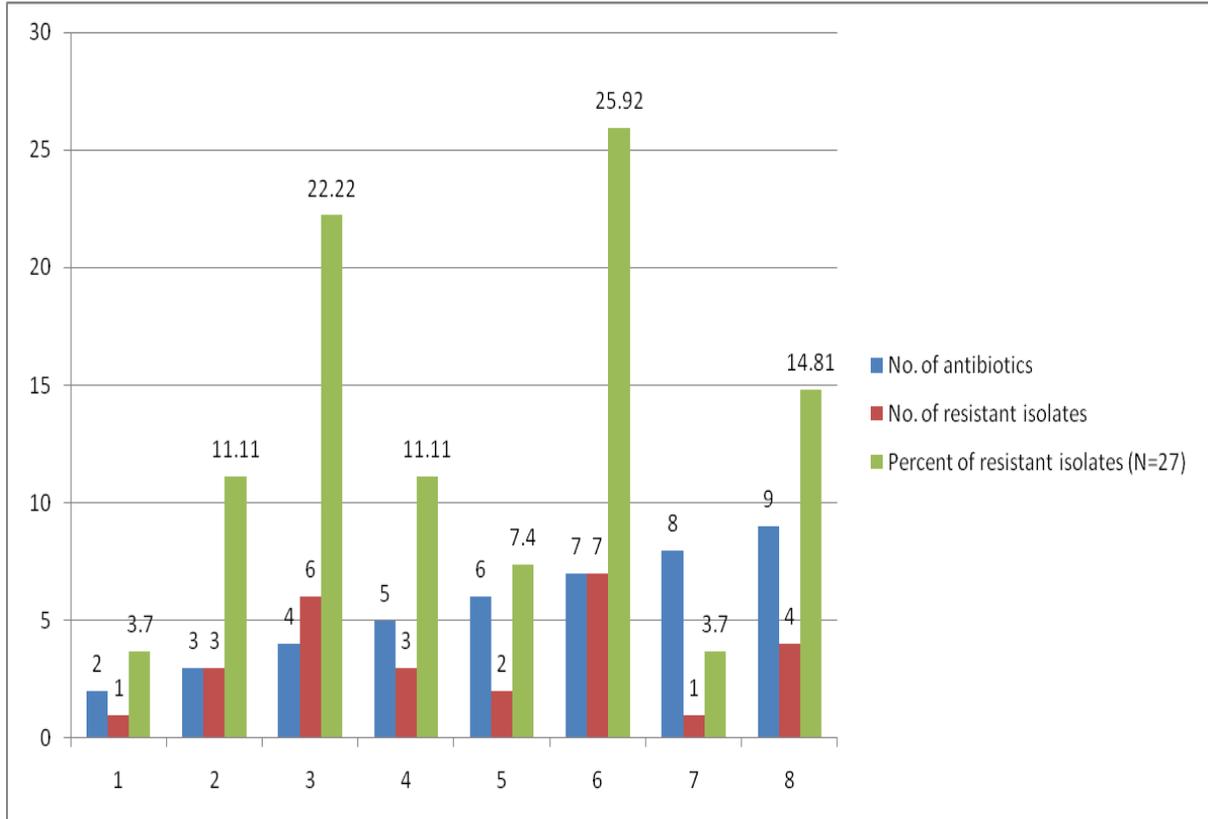
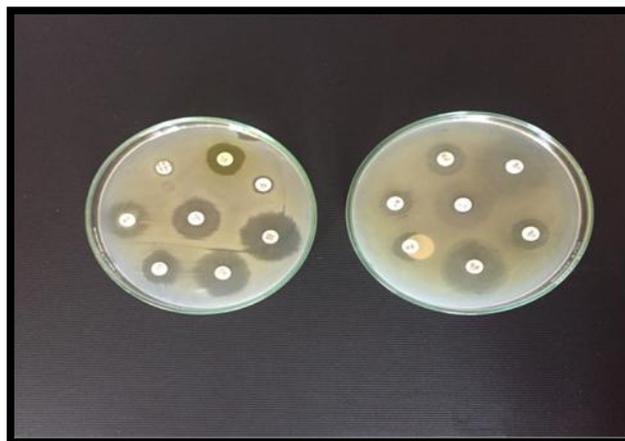


Plate.1 Results of antibiotic sensitivity test



The highest resistance was attributed towards antibiotic Kanamycin (85.18%) followed by Cefotaxime (70.37%), Trimethoprim (55.55%), Cefotaxime/clavulanic acid (55.55%), Amoxicillin (51.85%), Cefixime (37.03%), Tetracycline (37.03%), Ampicillin

+ salbactam (33.33%), Amikacin (29.62%), Chloramphenicol (25.92%), Nitrofurantoin (22.22%), Gentamicin, Cefoperazone and Ceftriaxone (18.51%) and Ciprofloxacin (14.81%). The highest sensitivity of *E. coli* was towards Ciprofloxacin antibiotic (Figure

1). The *E. coli* isolates simultaneously resistant to 2 to 9 antibacterial agents, was observed in 27 (100%) out of total 27 isolates. The maximum number of isolates was resistant to 7 drugs (Figure 2). Multidrug resistance in *E. coli* from milk sample was also reported by Zdolec *et al.*, (2016). The level of resistance of organism to a particular drug might be due to the indiscriminate use of the respective drugs. Hence, antibiotic should be used at recommended dosage for appropriate time course preferably after performing the *in vitro* sensitivity testing.

Various antibiotics used for Antibiotic sensitivity test (AST) in this study has also been reported by other workers viz. Preethirani *et al.*, (2015), Farooq *et al.*, (2008), Ranjan *et al.*, (2011), Kisku and Samad (2013) and Verma *et al.*, (2018). There has been evidence of increase in resistance to wide range of drugs in *E. coli* from animals in India and abroad (Dhakal *et al.*, 2007, Sumathi *et al.*, 2008, Kalmus *et al.*, 2011 and Jeykumar *et al.*, 2013)

Khan *et al.*, (2004) reported that *E. coli* isolates were 100% sensitive to Gentamicin and Chloramphenicol and 93.3% to ciprofloxacin but sensitivity recorded for these three antibiotics in present study is lower in percentage i.e. 70.37 % for Gentamicin and Chloramphenicol and 77.77% for Ciprofloxacin. Verma *et al.*, (2018) reported that Gentamicin (65.96%) was the most effective antibiotic, our findings are in partial agreement with this report. Preethirani *et al.*, (2015) reported that *Escherichia coli* isolates of buffalo mastitis showed 100% resistance for Amikacin, Amoxicillin/sulbactam, Ampicillin, Cefotaxime, Ceftriaxone/sulbactam and completely sensitive to Chloramphenicol, which is not in corroboration to our findings. In studies by Ranjan *et al.*, 2011 and Charaya *et al.*, 2014, *E. coli* was found highly sensitive to

Chloramphenicol, Gentamicin, Ciprofloxacin which is similar to findings of present study.

AST is widely used clinical investigation with the sole purpose to select most appropriate antimicrobial agent for therapeutic use. However, it is difficult to judge the clinical efficiency of an antimicrobial agent solely on the basis of AST, as there are large variations in response among herds and within herds, due to type of organism involved, location of infected sites, degree of udder induration, physiochemical properties and kinetic behavior of antibiotics in udder and milk, site of injection and sensitivity of udder pathogens, liquid solubility and tissue protein binding of the drug, pH of milk and inflammatory exudates/barrier at the site (Srivastava, 2000).

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Conflict of Interest

All authors declare no conflict of interest.

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