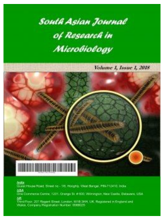

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Isolation of *Escherichia coli*, *Klebsiella* spp. and S*taphylococcus* spp. from Bovine Mastitic Milk in Nuwera Eliya District of Sri Lanka and their Sensitivity to Commonly Used Antibiotics

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JLPCR, TSPJJ, HAD Ruwanjith and HAD Ruwandeepika designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JLPCR, HGCLG, WUNTSE and NNU managed the analyses of the study. Author JLPCR managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Mastitis is one of the very important and most common diseases among dairy cattle globally which leads to severe economical losses in the dairy industry. For the sustainability of the dairy sector it is critical that efficient, economically feasible treatment regime is available for clinical cases

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of mastitis as a part of the control program with minimum risk for residues in milk. Antimicrobials are the most common drugs of choice for controlling and preventing this devastating condition. But the frequent use of antibiotics leads to the development of resistant bacteria which could have an adverse effect on human health as well. To mitigate this destructive constraint in the industry, identifying the etiology and their susceptibilities to remedial measures are of paramount importance. Hence this study was aimed at isolating and identifying the common bacterial etiology *Escherichia coli, Klebsiella* spp. and *Staphylococcus* spp. of mastitis and evaluating the antimicrobial susceptibility of the isolates in order to develop mastitis control strategies in the area.

Study Design: Milk samples were collected from mastitic cows in different stages including subclinical and clinical cases based on the results of California Mastitis Test

Place and Duration of Study: Samples were collected from dairy farms in Nuwera Eliya District, Sri Lanka and Laboratory investigations were carried out in the Laboratory of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Between Aug. 2017 and Nov. 2017.

Methodology: *E.coli*, *Klebsiella* spp. and *Staphylococcus* spp. were isolated from 31 milk samples and susceptibility to commonly used antibiotics (Trimethoprim, Oxytetracycline, Chloramphenicol, Cephalexin, Enrofloxacin and Ciprofloxacin) was determined by Kirby Bauer disk diffusion method.

Results: The study revealed that the most common isolate was the *Klebsiella* spp. and it is 54.8% and other two organisms *Staphylococcus* spp. had 51.6% and *Escherichia coli* 41.9%. Of all isolated pathogen, 97.1% exhibited resistant to Cephalexin and it was the highest while lowest resistance was to Chloramphenicol (31.4%). Among the other antibiotics, 54.3% of total isolates showed resistance to Trimethoprim followed by 42.9% to Oxytetracycline and Enrofloxacin, 34.3% was resistant to Ciprofloxacin. Resistance to at least one antibiotic was observed for the isolated microorganisms. All the three isolated pathogens are more resistant to Cephalexin. Both *E. coli* and *Klebsiella* spp. show 100% resistance to Cephalexin while *Staphylococci* had 92.9% resistance. This further revealed that *E. coli* (10%) and *Klebsiella* spp. (27.3%) are showing the least resistance to Chloramphenicol, None of *Staphylococcus* spp. (0%) isolated show resistance to Enrofloxacin.

Conclusion: Most common organisms isolated were *Klebsiella* spp. followed by *Staphylococcus* spp.*, E. coli* and there is a resistance of isolated organisms to some commonly used antibiotics.

Keywords: Bovine mastitis; causative agents; antimicrobial agents; resistance.

1. INTRODUCTION

Dairy farming has been a part of agriculture for thousands of years and it is a popular business which involves rearing animals such as cows, goats, sheep, camels, and buffaloes etc. for the purpose of harvesting their milk for human consumption. Among the other livestock animals, rearing of cows is very popular in many countries including Sri Lanka According to the International Dairy Federation (2016) [1], 29% of world cow's milk production is fulfilled by Asia. Bunch of constraints are found in dairy industry worldwide, diseases are one of the major constraints in dairy industry. Among the diseases, mastitis is one of the major economically important, most prevalent production diseases in dairy herds world-wide and it leads to a devastating economical loss due to the reduction in milk production, decrease in milk quality and poor hygiene in milk [2]. Mastitis causes to 30.0 percent less productivity per quarter and 15 percent production per cow [3], and loss of milk yield is estimated to range from 100 to 500 kg/cow per lactation. Mastitis accounts for about 38.0 percent of the total direct costs of the common production diseases [4] worldwide.

Prevention of this disease is a crucial fact in order to prevent losses in the industry and to maintain the quality of milk. Mastitis can be defined as an inflammation of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature [2]. It is a persistent, inflammatory reaction of udder tissues of the animal and it has been identified as a potentially fatal disease. Thus prevention of this constrain is must for the sustainable dairy industry in a country by understanding the predisposing factors including management aspects and moreover identifying the etiologies and their susceptibilities to remedial measures [4].

Mastitis is caused by wide variety of microorganisms including bacteria, mycoplasma, yeasts, algae etc. and among the etiologies; bacteria are playing a major role in causing mastitis [4,5]. It is a complex disease, numerous causative agents and the varied management and environmental practices that influence the severity of the disease [6]. However, almost any microbe can opportunistically invade udder tissue and can lead to mastitis. Whatever it is, most infections are caused by various species of *Streptococci*, *Staphylococci* and gram-negative rods, especially lactose-fermenting organisms of enteric origin, commonly termed coliforms [7]. Infection can spread either animal to animal as a contagious form or environmental. However, as it is a contagious disease, correct identification and practicing proper control measures including the correct therapeutic applications of drugs for the pathogen is crucial.

Antimicrobial therapy is one of the most effective and commonly practicing methods of controlling mastitis. In this situation the use of correct, effective drug is more vital because of the widespread use of antibiotics; resistance profile of microorganisms is increasing among bacteria. Antimicrobial resistance is a main public health concern now a day worldwide and public hazards associated with the consumption of antibiotic contaminated milk could be allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria [8,9]. The expansion of resistance both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals. Further transfer of antimicrobial resistant bacteria such as *S. aureus* to humans via the food chain has been reported [10]. Although there are many reasons which compromise antibiotic treatment of *S. aureus* mastitis, resistance of bacteria toward antibiotics is one of the most important [11,12].

During the process of controlling the disease, selection of most appropriate antibiotic for the purpose of inhibiting the growth and spread of the causative bacteria is an effective way in order to reduce the idle money wastes. It is important to prevent the disease going worst and to get a rapid cure of an infected animal as well. Selection of the appropriate antibiotic depends on the organisms which are responsible for the disease in a particular situation. However the organism which causes the mastitis can vary from country to country, region to region etc. due to the huge variation of preferable environmental conditions of different microorganisms. As well as the antibiotic resistance of the same species may vary from place to place and duration to duration as well.

Increasing antimicrobial resistance has become a serious concern throughout the world. Mastitis is said to be the most common reason for antibiotic use in dairy herd. Hence, antimicrobial resistance of mastitis pathogens has received recent attention [13,14]. With the development of antibiotic resistance by different microorganisms, most appropriate antibiotics to be used against them also vary from place to place and time to time [15,16,17].

Hence isolation and identification of mastitis-causing pathogens and testing their sensitivity to different antibiotics is vital to the management in order to select the most suitable antibiotics which can effectively control the causative agent. Ultimately it facilitates to control the spread of disease effectively. There are scanty of studies already done in Sri Lanka regarding this topic. This study was aimed at isolating and identifying the common bacterial etiology *Escherichia coli, Klebsiella* spp. and *Staphylococcus* spp. of mastitis and detecting the susceptibility of these isolates to six commonly used antibiotics.

2. MATERIALS AND METHODS

2.1 Sample Collection

Thirty one animals having either clinical or sub clinical form of Mastitis based on the California mastitis test (CMT) [18] were selected. Having cleaned the udder of selected cows with clean water and 75% alcohol, mid stream milk samples were aseptically collected in to cleaned sterile 50 ml falcon tubes. Samples were transported to the Laboratory of Livestock Production, Faculty of Agricultural Sciences, under cool conditions in ice and samples were processed as soon as the arrival.

2.2 Isolation and Identification of *E.coli*, *Klebsiella* spp and *Staphylococcus* spp.

Milk samples were subjected to Pre-enrichment in a non selective media (Peptone broth) and incubated for 24hours at $37^{o}C$. Subsequently samples were inoculated from preenrichment broth to selective/differential media (MacConkey agar, Eosin Methylene blue agar, Baird Parker Agar and Mannitol salt agar). Bacterial colonies were selected (five colonies from a plate) randomly and identified using traditional method which includes culture characteristic on selective media, gram-staining and biochemical reactions, according to Bergey's Manual of Determinative Bacteriology [19], [20,21,22]. The biochemical tests involved growth in Kligler's Iron (KIA), Lysine Iron agar (LIA), triple sugar iron agar (TSI), Citrate utilization, Motility test, Indole test, Urease production, Methyl Red (MR), Voges Proskauer (VP), carbohydrate fermentation test was done for lactose, sucrose, glucose and starch, DNAse test, nitrate reduction test, Oxidase, coagulase test and Catalase tests.

2.3 Antibiotic Sensitivity Test

Sensitivity to antibiotics was tested by using the Kirby Bauer disk diffusion method [23] which is recommended by the NCCLS for antimicrobial sensitivity testing. Bacterial sensitivity for five commonly used antibiotics (Trimethoprim 5 mcg, Oxytetracycline 30 mcg, Chloramphenicol 30 mcg, Enrofloxacin 10 mcg, Cephalexin 30 mcg and Ciprofloxacin 30 mcg) was checked by using commercially available antibiotic disks (Himedia, India). Overnight-grown cultures in Luria–Bertani broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were prepared as a lawn on Mueller Hinton agar. The antibiotics disks were placed aseptically on the lawn and incubated at 37°C for 16–18 h. Subsequently the growth inhibition zone was measured by directly measuring the magnitude of a zone of bacterial inhibition around the antibiotic-impregnated disks placed on Mueller Hinton agar plate surface. Diameters of inhibitory zones belong to each bacteria were compared with a standard chart which shows the performance standards [24] of each antibiotic used during the test for each bacteria.

3. RESULTS AND DISCUSSION

3.1 Isolated Organisms

In this study *Klebsiella* spp. *Staphylococcus* spp. and *Escherichia coli* organisms were isolated from collected milk samples. Out of 31 collected milk samples, 17 (54.84%) samples were positive for *Klebsiella* spp. 16 samples (51.61%) were positive for *Staphylococcus* spp. 13 (41.94%) samples were positive for *E. coli.* The most common isolate was *Klebsiella spp* followed by *E. coli* and *Staphylococcus spp*. (Fig. 1). Nineteen isolates of *Staphylococcus* spp. were present in the 16 milk samples; which were positive for *Staphylococcus* spp. and there were 12 coagulase positive isolates whereas 7 out of 19 were coagulase negative *Staphylococcus* spp.

Only one sample out of 31 samples detected having all these three (*Klebsiella* spp. *Staphylococcus* spp. and *Escherichia coli*) organisms. However, 16 samples out of 31 (51.61%), were positive for at least two organisms among three isolated organisms. However, among the collected 31 milk samples, 29 samples (93.54%) were positive at least for one organism among three isolated organisms. Only two samples out of 31 (6.45%) collected samples were detected not to be having any of above microorganisms. One of them was a sample obtained from a cow having clinical mastitis. Hence it proves that there are some other organisms which are capable of causing mastitis without any contribution of above isolated pathogens are also available in this area.

In line with this study, the study done by Munoz et al. [25] on a dairy farm in USA found that *Klebsiella pneumoniae* as the most prevalent organism causing mastitis. Another molecular epidemiological study conducted in Newfoundland has been shown the *Klebsiella* spp associated with bovine mastitis [26]. Study by Ahamed et al. also found that out of the 172 sub clinical mastitic samples, 34. 3% contaminated with *Klebsiella* spp.; 16.3% *K. pneumoniae*, 8.1% *K. oxytoca* and 9.9% other unclassified *Klebsiella* spp. Some other studies found that presence of *E. coli* in the mastitic milk, this study also showed the presence of *E. coli* [18]. Other studies also revealed that many microorganisms in mastitic milk samples and the common microorganisms associated were *Pseudomonas*, coagulase negative *Staphylococci*, *E.coli*, *Streptococci* and etc [27,28]. Further, another study showed, the prevalence of major pathogens isolated in Bangalore, India was 24% *for Staphylococcus aureus*, 20% for *E. coli* followed 16% for *S. epidermidis* and *Streptococcus* spp and 10% for *Klebsiella* spp [5]. The study done in a separate geographical location in Sri Lanka showed that high prevalence of *Staphylococcus* spp*, E.coli* and *Streptococcus* spp [29]. In many studies, *Staphyloccocus* spp, *E.coli* and *Klebsiella* spp has been found as the common etiologies in line with the current study [30,31,32,33,34].

3.2 Antibiotic Sensitivity Test

Forty percent of *E. coli* (4/10) isolates, of *Klebsiella* spp 63.6% (7/11) and 57.1% (8/14) of *Staphylococcus* spp. isolates showed resistance to Trimethoprim. Whereas for the Oxytetracycline, *Staphylococcus* spp. showed the highest resistance compared to the other two isolates and it was 57.1% resistance (8/14) followed by *Klebsiella* spp (45.5% resistance; 5/11) and *E. coli with* 20% resistance (2/10).

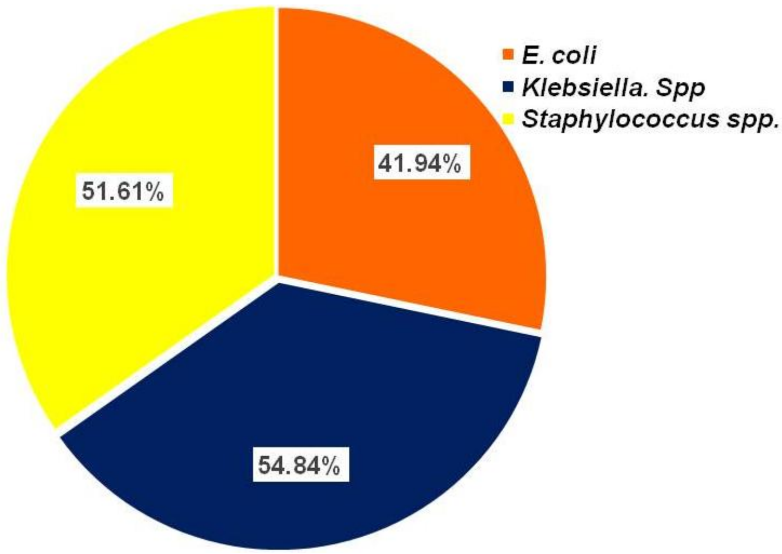

Fig. 1. Distribution of isolated organisms (*E. coli*, *Klebsiella* spp and *Staphylococcus* spp.) in bovine mastitic milk samples

Further, this study revealed that for Chloramphenicol, *E. coli* (1/10). *Klebsiella* spp (3/11) and *Staphylococcus* spp (7/14) showed 10%, 27.3% and 50% resistance respectively. All three organisms exhibited the highest resistance to Cephalexin with the rates of 100 (10/10) %, 100 (11/11) % and 92.9 % (13/14) for *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp., respectively. *E. coli* showed 60% (6/10) and 30% (3/10) resistance against enrofloxacin and ciprofloxacin whereas *Klebsiella* spp showed 81.8% (9/11) and 72.7% (8/11) resistance to both the antibiotics respectively. But the all isolates of *Staphylococcus* spp showed 100% sensitivity to enrofloxacin and for the ciprofloxacin it was 92.9% (13/14). More interestingly there was an isolate of *Klebsiella* spp which had resistance to all six antibiotics tested in the study (Fig. 2).

Among the *E. coli* isolates which used for antibiotic sensitivity testing (10 isolates), five isolates have been shown resistance to more than one antibiotic used in the study (Table 1). Isolate E10 showed resistance to Oxytetracycline, Cephalexin, Enrofloxacin and Ciprofloxacin. E67 and E70 had resistance to Trimethoprim, Cephalexin, Enrofloxacin and Ciprofloxacin whereas the isolate E80 showed resistance to these antibiotics except Ciprofloxacin. E75 isolate of *E. coli* exhibited resistance against Trimethoprim, Oxytetracycline, Chloramphenicol and Cephalexin (Table 1).

Out of the 11 isolates of *Klebsiella* spp. checked for antibiotic sensitivity, 8 isolates have shown resistance to more than one antibiotic. K32, K23 and K3 had similar kind of resistance, having resistance to Trimethoprim, Cephalexin, Enrofloxacin and Ciprofloxacin while K29 showed resistance against Oxytetracycline, Cephalexin, Enrofloxacin and Ciprofloxacin. K8 was resistant to four antibiotics namely Trimethoprim, Oxytetracycline, Cephalexin and Enrofloxacin. K72 was resistant to all the antibiotics tested whereas the isolate K55 showed resistant to all antibiotics except the Chloramphenicol. K52 was sensitive to Enrofloxacin and Ciprofloxacin while it was resistant to other four antibiotics. K78 showed sensitivity to Trimethoprim, Oxytetracycline and resistant against Cephalexin, Enrofloxacin and Ciprofloxacin (Table 1).

Staphylococcus isolates, S88, S89 (coagulase positive isolates) showed resistant to Trimethoprim, Oxytetracycline, Cephalexin and Chloramphenicol while S90 had the similar pattern of resistant but not against the Chloramphenicol. Isolate S16 which is a coagulase positive isolate has shown resistant against Oxytetracycline, Cephalexin and Chloramphenicol. Coagulase negative isolates of S91, S85 and S87 have displayed the resistance against Trimethoprim, Oxytetracycline, Chloramphenicol and Cephalexin and in addition, S85 had resistant to ciprofloxacin as well (Table 1).

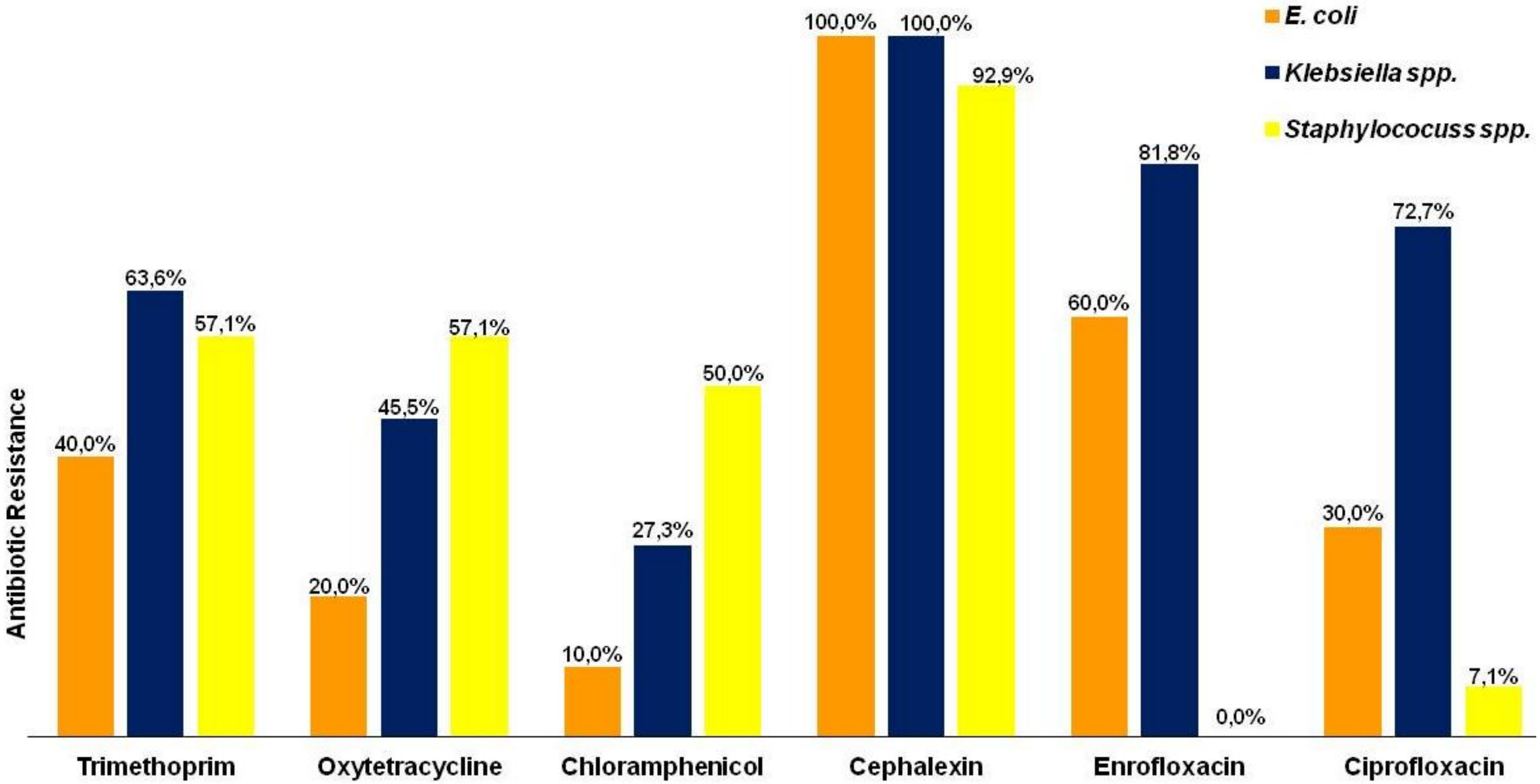

Fig. 2. Antibiotic resistance patterns of bacterial isolates (*E. coli*, *Klebsiella* spp and *Staphylococcus* spp.) from bovine mastitic milk to antibiotics; Trimethoprim, Oxytetracycline, Chloramphenicol, Cephalexin, Enrofloxacin and Ciprofloxacin

Table1. Sensitivity pattern of *E.coli*, *Klebsiella* and *Staphylococci* isolates to different antibiotics; Trimethoprim, Oxytetracycline, Chloramphenicol, Cephalexin, Enrofloxacin and Ciprofloxacin

	Trimethoprim		Oxytetracycline		Chloramphenicol		Cephalexin		Enrofloxacin		Ciprofloxacin	
Isolate	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**
E. coli												
E50	22	S	19	S	24	S	13	R	32	S	33	S
E10	23	S	11	***R***	24	S	10	***R***	16	***R***	15	***R***
E14	25	S	19	S	26	S	12	R	31	S	33	S
E19	23	S	20	S	27	S	14	R	15	R	30	S
E34	23	S	18	S	25	S	13	R	14	R	32	S
E80	6	R	19	S	22	S	12	R	13	R	30	S
E67	6	R	21	S	28	S	6	R	15	R	11	R
E62	26	S	22	S	29	S	14	R	33	S	36	S
E75	6	*R*	10	***R***	12	***R***	6	***R***	42	S	44	S
E70	6	*R*	23	S	30	S	6	***R***	16	***R***	15	***R***
Klebsiella												
K32	9	***R***	18	S	22	S	13	***R***	8	***R***	14	***R***
K29	21	S	10	***R***	21	S	6	***R***	15	***R***	15	***R***
K42	24	S	19	S	23	S	6	***R***	12	***R***	15	***R***
K8	8	***R***	9	***R***	21	S	6	***R***	12	***R***	30	S
K23	8	***R***	19	S	21	S	14	***R***	16	***R***	10	***R***
K3	9	***R***	19	S	24	S	6	***R***	16	***R***	12	***R***
K78	21	S	18	S	23	S	14	***R***	15	***R***	15	***R***
K79	22	S	21	S	10	R	6	R	32	S	33	S
K72	8	***R***	7	***R***	12	***R***	14	***R***	11	***R***	13	***R***
K55	8	***R***	10	***R***	21	S	14	***R***	12	***R***	13	***R***
K52	6	***R***	11	***R***	10	***R***	6	***R***	34	S	37	S
Staphylococci												
S16	28	S	8	***R***	12	***R***	14	***R***	34	S	37	S
S38	22	S	23	S	20	S	19	R	24	S	28	S
S17	8	R	25	S	28	S	30	S	26	S	30	S

	Trimethoprim		**Oxytetracycline**		**Chloramphenicol**		**Cephalexin**		**Enrofloxacin**		**Ciprofloxacin**	
Isolate	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**	**Diameter mm**	**S / R**
S18	24	S	6	R	25	S	12	R	27	S	33	S
S7	7	R	28	S	24	S	10	R	31	S	36	S
S89	6	***R***	11	***R***	11	***R***	6	***R***	30	S	33	S
S82	20	S	6	R	28	S	13	R	25	S	27	S
S88	6	***R***	12	***R***	12	***R***	6	***R***	39	S	44	S
S92	27	S	20	S	31	S	14	R	32	S	35	S
S91	6	***R***	9	***R***	8	***R***	6	***R***	30	S	36	S
S90	6	***R***	20	S	10	***R***	6	***R***	39	S	46	S
S85	6	***R***	14	***R***	12	***R***	6	***R***	26	S	15	***R***
S87	6	***R***	12	***R***	11	***R***	6	***R***	34	S	35	S
S86	19	S	30	S	28	S	14	R	28	S	30	S

**Inhibitory zone diameters (In nearest millimeter) and S/R indicate the Sensitive or Resistant to each antibiotic*

***Organisms with multiple resistance to antibiotics are bold, italic and colored; pink colour indicate the coagulase positive multiple resistant staphylococci spp. and blue colour indicate the coagulase negative.*

A study was done in Thailand shown that *E. coli* isolated from cows with subclinical mastitis, had resistant to ampicillin, carbenicillin, and sulfamethoxazole-trimethoprim [35]. A study by Das and his colleagues found the occurrence of ESBL (extended-spectrum β-lactamase) producing Gram-negative isolates in subclinical mastitic cattle West Bengal [36]. Further supporting the findings of the current study, multidrug resistance (≥3 to 6 antimicrobials) was seen in 40% of *E. coli* isolates from healthy lactating cattle in a study done by Sawant and his group [37]. The metagenomics study revealed the presence of multiple resistant genes in cow manure [38] and it has shown the possibility of possessing a phenotype of multidrug resistant in agreement with this study. A study conducted to determine the molecular characteristics, antibiogram and prevalence of multidrug resistant *S. aureus* from culled dairy cows and from cows with acute clinical mastitis, revealed that *S. aureus* isolates were resistant to erythromycin, penicillin, streptomycin, doxycyclin and Trimethoprim/sulpha and also it has shown that MRSA isolates were completely resistant to ten commonly used antibiotics such as, ampicillin, penicillin, streptomycin, gentamycin, erythromycin, ciprofloxacin, Oxytetracycline, Trimethoprim/sulphamethoxazole, enrofloxacin and doxycyclin [39], in line with this study. Moreover, in comparison with the study, several other researches also have shown the presence of multidrug resistant organisms in bovine milk samples [40,41,42,43,44].

While considering the overall resistance for all the individual organisms (*E. coli*, *Klebsiella* spp and *Staphylococcus* spp.) the isolates showed 97.1% resistance (34/35) against Cephalexin, was the maximum resistance observed among six antibiotics. 54.3% resistant (19 out of all 35 isolates) was observed to Trimethoprim whereas 42.9% resistance (15 out of 35) was observed against both Oxytetracycline and Enrofloxacin. For Ciprofloxacin there was 31.4% resistance (11 out of 35) while the least resistance among six antibiotics 31.4% (11/35) was against Chloramphenicol (Fig. 3).

In a similar study conducted in Finland and Israel using bovine mastitic milk samples in year 2003, *E. coli* had shown a 16% resistance to Cephalexin, 4% and 2% resistance to Trimethoprim 0% resistance to Ciprofloxacin [45]. In this study also the least resistance of *E. coli* was observed for Ciprofloxacin while 100% *E. coli* resistance was observed against Cephalexin. It may be due to the increase in the use of particular antibiotic. However, Ciprofloxacin seems to be still an effective antibiotic in other countries as well.

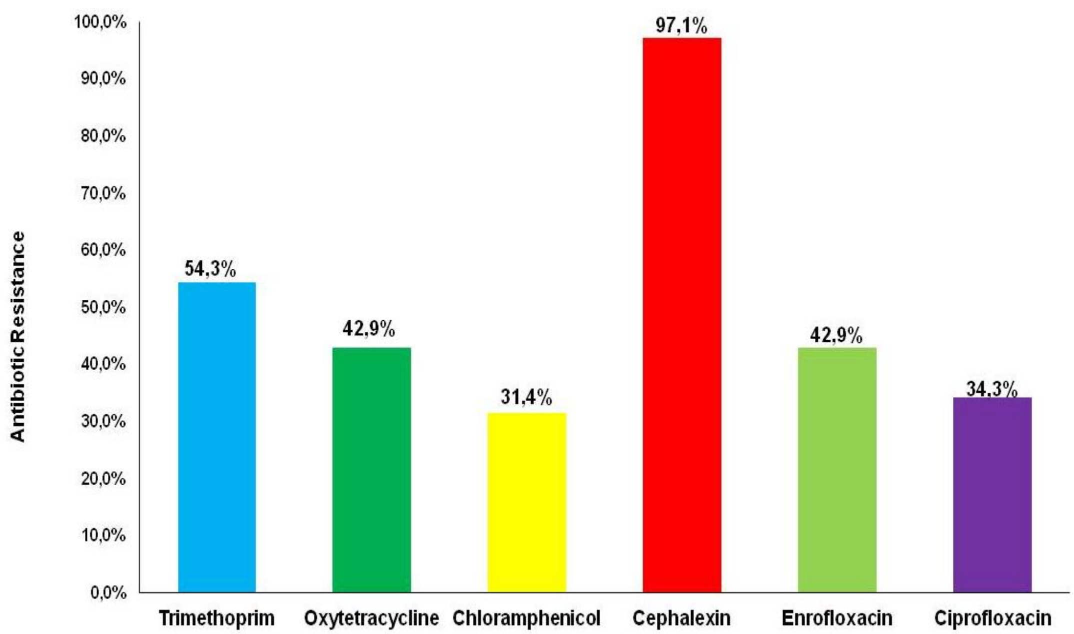

Fig. 3. Overall resistance of isolated organisms from bovine mastitic milk to antibiotics; Trimethoprim, Oxytetracycline, Chloramphenicol, Cephalexin, Enrofloxacin and Ciprofloxacin

Another study done in the United States had revealed that there was a 5.5% resistance of an *E. coli* strain which causes urinary tract infection [46]. However, Murray and his research group [47,48], found that *E. coli* as the predominant organism it has shown the resistance to Trimethoprim having 96% resistance.

Thaker et al. has shown that the *S. aureus* isolated from bovine mastitic milk possess highest sensitivity towards cephalexin (100%) whilst 80% resistance for Ciprofloxacin 20% for Oxytetracyclin [49]. In this study *Staphylococci* spp showed a 92.9% resistance whilst other two genera showed 100% resistance against Cephalexin. This again shows the low efficiency of Cephalexin. These results may be some shreds of evidence to say that Cephalexin has already become less efficient in many countries. But in that study 80% resistance had been observed against Ciprofloxacin. But in this study Ciprofloxacin had performed better than other antibiotics against *Staphylococci* a resistance as low as 7.1%. This shows the variation of the antibiotic resistance in different geographical locations and with the time even within the same genera.

However, when the results of all these studies are compared, it is crystal clear that there can be a huge variation of the resistance pattern within the same genera against the same antibiotic when the study area, time etc. differ. Hence it is important to conduct similar studies further in order to identify the variation of mastitis etiologies and their antibiotic sensitivity. This study demonstrated that the antibiotic susceptibility tests (ABST) are vital for the selection of the most effective treatment for mastitis caused by *Escherichia coli, Klebsiella* spp., and *Staphylococcus* spp. organisms.

4. CONCLUSION

Among the three isolated organisms, the most common organism isolated was *Klebsiella* spp. followed by *Staphylococcus* spp., *E. coli* and there is a resistance of isolated organisms to some commonly used antibiotics.

ETHICAL APPROVAL

As per international standard or university standard, written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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