

Clinical mastitis in cows and their response to *Invitro* sensitivity

Ahmed Shaban Kassem Agha ¹, Otman Nasser Ermithi ¹, Alarbi Abdulmajed Belgasim ^{1,*}, Ahmed Zaghdani ¹, Husain Abuhilala ¹, Salah Abdulhadi Bshina ² and Khalid Mohammed Naffati ¹

¹ Department of Microbiology, Libyan Center for Biotechnology Research, Tripoli, Libya.

² Department of Medicine, Faculty of Veterinary Medicine, Azzaytuna University, Libya.

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Abstract

A total of 50 cases of bovine clinical mastitis in Tripoli were subjected to microbiological examination. Thirty-five bacterial isolates were obtained and further identified by using the biochemical tests. *Staphylococcus spp.* (36%) was the predominant causative organism, then *E. coli* (24%) and *Streptococcus spp.* (6%) Additionally, twelve cases were caused by nonbacterial agents. The bacteria isolates were tested for their *in-vitro* susceptibility to different antimicrobial agents that are used in commercial intramammary infusion products. Antibacterial susceptibility testing showed that the bacteria isolates were sensitive to ciprofloxacin (100%), enrofloxacin (96%), cefotaxime (90%), deoxicillin (88.8%), clorophenicol (66.5), ampicillin (62.5%), amoxicillin (50%), vancomycin (42%) and fusidic acid (33.3%).

According to these results, the ciprofloxacin was proved to be the drug of choice.

Keywords: Bovine; Clinical mastitis; Milk samples; Antimicrobial agents

1. Introduction

Mastitis, which is an inflammation of the milk gland, is the most important disease of dairy animals [1, 11], and it causes losses of about two billion dollars every year in the United States alone [6]. It is classified as subclinical or clinical [18].

Subclinical mastitis is characterized by no visible sign of disease, apparently normal milk, and an increase in somatic cell count (SCC) that is bacteriologically positive. Diagnosis of mastitis needs special screening tests, such as the California mastitis test (CMT) [17].

Clinical mastitis is characterized by abnormal secretion containing clots [3, 9]. Sudden onset of clinical mastitis (acute clinical mastitis) is accompanied by swelling, hardness and increased temperature, and it may also be accompanied by systemic signs such as loss of appetite, fever, dehydration and depression [8].

More than 135 different microorganisms have been isolated from bovine mastitic milk samples [12]. The causative organisms of mastitis are divided into:

- Contagious pathogens that are spread from infected quarter to other quarters and cows. These include *Staphylococcus aureus*, *Streptococcus agalactia*, and *Mycoplasma bovis*.

*Corresponding author: Alarbi Abdulmajed Belgasim
Microbiology Department, Libyan Center for Biotechnology Research, Tripoli, Libya.

- Environmental pathogens which are usually present in the cow's environment and reach the teat from that source such as environmental *streptococcus* species (e.g., *Streptococcus dysagalactia* and *Streptococcus uberis*), and the enterobacteriaceae.
- Minor pathogens rarely cause clinical mastitis. They include coagulase-negative *Staphylococcus* spp. (e.g., *Staphylococcus hyicus* and *Staphylococcus chromogenes*).
- Uncommon pathogens can cause severe mastitis which is usually sporadic and affects only one cow or few cows in the herd. These include *Arcanobacterium pyogenes*, *Nocardia* spp., *Pasteurella* spp., *Mycobacterium bovis*, fungi and yeasts [13].

Most infections are caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, and by the environmental pathogens *Streptococcus uberis* and *Escherichia coli*. [4,5, 10,7].

Clinical mastitis is one of the most costly diseases in dairy cattle.

In the National Animal Health Monitoring System of dairy herds in the US, clinical mastitis alone was the most costly disease identified, with a loss to the producer of \$27-50 per cow per year. Prevention of mastitis cost \$14.50/cow-year. Lost milk production was estimated \$14.85/cow-year which does include the losses associated with subclinical mastitis. (13).

These losses could even be higher in Tripoli because mastitis prevention practices, such as teat dipping and dry period antibiotic therapy, are not used.

The objectives of this study were to isolate and identify mastitis associated bacteria in Tripoli district, and to determine the susceptibility of the isolated bacteria to antimicrobial agents used in commercial intramammary infusion product.

2. Material and methods

2.1. Sample collection

Milk samples were collected from 50 cows affected with mastitis in Tripoli. Mastitis was identified by swelling, hardness, warmth and/or abnormal secretions (abnormal color or consistency and/or presence of clots or flakes).

Sampling of milk was performed as following:

- The udder was carefully washed, dried and the first few squirts of milk from each quarter were discarded.
- The milk samples were collected in sterile screw capped vials.
- Collected samples were immediately kept in an insulated container and transferred to the laboratory for bacterial culturing.

2.2. Bacterial Isolation and identification

Milk samples were brought to room temperature and mixed thoroughly. Each sample was streaked on blood agar, MacConkey's agar and nutrient agar (Schalan Barcelona, Spain). The plates were incubated aerobically at 37°C and examined for growth after 24 and 48 hr. The gram stain according to Quinn *et al* (12) was performed to distinguish Gram-positive and negative organisms and to reveal the bacterial.

The type of bacterial haemolysis (alpha, beta or none) was determined on blood agar plates. Catalase test was performed to distinguish *streptococci* and *staphylococci*. (Agropharm, Buckingham, UK).

Oxidase test used to distinguish the *Enterobacteriaceae* spp. from Gram-negative non-Enterobacteriaceae organisms. (Leicestershire, UK).

2.3. Susceptibility testing

Finally, the isolated bacteria, from the milk samples showed sensitivity to antibiotics, were tested using Mueller Hinton agar and the antibiogram was determined by the disc diffusion method (Schalan Barcelona, Spain). The used antibiotics were ciprofloxacin, enrofloxacin, cefotaxime, deoxicillin, chlorphenicol, ampicillin, amoxicillin, vancomycin and fusidic acid (Oxide Ltd, Hampshire, England). After 18-24 hr. of aerobic incubation at 37°C, the diameter of the zone of inhibition

was measured by a ruler and classified as resistant, intermediate, or susceptible, according to the Quinn *et al* procedure (12).

3. Results and discussion

The isolation frequency of the bacterial strains is summarized in table (1).

Of the fifty milk samples examined in the present study, five samples were considered contaminated. On the other hand, twelve showed no bacterial growth. The remaining thirty-three samples revealed high prevalence of *Staphylococcus spp.* induced clinical mastitis (36%). This is consistent with the findings of other authors (14, 2) who considered *Staphylococcus spp.* As a major etiological agent of clinical mastitis. *Escherichia coli* is the second at (24%) and *Streptococcus species* were also encountered in a high prevalence in this study (6 %). Thus, twelve culture–negative milk samples encountered in the present study may be attributed to other intramammary pathogens could not be detected in the present study such as mycoplasmas, fungi, yeasts, and chlamydia.

The susceptibility of the bacterial species isolated in this study to the antimicrobial agents used in intramammary infusion products (Table 2) revealed that 100% of isolated bacteria were sensitive to ciprofloxacin whereas 96%, 90% and 88.8% were sensitive to Enrofloxacin, Cefotaxime and Deoxicillin respectively. Sensitivity of the other antibiotics ranged from 33.3% to 66.5% (Table 2).

Table 1 Bacterial isolates from milk samples obtained from the mastitic quarters

Bacteria	Number of isolates	%
<i>Staph.</i>	18	36
<i>E. coli</i>	12	24
<i>Strepto.</i>	3	6
No bacterial growth	12	24
Mixed cultures	5	10

Table 2 Sensitivity pattern of mastitis milk samples

Antibiotic	Percent sensitivity
ciprofloxacin	100
enrofloxacin	96
cefotaxime	90
deoxicillin	88.8
clorophenicol	66.5
ampicillin	62.5
amoxicillin	50
vancomycin	42
fusidic acid	33.3

4. Conclusion

Mastitis not only reduces the productive capacity of the cows, it's also expensive to treat. The results showed that the most common agent was *Staphylococcus spp.* The best treatment was Ciprofloxacin

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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