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# Antimicrobial susceptibility of coagulase negative *staphylococci* Isolated from bovine mastitis

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## Abstract

**The present study aimed to determine the *in vitro* susceptibility of coagulase negative *staphylococci* (CNS) isolated from milk samples in cases of clinical and subclinical bovine mastitis to some antimicrobial drugs. The antibacterial sensitivity was tested using disk diffusion method and performed according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines using Mueller-Hinton agar. A total of 180 milk samples from apparently healthy cows and 130 milk samples from cows suffering from bovine mastitis were collected. Subclinical mastitis was detected using California test and bacterial isolation and identification of CNS isolates were carried out depending on the morphology of colony and biochemical tests. The results showed that a total of 368 (45.77%) isolates of CNS were obtained from clinical and subclinical mastitis. Coagulase negative *staphylococci* were dominant in 366 (%54.3) isolates from subclinical mastitis and only 2 isolates from clinical mastitis. The highest number of CNS isolates was susceptible to chloramphenicol (84.78%), followed by tetracycline (80.43%) and kanamycin (74.46%). Whereas, 40.76 % of CNS isolates was resistant to penicillin. Before starting the treatment of mastitis, the antibiogram for different antibacterial agents is necessary to attain the maximum efficacy of used antibiotic and to prevent emergence of bacterial resistance.**

**Keywords**: Antimicrobial susceptibility, Coagulase negative *staphylococci*, Bovine mastitis
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## Introduction

Bovine mastitis is an inflammation of the mammary glands of dairy cows accompanied by physical, chemical, pathological and bacteriological changes in milk and the glandular tissue. It is the most common infectious diseases of dairy cows all over the world which adversely affects dairy industry (Halasa *et al.*, 2009 and Pachauri, *et al.*, 2013). Mastitis may be caused by a wide variety of microorganisms including bacteria, fungi, yeast and *mycoplasma.* However, bacteria are the most frequent pathogens causing this disease (Halasa *et al.*, 2007). Mastitis is continuously the most frequent and most expensive disease of dairy cows (Halasa *et al.*, 2009). It is one of the most prevalent and costly diseases in the dairy industry with losses attributable to reduced milk production, discarded milk, early culling, veterinary services, and labor costs (Thompson-Crispi *et al.*,2014). In most cases, mastitis results from bacteria migrating into the mammary gland through the teat canal. The invaded bacteria interact with the host tissue and activate the cow's immune system, promoting neutrophil migration from the blood into the milk, so leading to increase in somatic cell count (SCC) in milk (Veerle, 2011 and Archer *et al.*, 2014). The increase in milk SCC of an individual cow or quarter above threshold is indicative for the presence of an intramammary infection (IMI)

and is inversely correlated with milk production and quality (Archer *et al.*, 2014). The cases of mastitis vary from severely clinical with visible signs of inflammation to subclinical without signs, but with an elevation of SCC. Not only clinical mastitis, but also subclinical form can result in reduction of milk production (Halasa *et al.*, 2009 and Fuenzalida, *et al.*, 2015).

Coagulase-negative *staphylococci* (CNS) have been traditionally considered to be the minor mastitis pathogens, but now it is the most bacteria commonly isolated from mastitis (Pitkälä *et al.*, 2004; Roberson *et al.*, 2006 and Tenhagen *et al.*, 2006). Mastitis caused by CNS in most cases remains subclinical, or the clinical signs are mild (Taponen, 2008). One of the most important reasons for failure of treatment of mastitis is attributed to the indiscriminate use of antibiotics without testing the *in vitro* sensitivity of causative bacterial strains. The practice at one hand increases economic losses and on the other results in the development of bacterial resistance to commonly used antimicrobial drugs (Tremblay *et al.* , 2014). For suitable and successful antibiotic therapy, the bacterial isolation and antibiotic sensitivity tests are always essentials. Antimicrobial susceptibility tests help to guide the veterinarians in selecting the most appropriate and effective antimicrobial agent for the treatment of bovine mastitis (Rüegsegger *et al* , 2014). Therefore, the present investigation was carried out to determine the in *vitro* susceptibility of CNS isolated from bovine mastitis to some antimicrobial drugs.

## Material and Methods

Milk samples for bacteriological examination were collected aseptically from 180 apparently healthy cows and 130 cows suffering from clinical and subclinical mastitis. Samples from subclinical mastitis were detected by California test. From each animal, 15ml of milk samples were collected, cooled and immediately transported to the laboratory on ice. The bacteriological examination was performed according to Devriese *et al.*, (1994)

Antimicrobial sensitivity of CNS isolated from milk samples was tested using disk diffusion method and performed according to guidelines of NCCLS (2013). The plates were prepared and checked for sterility by incubating the plates overnight at 37°C. The antibiotics discs were kept at room temperature for one hour then the agar plates were overlaid with different inoculums of coagulase negative *staphylococcus* isolates that showed turbidity equivalent to that of 0.5 McFarland turbidity tube as a standard.

## Results

The sensitivity of coagulase negative *staphyloccoci* (CNS) isolated from milk samples to antimicrobial drugs are presented in Table (1) and illustrated in Figs (1) and (2).

The results of antibiotic sensitivity test of CNS isolated from mastitis are shown in Fig. (1). It clear that the prevalence of bovine mastitis was found to in 368 (45.77%) isolates of CNS which obtained from clinical and subclinical mastitis. The CNS was dominant in 366 isolates (%54.3) from subclinical mastitis, and 2 isolates from clinical mastitis. The CNS were belonging to

8 species as follows: S. *chromogenes* 80 (21.86%); S. *xylosus* 86 (23.5%); S. *epidermidis* 65 (17.76%); S.*haemolyticus* 64 (17.49%); S. *sciuri* 32 (8.74%); S. *saprophyticus* 19 (5.19%); S. *hominis* 18 (4.91%) and S. *lentus* 2 (0.55%).

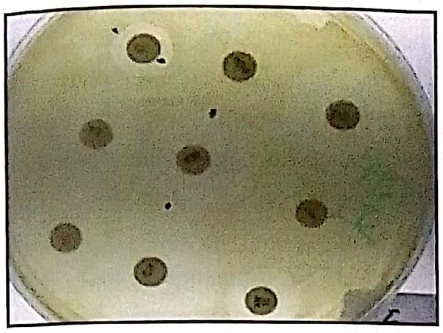
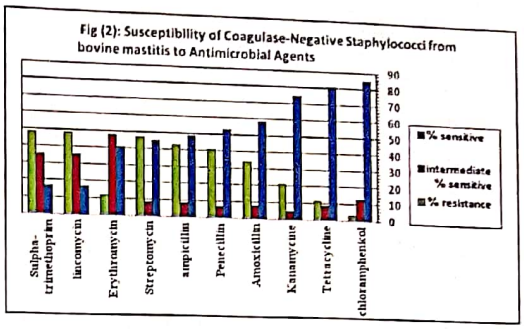


Fig.(1): Showing antibiotic sensitivity test of CNS isolated from mastitis.

As recorded in table (1) and shown in Fig. (2), the susceptibility of coagulase negative *staphyloccoci* (CNS) to the tested antimicrobial drugs on the isolates were in the following order: the highest numbers of CNS were susceptible to chloramphenicol (84.78%), followed by tetracycline (80.43%), kanamycin (74.46%), amoxicillin (58.15%), penicillin (52.99%), ampicillin (48.64%), streptomycin (44.84%), erythromycin (40.49%). The lowest susceptibility was shown to lincomycin (16.58%), and sulpha-trimethoprim (16.58%).

Table (1): Susceptibility of CNS isolated from mastitis to some antimicrobial agents.

| Antimicrobial | High sensitive | | Intermediate sensitive | | Resistant | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Chloramphenicol | 312 | 84.78 | 46 | 12.5 | 10 | 2.72 |
| Tetracycline | 296 | 80.43 | 31 | 8.41 | 41 | 11.14 |
| Kanamycin | 274 | 74.46 | 17 | 4.62 | 77 | 20.92 |
| Penicillin | 195 | 52.99 | 23 | 6.25 | 150 | 40.76 |
| Ampicillin | 179 | 48.64 | 31 | 8.42 | 158 | 42.93 |
| Streptomycin | 165 | 44.84 | 30 | 8.15 | 173 | 47.01 |
| Lincomycin | 61 | 16.58 | 130 | 35.33 | 177 | 48.10 |
| Erythromycin | 149 | 40.49 | 175 | 47.55 | 44 | 11.96 |
| Amoxicillin | 214 | 58.15 | 29 | 7.88 | 125 | 33.97 |
| Sulpha-trimethoprim | 61 | 16.58 | 130 | 35.33 | 177 | 48.10 |




## Discussion

The present study was performed to evaluate the in *vitro* susceptibility of coagulase negative *staphylococci* (CNS) isolated from bovine mastitis to some antimicrobial drugs.

Previous studies have been reported that the response rate of CNS to different antimicrobial drugs was qualified as poor when cure rate of mastitis less than or equal to 25% and as favorable when it attained 75% or above (Silley, *et al.*, 2012 ; Moser, *et al.*, 2012 and Tremblay et al , 2014). It has been also reported that the highest sensitivity of CNS isolated from bovine mastitis was to chloramphenicol, tetracycline and kanamycin (Basappa *et al.*, 2011). The result of our study that CNS was highly sensitive to chloramphenicol followed by tetracycline and kanamycin was closely similar to the results reported by Abdulla *et al.*, (2011); Basappa *et al.*, (2011); Rüegsegger, *et al.*, (2014) and Tremblay, *et al.*, (2014).

The results of this present study indicated the existence of CNS resistance for penicillin at a high rate (40.76%). The most significant factors affecting the cure rates from coagulase-negative *staphylococci* (CNS) causing bovine mastitis were the ability of CNS to produce β-lactamase (Pyorala *et al.*, 2000 and Veerle, 2011) which inactivate

penicillin. Other mechanism of bacterial resistance of CNS to penicillin might be due to its slime-producing ability i.e. biofilm formation (Tremblay *et al.*, 2014). The emergence of bacterial resistance to antimicrobial drugs among pathogens that affects animal health is of growing concern in veterinary medicine. Antimicrobial-resistant pathogens in animals have also been considered as a potential health risk for humans (Basappa *et al.*, 2011). However, the effectiveness of antibacterial therapy against udder pathogens depends not only on its exogenous administration into intramammary gland tissues, but it is also related to the various indigenous inhibitors in milk such as immunoglobulin (Ig), complement, lactoferrin, lactoperoxidase, etc. Many of these are activated by the inflammatory process. Interactions between antibiotics and immunological factors could lead either to augmenting the antibacterial effect on the target tissue or diminishing it (Saran and Leitner, 2000 and Basappa *et al.*, 2011). Therefore, antimicrobials for mastitis therapy should be selected not only on bacterial sensitivity, but also for their positive interactions with the innate immune response of the mammary gland (Mazzilli and Zecconi, 2010 and Leitner *et al.*, 2013).

## Conclusion

Before starting the treatment of mastitis, the antibiogram for different antibacterial agents is necessary to attain the maximum efficacy of used antibiotic and to prevent emergence of bacterial resistance. Appropriate elimination of bacteria in mastitis requires both effectiveness of the used antimicrobial drug and well functioning immune defense system of the animal.

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# حساسية العنقوديات السالبة لانزيم التخثر المعزولة من التهاب ضرع الابقار للمضادات الميكروبية

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## الملخص

استهدفت هذه الدراسة قياس حساسية العنقوديات السالبة لانزيم التخثر المعزولة من التهاب الضرع السريرى وتحت السريرى في الابقار لبعض المضادات الميكروبية. وتم اجراء اختبار حساسية العنقوديات للمضادات الميكروبية بطريقة الأقراص على بيئة مولر هنتون. ولقد تم تجمع عينات الحليب من ١٣٠ بقرة مصابة بالتهاب الضرع السريري ومن ١٨٠ بقرة سليمة ظاهرياً. وقد تم اجراء اختبار كاليفورنيا لتحديد التهاب الضرع تحت السريرى. وتم التعرف على المعزولات اعتماداً على الفحص الميكروسوبى والاختبارات الكيميائية. وتم زرع عينات الحليب على الأوساط الجرثومية للفحص البكتريولوجى .. وأظهرت النتائج أن العنقوديات السالبة لانزيم التخثركانت موجود في ٣٦٨ معزولة من مجمل حالات التهاب الضرع بنسبة ٤٥,٧٧%, وكانت منها ٣٦٦ معزولة من حالات التهاب الضرع تحت السريري بنسبة ٥٤,٢٣% وعينتين من حالات التهاب الضرع السريري. واثبتت النتائج أن حساسية العنقوديات السالبة لانزيم التخثر للمضاد الحيوى كلورامفينيكول كانت بنسبة ٨٤,٧٨ % ثم التتراسيكللين بنسبة ٨٠,٤٣ % ثم الكاناميسين بنسبة ٧٤,٤٦%. بينما كانت هذه العنقوديات مقاومة للبنسيللين بنسبة ٤٠,٧٦ %. واختبار حساسية البكتيريا للمضادات الميكروبية ضرورى للحصول على أعلى كفاءة فى علاج التهاب الضرع ولتجنب ظهور مقاومة البكتيريا للمضاد الميكروبى.