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Mastitogenic Bacteria Isolated from Clinical Mastitis Cases Associated with Teat Lesions and their Antimicrobial Sensitivity

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ABSTRACT

Mastitis is the most common and most expensive disease that impacts dairy farms in Egypt. In this study, we investigated bacterial mastitis cases that did not respond to antimicrobial treatment from buffaloes and cows. Milk samples (11) were collected from diseased animals (5 buffaloes and 6 cows). The samples were tested microbiologically to isolate and identify the causative bacteria and antimicrobial susceptibility. The antimicrobial sensitivity of the isolated bacteria was tested by using minimum inhibitory concentration technique according to the clinical national laboratory standards. The total number of bacterial isolates from the cases was 15 isolates. *Staphylococcus aureus* (*S. aureus*) was isolated alone in two cases (13.33% of the isolates), and *S. aureus* isolated in combination with *Arcanobacterium pyogenes* (*A. pyogenes*) in one case (6.67%), and *S. aureus* isolated in combination with both *A. pyogenes* and *Streptococcus agalactiae* (*S. agalactiae*) in two cases (13.33%), and Coagulase negative staphylococci (CNS) isolated alone in one case (6.67%), and CNS isolated in combination with *Klebsiella pneumoniae* in one case (6.67%), and *A. pyogenes* isolated alone in two cases (13.33%). In two cases were not able to isolate any bacterial pathogen. In terms of susceptibility to antibiotics, the isolated strains were sensitive to clindamycin with MICs ranging from 0.125 to <0.03125 µM. The same isolates exhibited medium sensitivity to gentamicin and ciprofloxacin with MICs ranging from 0.5 to 8 µM. Most *S. aureus* isolates were resistant to oxytetracycline with a MIC of 128 µM. Most of the isolated bacteria were resistant to polymyxin B with a MIC > 128 µM. With the exception of the *A. pyogenes* isolates, all the isolated bacteria were sensitive to enrofloxacin with a MIC <1 µM.

Introduction

Mastitis is the most frequent and costly disease that affects dairy cows. Viral infections, such as those caused by the pseudo cowpox virus, can induce teat lesions that negatively reduce the natural defense mechanisms of the udder and indirectly lead to infiltration of bacterial pathogens that cause bovine mastitis (Turner *et al*., 1976; Francis, 1984; Scott and Holliman 1984). Thus, viral infections may play an indirect role in bovine mastitis, due to their immunosuppressive properties (Wellenberg *et al*., 2002). Although multiple pathogens are associated with bovine mastitis, *Staphylococcus aureus* is a common and important cause of the disease (Cao and Hu, 2010) and has proved more difficult to control and treat despite the best possible antimicrobial treatments utilized (Barkema *et al*., 2006). Achieving a cure for a mastitis case is highly dependent on host immunity and specific characteristics of the infective organism; thus, antimicrobial susceptibility tests are not always accurate in predicting clinical outcomes (Apparao *et al*., 2009;

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Hoe and Ruegg, 2005). However, determination of antimicrobial sensitivity of mastitis pathogens is an important tool for monitoring the development of antimicrobial resistance (Pol and Ruegg, 2007). There are limited published national surveys of the antimicrobial susceptibilities of pathogens isolated from dairy cattle in Egypt. The objective of this study was to assess the antimicrobial susceptibility of bacterial bovine and bubaline mastitis pathogens isolated from cases admitted to the Veterinary Teaching Hospital in Assiut, Egypt. The antibiotics studied included gentamicin, polymyxin B, clindamycin, oxytetracycline, enrofloxacin and ciprofloxacin.

Materials and methods

Clinical examination

Eleven cases suffering from mastitis were admitted to the Veterinary Teaching Hospital- Faculty of Veterinary Medicine- Assiut University, Assiut, Egypt, during the months of January and February (2014). The attending veterinarian examined each case clinically and clinical symptoms (local signs such as swelling and pain in the udder, milk appearance, and systemic signs such as rectal temperature, general attitude, and appetite) were recorded. These cases included 5 buffaloes and 6 cows with teat lesions that resembled those of pseudo cowpox disease. All the studied cases were between the second and fourth lactation seasons, cows were all native breed and buffaloes were the Egyptian *Bubalis bubalis*. Some of these cases showed lesions in one quarter, others in two quarters and some with all quarters affected. The disease, in most cases, initially began with lesions in the teats resembling those observed with pseudo cowpox disease. Next, milk from the affected quarter changed complexion to form a pussy material. The affected quarter did not respond to treatment, became blackish in color, and eventually ended with sloughing of the teat. These cases were treated with oxytetracycline and penicillin; however there was no response to the treatment. In several cases, the infection extended to more than one quarter.

Sample collection and bacterial isolation

For milk collection, udders of the clinical mastitis cow were washed with clean water and dried. Cotton swabs soaked with 70% ethanol were used to disinfect the surfaces of teats. The first few streams of milk were discarded before a milk sample was collected into a 10-ml sterile plastic tube. Samples sent directly to the laboratory in an ice tank, and cultured at the same day of collection.

Milk samples were inoculated on 5% sheep blood agar plates and incubated at 37ºC for 24 h. *S. aureus* identification was confirmed via gram staining, morphology, and traditional biochemical tests, including catalase, coagulase and mannitol fermentation tests (Turk and Porter, 1978; Bannerman, 2003). Other pathogens were identified using laboratory procedures as defined by the National Mastitis Council guidelines (NMC, 1999).

Antibacterial assays

The selected antimicrobial agents (gentamicin and oxytetracycline (aminoglycosides), clindamycin (lincosamide), ciprofloxacin and enrofloxacin (fluoroquinolones) and polymyxin B (polypeptide)) were provided as powders by VWR, USA. The antimicrobial agents were subsequently dissolved in suitable solvents to make stock of 10 mM solutions.

The minimum inhibitory concentration (MIC) of each antibiotic was evaluated using the broth microdilution technique, in Mueller-Hinton broth (MHB) with an initial bacterial inoculum of 5×105 CFU/mL in non-treated Polystyrene microtiter plates, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2007). The MIC was interpreted as the lowest concentration of antibiotic that completely inhibit the growth of bacteria after 16 h incubation of the plates at 37°C. Each agent was tested in triplicate in at least two independent experiments. The highest MIC value was reported.

Results

Clinical findings

The disease commenced with vesicle and ulcerative lesions in the teats resembling those of pseudo cowpox disease (Fig. 1). Next, milk from the affected quarter changed to a pussy material; in some cases, the lesions resulted in the loss of milk secretion. Then the affected quarter did not respond to treatment with antibiotics and the skin color

changed to blue or black in color. Eventually, the disease progressed to sloughing of the teat (Fig. 2). These cases were treated in the field with oxytetracycline and penicillin with no response. In some cases, the infection started in more than one quarter, but in other cases, it extended from the diseased quarter to other healthy quarters, resulting in sloughing of the skin of the teat in all quarters especially in buffaloes (Fig. 3).

Fig. 1. Vesicles and ulceration in the teat with bluish discoloration of some areas on the teat.

Fig. 2. Sloughing of the dead tissue of the teat and no milk secretion.

Isolated bacteria

Fifteen bacterial isolates were identified from the cases, presented as 5 isolates of *S. aureus* (33.33%), 5 isolates of *Arcanobacterium pyogenes*

Fig. 3. Infection extended to all quarters with blackish discoloration of the teat skin and sloughing of the dead tissue.

(33.33%), 2 isolates of *Streptococcus agalactiae* (13.33%), 2 isolates of coagulase negative staphylococci (13.33%) and one *Klebseilla pneumoniae* isolate (6.67%) as shown in Fig. 4. *S. aureus* was isolated alone in two cows (13.33% of the isolates). *S. aureus* was isolated in combination with *A. pyogenes* in one buffalo (6.67%), and was isolated in combination with both *A. pyogenes* and *S. agalactiae* in two buffaloes (13.33%). Coagulase-negative staphylococci (CNS) were isolated alone in a single buffalo case (6.67%) and were isolated in combination with *Klebsiella pneumoniae* in one cow case (6.67%). *A. pyogenes* was isolated alone in two cows (13.33%). In two cases (one cow and one buffalo), we were not able to isolate any bacterial pathogens in spite of all quarters were affected in these two cases and discharging pussy milk.

Antimicrobial susceptibility

The antimicrobial susceptibilities of the analyzed bacterial isolates are presented in Table 1. The isolated strains were sensitive to clindamycin with a MIC between 2 to <0.03125 µM and showed medium sensitivity against gentamicin and ciprofloxacin with MICs ranging from 0.5 to 8 µM. Most *S. aureus* isolates were resistant to oxytetracycline with a MIC of 128 µM., additionally, most of the isolated bacteria were resistant to polymyxin B with a MIC > 128 µM. Nearly all the isolated bacteria were sensitive to enrofloxacin with a MIC <1 µM, some strains of *A. pyogenes* that only exhibited medium sensitivity.

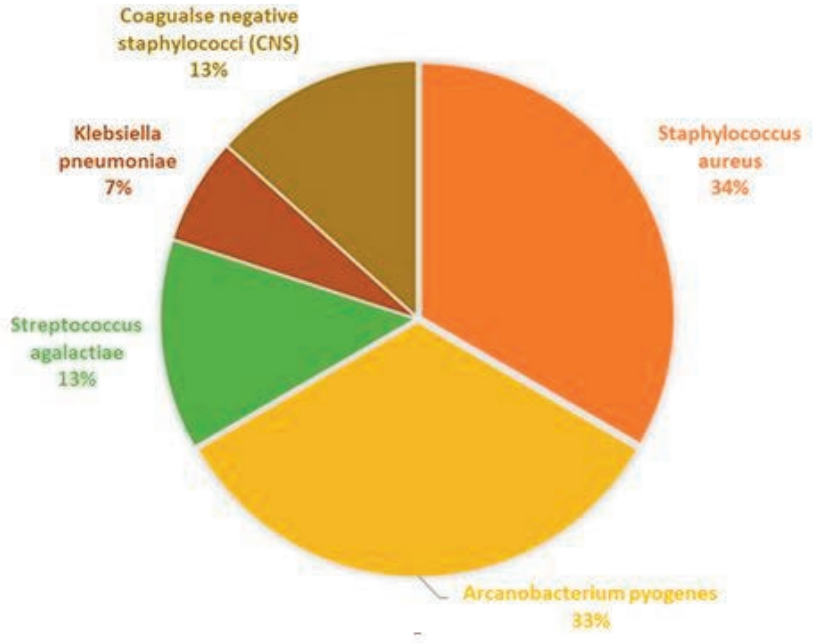

Fig. 4. Percentage of the isolated bacteria from the bovine mastitis cases.

Table 1. Minimum inhibitory concentration (MIC) of antibiotics against 15 bacterial isolates that were isolated from 11 mastitis cases.

Animals	Cases	Bacterial isolates	MIC/MBC(μM)					
			Gentamicin	Polymyxin B	Clindamycin	Ciprofloxacin	Oxytetracycline	Enrofloxacin
Buffaloes	Case No.1	Coagulase -ve staphylococci	<1	8/8	0.0625/0.0625	0.5/0.5	<1	<1
		Staph. aureus	8/8	128/128	<0.03125	2/2	<1	<1
	Case No.5	*Arcanobacterium pyogenes*	4/4	4/4	<0.03125	2/2	<1	4/16
		Streptococcus agalactiae	4/4	>128	<0.03125	4/4	<1	<1
	Case No.6	*Staph. aureus*	2/16	>128	<0.03125	0.5/0.5	128/128	<1
		Arcanobacterium pyogenes	4/4	2/4	<0.03125	2/2	<1	2/2
	Case No.8	*Staph. aureus*	8/8	>128	<0.03125	0.5/0.5	128/128	<1
		Arcanobacterium pyogenes	2/4	2/2	<0.03125	2/2	8/8	2/2
		Streptococcus agalactiae	4/4	>128	<0.03125	4/4	<1	<1
Cows	Case No.2	*Arcanobacterium pyogenes*	2/2	16/16	<0.03125	2/2	16/16	2/2
	Case No.3	*Arcanobacterium pyogenes*	2/4	2/2	<0.03125	<0.03125	8/16	2/4
	Case No.7	Coagulase -ve staphylococci	<1	2/128	<0.03125	<0.03125	<1	<1
		Klebseilla pneumoniae	8/8	2/2	2/2	<0.03125	<1	<1
	Case No.10	*Staph. aureus*	8/8	128/>128	<0.03125	0.5/0.5	128/128	<1
	Case No.11	*Staph. aureus*	>128	128/>128	<0.03125	0.5/0.5	128/128	<1

Discussion

Mastitis is the most prevalent disease of dairy cows and remains one of the most common reasons to remove cows from production (Pol and Ruegg, 2007). After developing mastitis, the probability of a successful cure depends on multiple factors including the immune system of the cow, virulence characteristics of the pathogen, and the efficacy of the treatment protocol. The development of antimicrobial resistance in mastitis pathogens is one factor that reduces the probability of cure (Oliveira *et al*., 2012). Bacteria may be intrinsically resistant to a specific class of antimicrobial due to a lack of an appropriate binding site/target, other pharmacological characteristics, or bacteria may acquire resistance to antimicrobials by mutations in their chromosomal genes or by exchange of genetic material via plasmids or transposons (Neu, 1992).

Bacteriological examination of milk samples has revealed that *S. aureus* is the predominant isolate in most mastitis cases especially in buffaloes. This may support the veterinary importance of *S. aureus* as a major cause of mastitis of dairy cows and buffaloes. Several authors in previous studies (Ali *et al*., 2008; Zaitoun, 2008; Tollersrud *et al*., 2006) concluded that *S. aureus* is an extremely important etiologic agent responsible for serious intramammary infection of all dairy ruminants, including buffaloes.

We did not test the MIC for penicillin against the isolated bacteria from our cases due to the fact that these animals were previously treated with penicillin were not responsive to this treatment. Therefore, the causative bacteria seem to be resistant to penicillin. Many studies from different countries have demonstrated that *S. aureus* strains isolated from bovine mastitis cases tend to be highly resistant to penicillin, a β-lactam antibiotic, while resistance to other antimicrobials was usually low (Erskine *et al*., 2004; Sabour *et al*., 2004; Vintov *et al*., 2003; Erskine *et al*., 2002; Aarestrup *et al*., 1995).

The second-highest degree of resistance to treatment of mastitis was observed with oxytetracycline (Guler *et al*., 2005). Our results showed high resistance of *S. aureus* to oxytetracycline; these results are similar to published reports in Finland (Myllys *et al*., 1998; Gianneechini *et al*., 2002). One possible explanation for this phenomenon could be that for many years tetracyclines have been the most widely used antimicrobial class utilized by farmers to treat any infection in their herd (Gao *et al*., 2012). Resistance to tetracycline comes in agreement with Pinto *et al*. (2013), and were described previously in other countries (Savoia *et al*., 2008; Martins *et al*., 2011), including in Brazil (Corrêa *et al*., 2011). The rise of tetracycline resistance is of concern since it is one of the main antimicrobials used in mastitis control (Keefe, 1997; Tenhagen *et al*., 2006). Overuse and misuse of antibacterial agents have been incriminated as the major selective forces encouraging the development of resistance in bacteria (WHO, 2002).

As resistance to penicillins and tetracycline amongst bacteria isolates from mastitis cases, it is critical to identify other antimicrobials that can be utilized for treatment. Potential options which warrant further investigation include polymixin B, gentamicin, and enrofloxacin. Polymyxin B is very effective in treatment of infections caused by Gram-negative bacteria. The mechanism of bactericidal activity of this agent is based on destabilization of outer and inner cell membranes surrounding the cells of Gram-negative bacteria (Galizzi *et al*., 1975). Gram-positive bacteria are protected with a thick cell wall composed of peptidoglycan (Galizzi *et al*., 1975). Therefore, they are more resistant to polymyxin B. Our findings confirmed that polymyxin B could kill all the isolated bacteria with the exception of *S. aureus*. These findings come in contrast with (Boyen *et al*., 2012; Szweda *et al*., 2014). Meanwhile most isolated strains showed mild sensitivity to gentamicin, which is in agreement with Boyen *et al*. and Szweda *et al*.'s results. Less data are available about trials using polymyxin B as a therapeutic agent for treating mastitis. Our investigation revealed low anti-staphylococcal activity of this compound, indicating it may not be a suitable treatment for mastitis cases caused by *S. aureus*.

Yoshimura *et al*. (2002) stated that gentamicin was the most active antimicrobial against *S. aureus* isolated from bovine intramammary infections with a MIC of 0.2 μg/ml. However, several of the tested isolates were resistant to gentamicin. Additionally, they found no isolates were resistant to clindamycin. Collectively, these results are similar to our findings. Clindamycin was used in our study to test for bacterial resistance against lincosamide antibiotics; as with Yoshimura *et al*'s study, none of the isolates we tested were resistant. Similar results were obtained by Gianneechini *et al*. (2002).

Enrofloxacin, a fluoroquinolone compound, inhibits DNA gyrase (Shen, 1993). Enrofloxacin, like other compounds in the quinolone class, has moderate activity against the staphylococci, fair activity against the streptococci, and good to excellent activity against Gram-negative bacilli (Green and Budsberg, 1993). Enrofloxacin was active against all groups of organisms used in the present study. The high susceptibility level of the isolated bacteria to enrofloxacin may be due to its infrequent use in cattle, in either intramammary or systemic administration, for treatment of mastitis. (Myllys *et al*., 1998; Gianneechini *et al*., 2002) found similar results.

Conclusion

All the isolated mastitogenic bacteria were sen-

sitive to enrofloxacin and clindamycin, which may suggest those antibiotics for dealing with the mastitic cases.

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References

Aarestrup, F.M., Wegener, H.C., Rosdahl, V.T., 1995. Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. Veterinary Microbiology 45,139–150.

Ali, L., Muhammad, G., Arshad, M., Saqib, M., Hassan, I.J., 2008. Bacteriology of mastitis in buffaloes in Tehsil Samundri of district Faisalabad, Pakistan. Pakistan Veterinary Journal 28, 31-33.

Apparao, D., Oliveira, L., Ruegg, P.L., 2009. Relationship between results of in vitro susceptibility tests and outcomes following treatment with pirlimycin hydrochloride in cows with subclinical mastitis associated with gram-positive pathogens. Journal of the American Veterinary Medical Association 234, 1437–1446.

Bannerman, T.L., 2003. Staphylococcus, Micrococcus, other catalase-positive cocci that grow aerobically. In Manual of Clinical Microbiology, (Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R.H., eds). Washington, DC: ASM Press. pp. 384–404

Barkema, H.W., Schukken, Y.H., Zadoks, R.N., 2006. Invited Review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science 89, 1877-1895.

Boyen, F., Verstappen, K., De Bock, M., Duim, B., Weese, J.S., Schwarz, S., Haesebrouck, F., Wagenaar, J.A., 2012. In vitro antimicrobial activity of miconazole and polymyxin B against canine meticillin-resistant *Staphylococcus aureus* and meticillin-resistant *Staphylococcus pseudintermedius* isolates. Veterinary Dermatology 23, 381–385.

Cao, L.T., Hu, S.H., 2010. Distribution of major pathogenic bacteria of cow mastitis in milk. Journal Anhui Agricultural Sciences 38, 19541–19542.

CLSI, 2007. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A7. CLSI, Wayne, PA.

Corrêa, A.B.A., Silva, L.G., Pinto, T.C.A., 2011. The genetic diversity and phenotypic characterisation of *Streptococcus agalactiae* isolates from Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz 106, 1002–1006.

Erskine, R.J., Cullor, J., Schaellibaum, M., Yancey, B., Zecconi, A., 2004. Bovine mastitis pathogens and trends in resistance to antibacterial drugs. Proc. NMC 43rd Annu. Mtg., Charlotte, North Carolina. National Mastitis Council, Madison, WI. pp. 400–403.

Erskine, R.J., Walker, R.D., Bolin, C.A., Barlett, P.C., White, D.G., 2002. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. Journal of Dairy Science 85, 1111–1118.

Francis, P.G., 1984. Teat skin lesions and mastitis. British Veterinary Journal 140, 430–436.

Galizzi, A., Cacco', G., Siccardi, A.G., Mazza, G., 1975. Mode of Action of Polymyxin B: Physiological Studies with a Bacillus subtilis-Resistant Mutant. Antimicrobial Agents And Chemorherapy 8, 366-369.

Gao, J., Ferreri, M.F., Yu, X., Liu, L., Chen, J., Su, B. H., 2012. Molecular types and antibiotic resistance of *Staphylococcus aureus* isolates from bovine mastitis in a single herd in China. Veterinary Journal 192, 550–552.

Gianneechini, R.E., Concha, C., Franklin, A., 2002. Antimicrobial Susceptibility of Udder Pathogens Isolated from Dairy Herds in the West Littoral Region of Uruguay. Acta Veterinaria Scandinavica 43, 31-41.

Green, C.E., Budsberg, S.C., 1993. Veterinary use of quinolones. Page 473 in Quinolone Antimicrobial Agents. 2nd Edition (D.C. Hooper and I. S. Wolfson, eds). Am. Soc. Microbiol., Washington, DC.

Guler, L., Ok, U., Gundu, K., Gulcu, Z.Y., Hadimli, H.H., 2005. Antimicrobial Susceptibility and Coagulase Gene Typing of *Staphylococcus aureus* Isolated from Bovine Clinical Mastitis Cases in Turkey. Journal of Dairy Science 88, 3149–3154.

Hoe, F.G.H., Ruegg, P.L., 2005. Relationship between antimicrobial susceptibility of clinical mastitis pathogens and treatment outcome in cows. Journal of the American Veterinary Medical Association 227, 1461–1468.

Keefe, G.P., 1997. *Streptococcus agalactiae* mastitis: a review. Canadian Veterinary Journal 38, 429–37.

Martins, E.R., Andreu, A., Correia, P., 2011. Group B streptococci causing neonatal infections in Barcelona are a stable clonal population: 18-year surveillance. Journal of Clinical Microbiology 49, 2911–2918.

Myllys, V., Asplund, K., Brofeldt, E., Hirvelä-Koski, V., Honkanen-Buzalske, T., Junttila, J., Kulkas, L., Myllykangas, O., Niskanen, M., Saloniemi, H., Sandholm, M., Saranpää, T., 1998. Bovine mastitis in Finland in 1988 and 1995 - Changes in prevalence and antimicrobial resistance. Acta Veterinaria Scandinavica 39, 119-126.

National Mastitis Council (NMC), 1999. Laboratory Handbook on Bovine Mastitis (Revised Edition). National Mastitis Council Inc, Madison. pp. 64–83.

Neu, H.C., 1992. The crisis in antibiotic resistance. Science 257, 1064–1073.

Oliveira, L., Langoni, H., Hulland, C., Ruegg, P.L., 2012. Minimum inhibitory concentrations of *Staphylococcus aureus* recovered from clinical and subclinical cases of bovine mastitis. Journal of Dairy Science 95, 1913–1920.

Pinto, T.C.A., Costa, N.S., Souza, A.R.V., da Silva, L.G., Corrêa, A.B.A., Fernandes, F.G., Oliveira, I.C.M., de

Mattos, M.C., Rosado, A.S., Benchetrit, L.C., 2013. Distribution of serotypes and evaluation of antimicrobial susceptibility among human and bovine *Streptococcus agalactiae* strains isolated in Brazil between 1980 and 2006. The Brazilian Journal of Infectious Diseases 17, 131-136.

Szweda, P., Schielmann, M., Frankowska, A., Kot, B., Zalewska, M., 2014. Antibiotic resistance in *Staphylococcus aureus* strains isolated from cows with mastitis in the eastern Poland and analysis of susceptibility of resistant strains to alternative non antibiotic agents: lysostaphin, nisin and polymyxin B. The Journal of Veterinary Medical Science 76, 355-362.

Pol, M., Ruegg, P.L., 2007. Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. Journal of Dairy Science 90, 262–273.

Sabour, P.M., Gill, J.J., Lepp, D., Pacan, J.C., Ahmed, R., Dingwell, R., Leslie, K., 2004. Molecular typing and distribution of *Staphylococcus aureus* isolates in Eastern Canadian dairy herds. Journal of Clinical Microbiology 42, 3449–3455.

Savoia, D., Gottimer, C., Crocilla, C., Zucca, M., 2008. *Streptococcus agalactiae* in pregnant women: phenotypic and genotypic characters. The Journal of Infection 56, 120–125.

Scott, F.M.M., Holliman, A., 1984. Serum antibodies to bovine mammillitis virus in pregnant heifers. The Veterinary Record 114, 19.

Shen, L.L., 1993. Quinolone-DNA interaction. In: Quinolone Antimicrobial Agents. 2nd ed. (D.C. Hooper and J. S. Wolfson eds). Am. Soc. Microbiol., Washington, Dc. p. 77.

Tenhagen, B.A., Köster, G., Wallmann, J., Heuwieser, W., 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. Journal of Dairy Science 89, 2542-2551.

Tollersrud, T., Kam pen, A.H., Kenny, K., 2006. *Staphylococcus aureus* enterotoxin D is secreted in milk and stimulates specific antibody responses in cows in the course of experimental intramammary infection. Journal of Infection and Immunity 74, 6, 3507-3512.

Turk, D.C., Porter, I.A., 1978. A Short Textbook of Medical Microbiology, 4th ed, London: Hodder and Stoughton.

Turner, A.J., Kovesdy, L., Morgan, I.R., 1976. Isolation and characterisation of bovine herpesvirus mammillitis virus and its pathogenecity for cattle. Australian Veterinary Journal 52, 166–169.

Vintov, J., Aarestrup, F.M., Zinn, C.E., Olsen, J.E., 2003. Association between phage types and antimicrobial resistance among bovine *Staphylococcus aureus* from 10 countries. Veterinary Microbiology 95, 133–147.

Wellenberg, G.J., van der Poel, W.H., Van Oirschot, J.T., 2002. Viral infections and bovine mastitis: a review. Veterinary Microbiology 88, 27-45.

WHO, 2002. Antimicrobial resistance. WHO Media Center Available: http://www.who.int/mediacenter/factsheets/fs194.

Yoshimura, H., Ishimaru, M., Kojima, A., 2002. Minimum inhibitory concentration of 20 Antimicrobial Agents against *Staphylococcus aureus* from Bovine intramammary infections in Japan. Journal of Veterinary Medicine, Series B 49, 457-460.

Zaitoun, A.M.A., 2008. Clinical mastitis caused by *Staphylococcus aureus* in dairy buffaloes. Assiut Veterinary Medical Journal 54, 289-310.