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RESEARCH ARTICLE

Assessing the Antimicrobial Resistance Patterns and in Vitro Efficacy of three Disinfectants as teat dip against Iodine (0.5%) Resistant Mastitogenic Pathogens

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Abstract

Dairy animals are facing a new challenge of increase bacterial resistance to some of the existing antibacterial agents. This study aimed to determine the antimicrobial resistance patterns and to evaluate in vitro efficacy of three teat dips, "chlorine dioxide, iodine glycerin (0.8%) and lactic acid /emollient (2%)" disinfectants against mastitogenic bacteria resistant to iodine (0.5%) in dairy herds. A cross sectional study was conducted in dairy cattle farm had a previous history of mastitis problem with high culling rate. All the udders of lactating cows were examined clinically and bacteriologically for isolation of mastitis causing bacteria then identified biochemically and serologically. All the bacteria isolated were subjected to antimicrobial susceptibility test by broth macro dilution method to iodine (0.5%) then forty five bacterial isolates showed iodine resistant were re-tested for their sensitivity to three tested disinfectants. Results revealed that the total prevalence of mastitis at cow and quarter levels were (30.0% and 18.67% resp.,). The most prevalent pathogens isolated were Coagulase negative staphylococci (CNS) (45.53%) followed by S.aureus (42.86%) and E.coli (O166) (11.61%). Regarding, antimicrobial sensitivity test, iodine (0.5%) showed the lowest bactericidal effect at different exposure times compared to tested disinfectants, Moreover, E.coli (O166) isolates showed highest resistant to iodine at 5 minutes exposure (50 %) followed by S.aureus and CNS & (30 % & 25% resp.,), Comparing the bactericidal effect of three tested disinfectants against bacterial isolates in vitro revealed that at 60 sec, both of iodine glycerin (0.8%) & lactic acid/emollient(2%) exhibited a highest lethal effect against CNS & S.aureus isolates (100%) compared to Chlorine dioxide (87% &85% resp.,) at 5 minutes exposure meanwhile, lactic acid/emollient (2%) had the highest effect against E.coli followed by iodine glycerin(0.8%) at 60 sec & 5 min then chlorine dioxide . In conclusion, in vitro testing of teat disinfectant resistance patterns against mastitogenic bacteria must be done regularly in dairy herds accompanied with application of disinfectant with lowest resistant such as lactic acid/emollient (2%) or iodine glycerin (0.8%) provide approaches to minimize the further development of antimicrobial resistance and improve the efficacy of bovine mastitis prevention programs.

Introduction

Mastitis is one of the most economically significant diseases for the dairy industry for backyard farmers in developing countries and high producing herds worldwide (Tiwari et al., 2013). Due to the involvement of multiple etiological agents it always remained a challenge to veterinarian all over the world (Vashney et al., 2012).

Awareness of the economic losses associated with mastitis is resulting in a desire for mastitis control programs. Control programs are focused on detection of mastitis, identification of the causative agents. Prevention of transmission by removing the source of the agent (milk contaminated fomites, bedding, persistently infected cows, etc.) and antibiotics/germicides is important in achieving effective mastitis control (Rajib Deb et al., 2013)

Post-milking teat disinfection is considered one of the most effective procedures for reducing the rate of subclinical and clinical cases of mastitis during lactation (El Behiry et al., 2012). More than ten different active compounds have been used in teat disinfectants worldwide over the last 20 years. The National Mastitis Council (NMC) (USA) reviewed and summarized nearly all the scientific literature on teat disinfectants published since 1980, and found that more than 10 different active ingredients as iodine and chlorhexidine have been used in teat disinfectants throughout the world (Pyörälä and Taponen, 2009)

Despite the universal acceptance of teat dipping as a method of mastitis control, a number of limitations are associated with most teat dips currently available. The most significant restriction is that teat dips do not provide equal protection against the huge amount of bacteria that cause bovine mastitis. Furthermore, prolonged in vitro exposure to germicidal teat dips has been shown to enhance the resistance of some bacteria to chemical disinfectants (El Behiry et al., 2012). Several passages of bacterial isolates with sub-lethal concentrations of disinfectants were found to either induce resistance or select for resistant variants (Yilmaz and Kaleta , 2009).

Some laboratory studies suggested that the development of resistances against biocides and antibiotics are linked whereas other studies failed to identify an association (McDonnell and Russell 1999; Russell et al., 1998) Microorganisms are limitlessly adaptable and have already demonstrated different mechanisms of resistance to different biocides; the concern is that these mechanisms may give cross-resistance to clinically important antibiotics. The current study was thus performed to determine the iodine resistance patterns of S. aureus , CNS and E.coli O166 isolated from cases of bovine mastitis and evaluate the in vitro efficacy of three different types of teat disinfectants (iodine glycerin (0.8%) , lactic acid /emollient (2%) and chlorine dioxide for tackling mastitiogenic bacterial resistant problem in dairy herds.

Materials and Methods

Study area and period: The study was conducted in a private dairy cattle farm had a previous history of mastitis problem with high culling rate at Beni-Suef district during the period from (August 2013 till March 2014).

Study animals and husbandry practices: The study population comprises of approximately 150 Holsteins lactating cattle managed under semi intensive system. The animals were kept in partially sheltered yards with bedded earthy floor at stocking rate 12 m2/ animal. The manure and soiled bedding were removed once every 3 months. Lactating cattle were milked three times daily in separated and well-constructed abreast parlour provided with 10 milking machines. All lactating cows were screened by California Mastitis Test (CMT), once /month for detection of subclinical mastitic cows. Udders of all lactating cows were dipped in Iodine 0.5% post-milking (once a day). Hygienic measures, prevailed in the farm were of moderate standard. No program admitted for prevention and control of subclinical mastitic animals.

Study design and sampling procedure: A cross-sectional type of study was carried out. A structural Questionnaire was prepared and information's regarding to mastitis prevalence, risk factors, previous history, preventive measures and teat dip uses were collected and analyzed. .Lactating cows were examined clinically, screened by CMT for detection of subclinically mastitic cows then quarter milk samples were collected from apparently health, sub-clinical and clinically mastitic cows for bacteriological examination..

Collection of milk samples: Udders of lactating cows were prepared before milk samples collection, teat ends of cows were cleaned with warm water and dried then 15 ml of fore milk was drawn and discarded. The teat ends were scrubbed with a cotton or paper towel containing 70% ethanol; one towel was used for each teat before the sample was collected. One hundred and forty quarter milk samples were collected from apparently health, sub-clinical and clinically mastitic cows using standard procedures described by the National Mastitis Council (2001). All collected milk samples were transported in ice box contain ice bag, maintained at 4 °C until analysis and transported as soon as possible to the laboratory at the Faculty of Veterinary Medicine, Beni-Suef University .

Time of sample collection: Samples for culture were collected before milking that was most convenient under the management conditions of the individual herd

Isolation and identification of mastitis causing bacteria: Bacteriological examination of quarter's milk samples was carried out following standard procedures of (National Mastitis Council, 2001). Preliminary identification of S. aureus strains was done by colony morphology, hemolysis, and Gram staining. Specific identification of S. aureus strains and CNS were done phenotypically by the tube coagulase test and the Staph ID 32 API systems ((API System, BioMe´rieux, Paris,France) while E.coli were isolated on MacConkey agar (Oxoid; CM 0115) and Eosine Methylen Blue agar (EMB; Oxoid; CM 69) plates, The suspected colonies were examined microscopically by Gram's methods, identification were done using the API 20E (Biomerieux, Crappone France) and purified for further serological tests and sent to central lab. belonged to the Ministry of Health, Cairo, Egypt, using techniques adopted by (Sojka, 1965) Serotyping was done by slide agglutination technique using diagnostic polyvalent and monovalent E.coli O & K antisera.

Disinfectant susceptibility test: The antimicrobial resistance patterns of the isolates were determined using the broth macro dilution technique as method described by (Costa et al., 1998)

Tested disinfectants: Iodine (0.5%) (Derma Kote,Westfalia-Surge); Chlorine dioxide (Bi-Sept,Westfalia-Surge,Naperville,IL) was provided as two separate parts , and each part was mixed in equal quantities before use . Part (1) was an activator containing (2.9%) lactic acid and (8%) glycerin, and it was mixed in equal quantity with part (2), which was a base containing (0.7%) sodium chlorite and (2%) glycerin ; iodine glycerin (0.8%) (polyalkylene glycol ether iodophor) (Les Produits Agro-B Inc) and lactic acid /emollient (2%) (96780, Biocel ltd).

Tested organisms: All Bacterial isolates were firstly tested in vitro for their sensitivity to iodine (0.5%) then forty five of bacterial isolates showed iodine resistant (n=15 of each) CNS ,S.aureus and E.coli (O166) were re-tested against three different types of disinfectants, the same active substances were used on both the pre- and post-milking teat dipping stages by dairy farmers

Test procedure: Minimum Inhibitory Concentration (MIC) for all bacterial strains was determined using the broth macro dilution method which is indicative of the guideline for examination of chemical disinfectants as recommended by (Costa et al. 1998), Homogeneous bacterial suspensions were prepared in BHI, and 1.2 mL aliquots were inoculated with 0.8 mL of disinfectant solutions, diluted in 0.2 ml of sterilize milk. The sensitivity pattern towards each disinfectant was then determined for different exposure periods (15, 30, 60 and 300 seconds). After wards, inocula were transferred to BHI-containing tubes, for incubation at 37^{o}C for 24 h. a positive sample is considered that exhibiting medium turbidity and formation of a surface thin skin or of a precipitate, in the bottom of the tubes. After incubation, the suspension was spiked in solid medium (blood agar) to confirm the presence or absence of microorganism tested against various teat disinfectants and exposure times. The absence of bacterial growth in plates indicated the effectiveness of the product in question.

Statistical analysis

The data collected and recorded on specifically designed forms and prepared for analysis was entered in the Microsoft excel spread sheet. The prevalence of mastitis (sub-clinical and clinical) and the frequent distribution of bacterial isolates from mastitic animals were calculated by using percentage values and analyzed by using Chi-square test (X^2) test as the proportion of affected cows out of the total examined, (Systat Version, 1997).

Results and Discussion

The prevalence of mastitis at both cow and quarter levels in examined farm based on bacteriological findings in **(Table 1)** were (30.0% and 18.67% resp.,) at X^2 =15.0, p value (< 0.001) and the highest rate of subclinical mastitis at cows and quarters level were (21.33% and 14%) compared to those showed clinical signs (8.67% and 4.67% resp.,) .These results are relatively similar to (Biffa et al., 2005) who recorded a lower incidence, 34.9% had mastitis, 11.9% clinical and 23.0% subclinical and lower than De Schepper et al., 2006 ; Mohammed et al., 1993 ; Gharagozloo et al., 2001; Schukken et al., 1989) concluded that clinical mastitis is only the 'tip of the iceberg'. Subclinical mastitis is by far the more costly disease in the majority of herds. The variability in the prevalence of

bovine mastitis between reports could be attributed to the difference in management of the farm, breeds, season of the study, agro climactic condition or diagnostic test employed.

Findings in (**Table 2)** revealed a high correlation between CMT score and bacteriological findings whereas the highest percentage of bacterial isolates were recovered from subclinically mastitic cow's quarters (65.63%) followed by clinically mastitic cow's quarters (53.85%), Moreover Coagulase negative staphylococci (CNS) was the most prevalent isolated agent from mastitic cows (45.53%) followed by S.aureus and E.coli (42.86% and 11.61% resp.,). The obtained results denoted that contagious pathogens primarily CNS followed by S.aureus were the most prominent cause of subclinical mastitis in dairy farm, meanwhile, S.aureus followed by E.coli were the main causative agent for clinical mastitis in dairy herd .Nearly similar findings were recorded by (Makaya et al., 1996; Kudinha & Simango, 2002; Petzer et al., 2009) they recorded that CNS is the most common agent isolated from sub-clinical mastitic animal. This highlights the importance of the role of CNS in sub-clinical mastitis and that some of them are more pathogenic than is generally assumed. Meanwhile (Mekibib et al., 2010) found that from 146 culture positive milk samples, a total of 153 bacteria were isolated, the most prevalent being S. aureus (47.1%) followed by Coagulase negative Staphylococcus (CNS) (30.1%). Other bacterial isolates included Streptococcus (7.2%), E.coli (4.6%) and Klebsiella pneumonae (3.3%).

Results in (**Fig. 1**) indicated that iodine (0.5%) had the lowest lethal effect (0.0%) against all tested bacterial isolates after 15 seconds exposure time that increased gradually with increased times of exposure (30"& 60" sec) to be moderately lethal against E.coli isolates (50%) and (75% &70%) for CNS & S.aureus resp., after 5 minutes. This result was in accordance with (Fox et al., 1991) who concluded that the bactericidal activity of iodine as teat disinfectant for control of bovine mastitis correlated positively with increasing \contact times up to 10 min. and (El Behiry et al., 2012) who found that prolonged in vitro exposure to germicidal teat dips has been shown to enhance the resistance of some bacteria to chemical disinfectants. Contrarily (Medeiros et al., 2009) carried out in vitro sensitivity of S. aureus isolates against different types of antiseptics. They found that the highest disinfectant activity in vitro was found to be with iodine (0.6%) for S. aureus

Comparing the bactericidal efficacy of three different types of disinfectants against bacterial isolates CNS ,S.aureus & E.coli in vitro as shown in **(Fig. 2,3&4)** . Results in **(Fig. 2)** revealed that both iodine glycerin (0.8%) and lactic acid (2%) exhibited a high bactericidal effect against CNS at 15 sec (87 & 84 % resp.,) to reach 100 % at 60 sec at ($P < 0.005$). Meanwhile the highest lethal effect for chlorine dioxide was (87%) at 5 min exposure only. Similarly obtained results in **(Fig. 3)** emphasized that S.aureus isolates exerted a high sensitivity to both iodine glycerin (0.8%) and lactic acid/emollient (2%) at 15 sec (87% & 82 % resp.,) to reach 100 % at 60 sec. meanwhile the highest lethal effect for chlorine dioxide by (85%) was at 5 min exposure. These results were in agreement with (Goldberg et al., 1994) who demonstrated that infected cell exposure time to (iodine glycerin) disinfectants is crucial and should be at least 60 seconds. (Boddie et al., 2000) revealed that the chlorine dioxide teat dip reduced the number of new intra mammary infection caused by staph aureus by 86.6% at ($p<0.001$). Regarding (Medeiros et al., 2009) observed that the Positive coagulase *Staphylococcus* (SCP) isolates were sensitive to lactic acid (2%) (72.7 %, 72.7 %, 90.9 % and 100.0 % resp.,) at different interval of 15, 30, 60 and 300 seconds exposure in vitro.

Regarding the bactericidal effect of tested disinfectant against E.coli. in **(Fig. 4)** it's evident that lactic acid/emollient (2%) exhibited a highest lethal effect at different exposure times as compared with the other two disinfectants to reach 90 % at 60 sec .on the other hand, although chlorine dioxide had a higher activity against E.coli (82%) at 5 minutes, Iodine glycerin (0.8%) excelled at different exposure times to equalized lactic acid efficacy at 60 seconds. From the obtained results in figures 2,3,4 it can be concluded that lactic acid /emollient (2%) showed a highest lethal effect against CNS, S.aureus (each100%) and E.coli O166 (90%) followed by Iodine glycerin (100%, 100% and 85% resp.,) after 60 second exposure. On the other hand, Chlorine dioxide revealed its efficacy against CNS, S.aureus and E.coli (87%, 85% and 82% resp.,) after 300 second exposure only.

Table 1: Prevalence of clinical and subclinical mastitis at cow and quarter levels based on clinical observation and culture.

Samples	Total examined (NO.)	Bacteriological findings			
		Normal (%)	Mastitis (%)		
			Subclinical	Clinical	Total
Cows	150	70	21.33	8.67	30
Quarters	600	81.5	14.0	4.67	18.67

X^2 =15.0, p value (< 0.001)

Table 2: Prevalence and distribution of bacteria isolated from mastitis cow's quarters based on CMT and culture

Findings	Quarters examined			Distribution of bacterial isolates (%)		
CMT results	Total (No.)	Infected		CNS	S. aureus	E.coli (O166)
		No.	%			
Normal	489	-	-	-	-	-
SCM	128	84	65.63	60.71	39.28	-
CM	52	28	53.85	-	53.57	46.43
Total	600	112	18.67	45.53	42.86	11.61

SCM: Subclinical mastitis **CM:** Clinical mastitis

Fig. 1: Relative sensitivity (%) of bacterial isolates to iodine (0.5%) at different exposure times / seconds

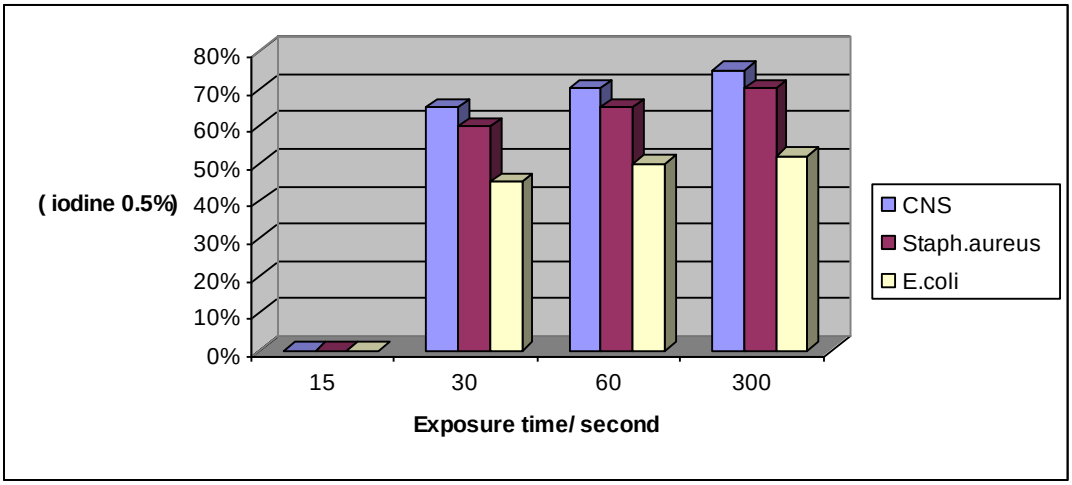


Fig. 2: Relative sensitivity (%) of Coagulase negative staphylococci (CNS) isolates to three tested disinfectants at different exposure times / seconds

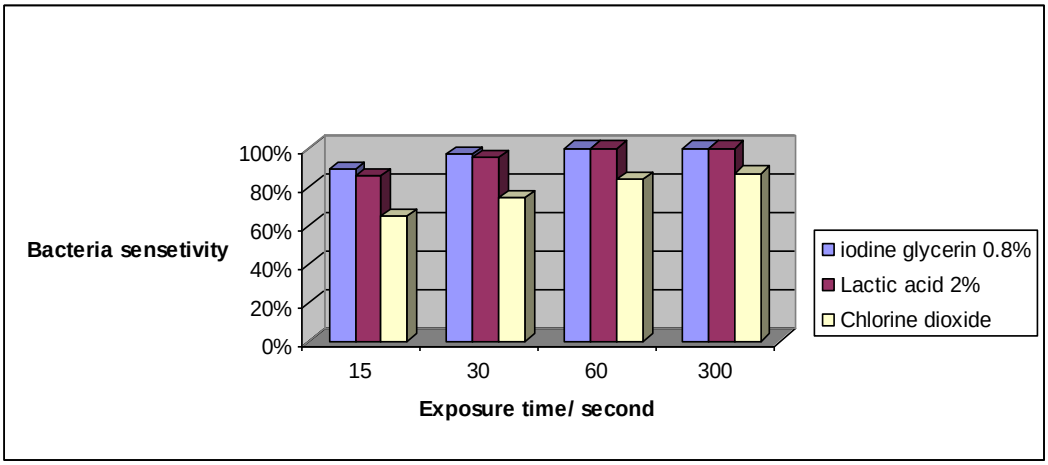


Fig. 3: Relative sensitivity (%) of S.aureus isolates to three tested disinfectants at different exposure times/seconds

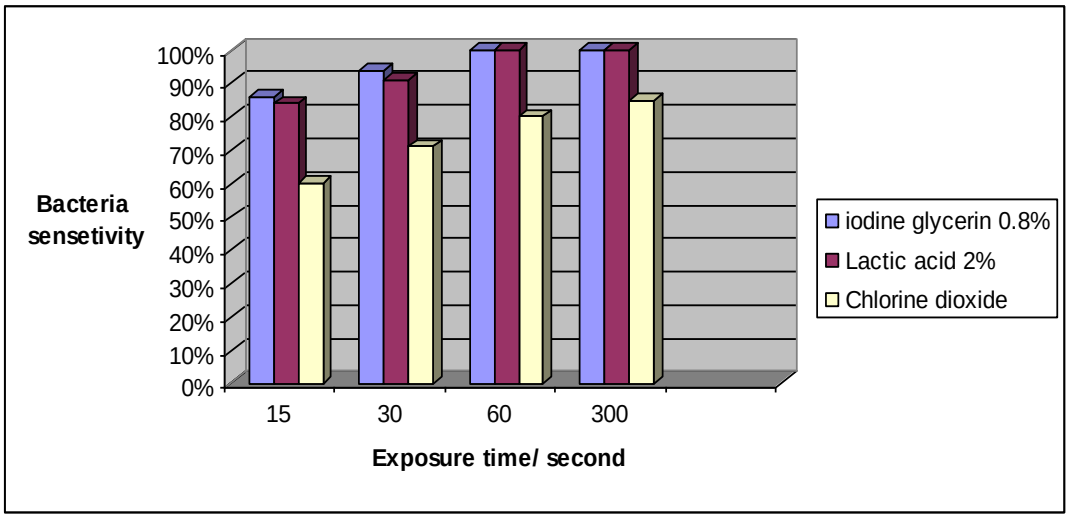
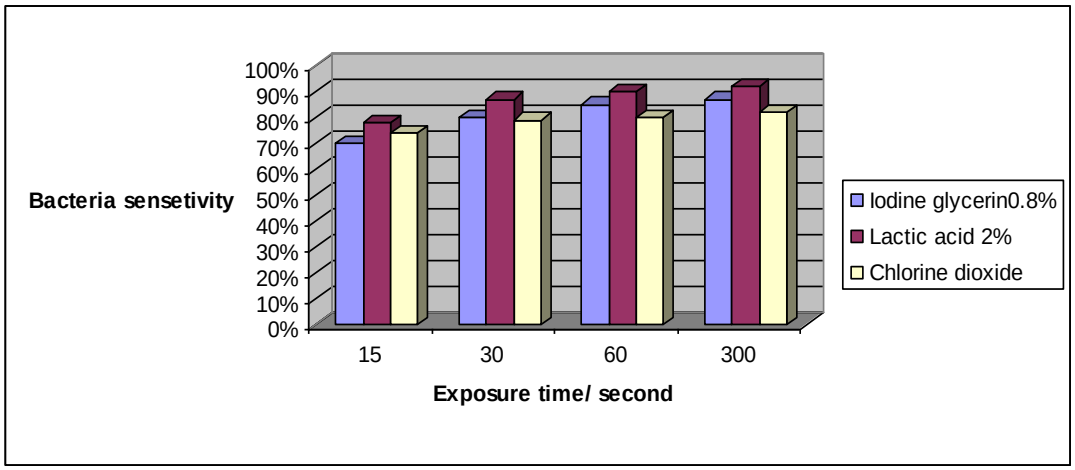


Fig. 4: Relative sensitivity (%) of E.coli (O166) isolates to three tested disinfectants at different exposure times / seconds



Conclusion

In vitro testing of teat disinfectant resistance patterns against mastitogenic bacteria must be done regularly in dairy herds accompanied with application of disinfectant with lowest resistant such as lactic acid/ emollient (2%) or iodine glycerin (0.8%) provide approaches to minimize the further development of antimicrobial resistance.

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References

Biffa, D., Debela, E. and Beyene, F. (2005). Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. International Journal of Applied Research in Veterinary Medicine, (3): 189-198.

Boddile, R.L., Nickerson, S.C. and Adkinson, R.W. (2000). Efficacies of chlorine dioxide and iodophors teat dips during experimental challenge with staphylococcus aureus and streptococcus agalactiae. Journal of Dairy Science, 83: 2975-2979

Costa, E.O., Ribeiro , A.R., Watanabe, E.T., Garino, J.R., Silva, F., JAB and Thiers, F.O .(1998). Avaliação *in vitro* dos desinfetantes utilizados na pós-ordenha (teat dipping) para controle da mastite bovina [*In vitro* evaluation of disinfectants used in post-dipping (teat dipping) for control of bovine mastitis]. Revta Napgama, 1: 18-22.

De Schepper , S., Bannerman, D.D., Paape, M.J. and Burvenich, C. (2006). Annual incidence of clinical mastitis in three dairy herds. Vet. Rec., 36: 14- 19

El Behiry, A.I., Schlenker, G., Szabo, I. and Roesler, U.(2012). In vitro susceptibility of Staphylococcus aureus strains isolated from cows with subclinical mastitis to different antimicrobial agents. Journal of Veterinary Science, 13(2): 153–161.

Fox, L.K., Nagy, J.A., Hillers, J.K., Cronrath, J.D. and Ratkowsky, D.A. (1991). Effects of post-milking teat treatment on the colonization of Staphylococcus aureus on chapped teat skin. American. Journal of Veterinary Research, 52:799- 802.

Gharagozloo, F., Erfanmanesh, A. and Bahonar, A.(2001). A survey of milk quality and occurrence of mastitis by bacteriological method and somatic cell count (SCC) in industrial farms around city of Karadj . Proceeding of 1st special committee of milk and dairy industry, PP: 95- 106.

Goldberg, J.J. ,Murdough, P.A., Howard, A.B. and Drechsler, P.A. (1994). Evaluation of 1 percent iodophor postmilking teat sanitizer. Journal of dairy science, 27 (33): 740-747.

Kudinha, T. and Simango, C.(2002).'Prevalence of coagulase-negative staphylococci in bovine mastitis in Zimbabwe', *Journal of the South African Veterinary Association,* 73(2): 62–65.

Makaya, P.V., Aarestrup, F.M. and Olsen, J.E.(1996). 'Distribution and antibiotic resistance patterns of common mastitis pathogens (Gram-positive cocci) in selected dairy herds of three farming dairy sectors in Zimbabwe', Zimbabwe Veterinary Journal,27(2): 65–75.

McDonnell, G. and Russell, A.D. (1999). Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999; 12: 147–179.

Medeiros, S., Elizabeth, A. and Sampaio, D. (2009).In vitro evaluation of the efficacy of commercial disinfectants used in pre and post-dipping against Staphylococcus spp. isolated from bovine mastitis. *Pesquisa* Veterinária Brasileira, 29 : 71-75

Mekibib, B., Furgasa, M., Abunna, F., Megersa, B. and Regassa, A.(2010). Bovine Mastitis: Prevalence, risk Factors and major pathogens in dairy Farms of Holeta Town, Central Ethiopia. Veterinary World, 3:397-403

Mohammed, A., Ibtisam, E. and Elowni, O.A.O. (1993). A study of the incidence and aetiology of bovine mastitis in Sudan. Second Sci , Cong. Egyptian society for cattle disease , Assiut, Egypt, pp. 326- 336.

National Mastitis Council, (2001). Summary of peer-reviewed publications on efficacy of pre-milking and post-milking teat disinfectants published since 1980, updated January 2001NMC website nmconline.org..

Petzer, I.M. , Karzis, J., Watermeyer, J.C., Van der Schans, T.J. and Van Reenen, R .(2009). 'Trends in udder health and emerging mastitogenic pathogens in South African dairy herds', *Journal of the South African Veterinary Association*, 80: 17–22

Pyörälä ,S. and Taponen,S.(2009). Coagulase-negative staphylococci-emerging mastitis pathogens. Veterinary Microbiology,134:3–8.

Rajib Deb, Kumar, A., Chakraborty, S., Verma, A.K., Tiwari, R., Dhama, K., Singh, U. and Kumar, S .(2013). Trends in Diagnosis and Control of Bovine Mastitis: A Review. Pakistan Journal of Biological sciences, 16 (23): 1633-1661

Russell, A.D. , Tattawasart, U., Maillard, J.Y. and Furr, J.R. (1998). Possible link between bacterial resistance and use of antibiotics and biocides. Antimicrobial Agents Chemotherapy, 42: 21-51.

Schukken, Y.H., Grommers, F.J., Van de Geer, D. and Brand, A. (1989). Incidence of clinical mastitis on farms with low somatic cell counts in bulk milk. Vet .Rec., 125(3):60-63.

Sojka, W.J.(1965) . E.coli in domestic animals and poultry. Fanham Royal Common ,Wealth Agriculture Bureax. PP.34-41

Systat Version 7.0.1, (1997). Statistics. SPSS Corporation.

Tiwari, J.G., Babra , C., Kumar, H., Tiwari, Williams, V ., De Wet, S. et al., (2013).Trends In Therapeutic and Prevention Strategies for Management of Bovine Mastitis: An Overview. Journal of Vaccines and Vaccination, 2013, 4:2

Vashney, S., Vashney, P., Dash,S.K., Gupta, M.K., Kumar, A., Singh,B .and Sharma, A.(2012). Antibacterial activity of fruits of Terminelia chebula and Terminalia belerica against mastitis field isolates. Med. Plants, 4: 167-169.

Yilmaz, A.1. and Kaleta, E.F. (2009). Suitability of two commercial preparations for disinfections against methicillin-resistant Staphylococcus aureus in veterinary medicine. Dtsch Tierarztl Wochenschr, 116(5):180 185.