Profile of Antimicrobial Susceptibility from Cattles's Milk Isolates Suffering from Mastitis in District Lahore

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Cover Page Footnote

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PROFILE OF ANTIMICROBIAL SUSCEPTIBILITY FROM CATTLES'S MILK ISOLATES SUFFERING FROM MASTITIS IN DISTRICT LAHORE

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ABSTRACT

Mastitis is highly prevalent infection in cattle causing cost-effective loss in dairy milk production. *Escherichia coli* is the most frequently isolated bacteria causing mastitis worldwide. The current study was performed to investigate the mastitis prevalence and effect of different antibiotics against pathogens causing it. In sum, 216 milk samples were collected randomly including 108 each from both cows and buffaloes. These samples were subjected to Surf Field Mastitis Test for prevalence of mastitis that found 18.50% and 23.14% in buffaloes and cows respectively. Mastitis was more prevalent both in buffaloes and cows at the age of 9-10 years that was reported in 35% and 32% respectively. The affected buffaloes showed 65% samples containg watery fluid having blood, pus and mucus whereas 36% samples of affected cows had mucus and watery fluid. Pus and blood content were found to be 12% and 16% respectively. Sub-clinical mastitis was prevalent as 56% and 55% in cows and buffaloes respectively while acute mastitis as 4% and 5% in similar fashion. In both groups Right-for part of udder was frequently affected than any other. Among commonly used four antibiotics, Norfloxacine was observed the most effective antibiotic to control mastitis as compared to Gentamycin, Penicillin and ciprofloxacin. The benefit of this study is to diagnose mastitis and use to isolate pathogens for further processing like antibiotic sensitivity and resistance. This study will help veterinarians in better selection of antibiotic to control mastitis.

Keywords: Mastitis, prevalence, cattle, udder, antibiotics.

INTRODUCTION

Livestock is playing an important role in the development of Pakistan just like other countries and facilitating the development and consumption of different items like meat, milk and various house products. There are 28.4 million buffaloes and 25.5 million cattle involved in the production of 1174.4 tons beef and 31294.4 tons milk respectively (Economic Survey of Pakistan 2005-2006). Mastitis is a common disease in livestock which make the milking animals useless (Sánchez-Macías et al., 2020). Mastitis is the redness, swelling, pain or hardness and heat in the udder of the animals (Zahid,

2004., Jamali et al., 2018). Field survey of Pakistan indicated that mastitis is one of the major diseases of dairy animals in livestock (Hussain et al., 2005). Mastitis causes the inflammation of the teat involving one/ two/three or full four quarters (Mc Dowell et al., 1995).

Worldwide, there is a great loss nearly of 35 billion dollars. Annual loss due to mastitis is about more than 2 billion dollars in United State. About 3 million animals linked to dairy farms show visible symptoms of mastitis (acute) whereas 3 lac die from endotoxins shock (Kivaria and Noordhuizen, 2007). In Punjab, Pakistan, the total annual loss caused by mastitis is about 240 million rupees. The incidence

and existence of pathogens connected to diseases (mastitis) differ from herd to herd, time to time and even place to place (Joshi and Gokhale, 2006). Mastitis from livestock and dairy animals cannot totally be eliminated but its effect can be reduces with the help of preventive practices against mastitis (Khan and Khan, 2006). Mastitis present in milking animals is of various types such as clinical and sub-clinical (Hillerton, 1999). It is more prevalent in cattle than buffaloes (Thepa and Kaphle, 2002). Mastitis causing agents include certain species algae, fungi, bacteria and some viral entities (Thomas et al., 1997). Among all mastitis causing strain of bacteria staphylococcus is one with broad geographic distribution throughout the world (Annemuller et al., 1999).

Pathogens which cause mastitis are mostly found in fecal matter and soil as well (Rodostits et al., 2000). The time of teat canal to remain open after milking is about 1-2 hours that facilitates the entrance of mastitis causing bacteria in to the teat canal (Jones, 2006). Under stress condition animal secretes adrenaline that causes constriction of blood vessels leading to hindrance the supply of oxygen to the mastitis suffering tissue (Rodostits et al., 2000). In animals suffering with mastitis cortisol level decreased while serum calcium, inorganic phosphorus and total protein also decreased (Zaki et al., 2008). Milk proteins such as transferrin, immunoglobulin and serum albumin pass into the milk as the result of vascular system permeability (Khan and Khan, 2006). Few antibiotics such as norfloxacin and gentamycine are more effective while pathogens are resistant against penicillin (Zahid, 2004).

Present study will help to enhance the dairy products with easy identification and control of mastitis at local level. The main objective of the designed study was to find the mastitis prevalence along the effect of various selected antibiotics on mastitis causing agents in buffaloes and cows in District Lahore.

MATERIALS AND METHODS

Milk samples from 216 lactating animals (108 cows and 108 buffaloes) were randomly collected and brought to the Microbiology Laboratory of GCU Lahore. Animals were grouped depending upon the age, part of udder infected, appearance of milk and condition of the disease. Prevalence of mastitis in animals was determined by Surf Field Mastitis Test (SFMT) (Muhammad et al., 1995). Buffaloes and cows were compared for prevalence of mastitis. Four different types of antibiotics i.e., penicillin, gentamycine, norfloxacine and ciprofloxacin discs were used to check which antibiotic is more effective to control mastitis in District Lahore.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar by the Kirby-Bauer disk diffusion method (Clinical and Laboratory Standards Institute, 2012). The 4 commercial antibiotic disks used in the experiment included Norfloxacin (10mg), Ciprofloxacin (5mg), Penicillin (10mg), Gentamycin (10mg). The test results were then recorded as susceptible or resistant based on the zone diameter (Clinical and Laboratory Standards Institute, 2013) The data were analyzed with percentage, one and two tail t- tests by using SPSS version 22.0 (SPSS, Chicago, IL, USA). The differences among buffalo and cow at different age groups were found.

RESULTS AND DISCUSSION

Results of current study indicated that mastitis was more prevalent in cows than buffaloes where 18.50% samples of buffaloes while 23.14% samples of cows were affected. More prevalence of mastitis

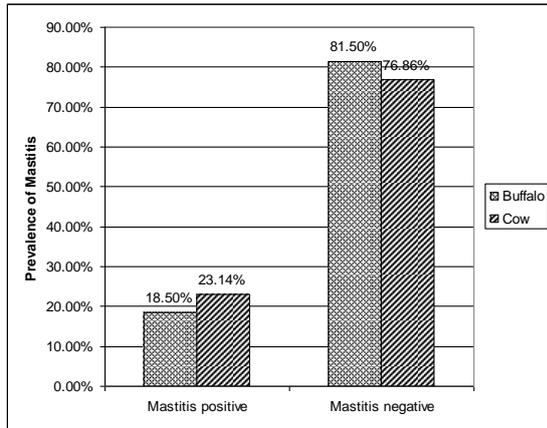in cows was due to the presence of soft udder sphincter (Uppal et al., 1994).

Figure 1: Comparison between buffaloes and cows for the prevalence of mastitis.
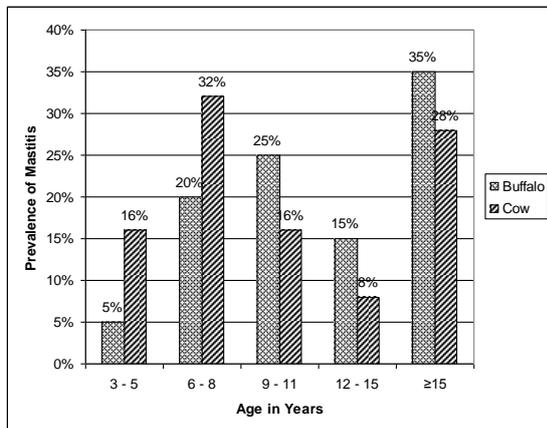

Figure 2: Comparison between cows and buffaloes for age wise prevalence of mastitis.

Over all the mastitis prevalence both in cows and buffalo was found more at the last stages of age that is about 9-10 years (Fig 1). In buffaloes at the age of 2 to 3 years, 4 to 6 years, 7 to 8 years, 9 to 10 and more than 10 years prevalence of mastitis was 1(5%), 3(15%), 4(20%), 7(35%) and 5(25%) respectively while in cows 2(8%), 4(16%), 7(28), 8(32%) and 4(16%) respectively. This disease became more prevalent in animals as they were getting old (Fig. 2) (Shawabkh and Aziz, 1987).

Table 1: t-Test: Two-Sample Summarizing Unequal Variances.

	Variable 1	Variable 2
Observations	2	2
Mean	0.5	0.5
Hypothesized Mean Difference	0	
Variance	0.19845	0.144292
Df	2	
P(T<=t) one-tail	0.5	
t Critical one-tail	2.919986	
t Critical two-tail	4.302653	
P(T<=t) two-tail	1	
t Stat	0	

Table 2: t-Test: Two-Sample Showing Unequal Variances

	Variable 2	Variable 1
Observations	5	5
Mean	0.2	0.2
Hypothesized Mean Difference		0
variance	0.0096	0.0125
df		8
P(T<=t) one-tail		0.5
t Critical one-tail		1.859548
t Critical two-tail		2.306004
P(T<=t) two-tail		1
t Stat		0

Over all milk composition and physical parameters change when an animal faces mastitis.

Current study indicated that among buffalos effected milk samples 2(10%) contained blood, 1(5%) contained pus, 4(20%) contained mucus while 13(65%) contained watery fluid whereas in cows these contents were 4(16%), 3(12%), 9(36%) and 9(36%) respectively (Fig 3).

As the quality and quantity of milk changes, milk composition and color also changes. Depending upon stage of disease sometimes water, sometimes mucus and sometimes blood appeared in mastitic milk (Mustafa et al., 2007).
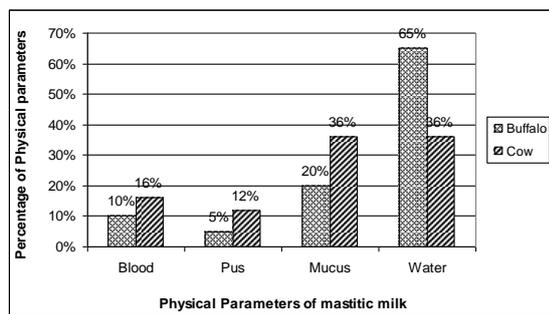

Figure 3: Comparison for physical parameters of mastitic milk both in buffaloes and cows.

Data indicated that infected animals passed through various stages of mastitis. In buffaloes 11(55%), 6(30%), 2(10%) and 1(5%) while in cows 14(56%), 4(16%), 6(24%) and 1(4%) animals were facing sub-clinical, clinical, chronic and acute stages of mastitis respectively (Fig 4). In developing countries like Pakistan sub-clinical mastitis had caused more loss due to lack of mastitis preventing practices (Khan and Muhammad, 2005).
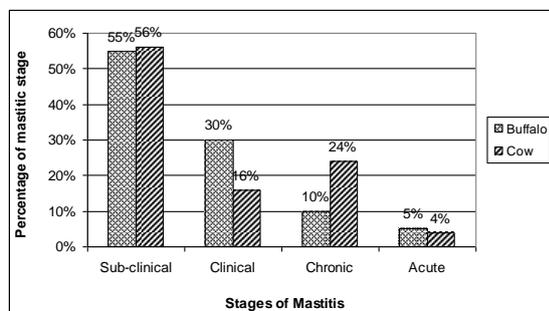

Figure 4: Comparison for various stages of mastitis both in buffaloes and cows.

Over all prevalence of mastitis was more in right fore teat of udder both in buffaloes and cows. Left hind teat, left fore teat, right hind teat and right fore teat in buffaloes were 4(20%), 2(10%), 5(25%) and 9(45%) whereas in cows were 1(4%), 9(36%), 12(48%) and 3(12%) effected respectively (Fig 5). According to previous results of various studies it is presented that right fore part is more susceptible for infection because usually it is used first during milking and microbes get entrance in this part of udder (Khan & Muhammad., 2005)
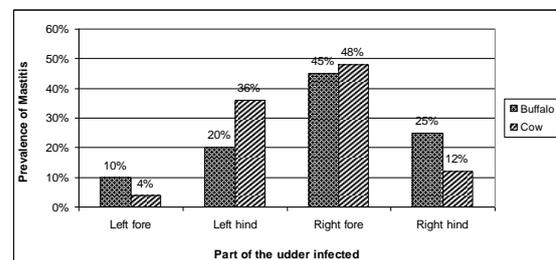

Figure 5: Comparison regarding quarter wise mastitis prevalence both in cows and buffaloes.

Data revealed that among different antibiotics available in market, Norfloxacine was highly sensitive, Ciprofloxacin was sensitive, Gentamycine was mild sensitive and Penicillin was ineffective against control of mastitis both in buffaloes and cows (Table 3). Pathogens are more susceptible against Ciprofloxacin, and Gentamycine whereas resistant against penicillin (Shabbir et al., 2009), because time by time penicillin is used as a medicine to control the infection of mastitis. The resistance against penicillin in mastitis causing agents may be due B-lactamase production that is used to breakdown ring of antibiotic called as B-lectam making antibiotic ineffective against microbial pathogens (Farooq et al., 2008).

Table 3: Antibiotics Sensitivity spectrum against microbial pathogens in Cows and Buffaloes in Lahore district

Subject	Type of Antibiotics used			
	Penicillin	Norfloxacine	Ciprofloxacin	Gentamycine
Cow	R	+ + + +	+ + +	+ +
Buffalo	R	+ + +	+ +	+

R = Resistant, +++ = effectively sensitive, ++ = mild sensitive, + = Sensitive.

Both in buffaloes and cows Right fore part of udder was frequently affected than any other part. Among commonly used four antibiotics, pathogenic bacteria were highly resistant against Penicillin while Norfloxacine was observed the most effective antibiotic to control mastitis as compared to Gentamycin and ciprofloxacin. Pathogens are more susceptible against Ciprofloxacin, and Gentamycin whereas resistant against penicillin, because frequent use of penicillin as a medicine to control the infection of mastitis. The resistance against penicillin in mastitis may be due to Beta-lactamase production which breakdown beta-lectam ring of antibiotic as making antibiotic ineffective against microbial pathogens.

CONCLUSION

The right fore teat canal both in buffalo and cows at the later age is more susceptible to mastitis causing agents those are more sensitive to Norfloxacine then other used antibiotics in District Lahore.

REFRENCES

Annemuller C, Lammler C, Zschock M (1999). Genotype of Staphylococcus aureus isolated from bovine mastitis. Vet Microbiol., 69: 217-224.

Bradley AJ, Leach KA, Breen JE, Green LE, Green MJ (2007). Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet Rec., 160: 253–7.

Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial disk susceptibility tests—approved standard. 11th ed. CLSI [461_TD$DIFF][445_TD$DIFF]Document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement. CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute.

Farooq AA, Inayat S, Akhtar MS, Mushtaq M (2008). Prevalence of mastitis and antibiotic sensitivity of bacterial isolates recovered from Nili Ravi buffaloes. J Anim Pl Sci., 18: 2-3.

Hillerton JE (1999). Balancing mastitis and quality. Proc. British Mastitis conference, Stonelergh, UK. Pp: 31-36.

Hussain M, Khan AM, Daraz B, Siddique U (2004). Bacteriology of mastitic milk and in vitro antibiogram of isolates. Pak Vet J., 24: 161-164.

Jamali H, Barkema HW, Jacques M, Levallée-Bourget E, Malouin F, Saini V, Stryhn H, Dufour S (2018). Invited review: Incidence, risk factors, and effects on clinical mastitis reccurence in dairy cows. J Dairy Sci., 101: 4729–4746.

Jones GM (2006). Understanding the basics of mastitis. Virginia Cooperative Extension No. 404-

233. Virginia State Uni, USA, Pp. 1-7.

Joshi S, Gokhale S (2006). Status of mastitis as an emerging disease in improved and puriurban dairy farms in India. Ann N Y Acad Sci., 1081:74-83.

Keane OM (2016). Genetic diversity, the virulence gene profile and antimicrobial resistance of clinical mastitis-associated Escherichia coli. Res Microbiol., 167: 678–84.

Keane OM, Budd KE, Flynn J, McCoy F (2013). Pathogen profile of clinical mastitis in Irish milk-recording herds reveals a complex aetiology. Vet Rec., 173:17.

Khan AZ, Muhammad G (2005). Quarter-wise comparative prevalence of mastitis in buffaloes and crossbred cows. Pak Vet J., 25 (1): 9-12.

Khan MZ, Khan A (2006). Basic facts of mastitis in dairy animals. Pak Vet J., 26 (4): 204-208.

Kivaria FM, Noordhuizen JP (2007). A retrospective study of etiology and temporal distribution of bovine clinical mastitis in smallholder dairy herds in Dar EI Salam region of Tanzania. Vet J., 173 (3): 617-622.

McDowell RE, Wilk JC, Shah SK, Balain DS, Metry G (1995). Potential for commercial dairying with buffaloes. North Carolina State Uni., USA.

Muhammad G, Ather M, Shakoor A, Khan MZ, Rehman FU, Ahmad MY (1995). Surf Field Mastitis Test: An inexpencive new tool for evaluation of wholesome of fresh milk. Pak J Food Sci., 5: 91-93.

Mustafa MY, Hassan SS, Ahmad M, 2007. Frequency of occurrence of mastitis in different quarters of udders and its cure-field study. Pak J Biol., 53 (1): 51-57.

Rodostits OM, Blood DC, Gay CC, Hincheliff KW (2000). Veterinary 9th ed. Billier Tindal, London.

Sánchez-Macías D, Hernández-Castellano LE, Morales-delaNuez A, Herra-Chávez B, Argüello A, Castro N (2020) Somatic cells: A potential tool to accelerate low-fat goat cheese ripening. Int Dairy J., 102: 104598.

Shabbir MZ, Rabbani M, Yaqub T, Ahmed A, Akhtar F, Khushi M, Hussain M, Ahmed M (2009). Trends in isolation and antimicrobial activities susceptibility of mastitis causing pathogens of Nili Ravi buffalo. Pak J Zool., 9: 273-279.

Shawabkh AK, Aziz AN (1987). Incidence of mastitis in dairy cows in Jordon. Vet J Bull Abst., 198-204.

Thomas CJ, Ronald DH, Norval WK (1997). Veterinary 6th ed. Maple vail Press USA. Pp 375-1196.

Uppal SK, Singh KB, Nauriyal DC, Bansal KB (1994). Natural defense mechanism against mastitis: A comparison histomorphology of buffalo and cow teat canal. Buffalo J., 2:125-131.

Zahid IA (2004). Studies on comparative incidence of sub-clinical and clinical mastitis and in-vitro antibiotic susceptibility of isolates from Holstein –Friesian and Jersy cows and buffaloes. Pak Vet J., 24:76-81.

Zaki SM, Sharaf EN, Mustafa OS, Fawzi OM, Batrawy NE (2008). Effect of sub-clinical mastitis on some biochemical and clinic-pathalogical parameters in buffalo. Am J Ag Env Sci., 3(2): 200-204.