



Short Communication

Prevalence and Antimicrobial Susceptibility Profile of *Mycobacterium bovis* Isolated from Egyptian Buffaloes' Mastitic Milk

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ABSTRACT

From the world's highlight on dangers of tuberculosis as a zoonotic and debilitating disease threatening animal and human health beside economy, this research aimed to determine the prevalence of *Mycobacterium bovis* from buffaloes' mastitic milk by isolation on Lowenstein-Jensen medium (LJ medium), Ziehl-Neelsen staining; identification by different biochemical tests and polymerase chain reaction (PCR) at 500bp diagnostic for *M. bovis*, as well as (2) to determine the therapeutic efficacy of the various antimicrobials (9 types) on the isolated *M. bovis* by using different antimicrobial sensitivity plate method. A total number of 100 samples of mastitic milk from native lactating buffaloes' breed were collected aseptically from 4 private farms in Cairo-Alexandria desert road in the north part of the Egypt and subjected to bacteriological and molecular examination for *M. bovis* followed by their antibiogram profile. Seven (7%) isolates were identified as *M. bovis* from totally examined 100 milk samples and isolated *M. bovis* showed resistance to ciprofloxacin; gentamycin and sulphamethoxazole-trimethoprim while were intermediate sensitive to both of erythromycin and norfloxacin but on the contrary were sensitive to amikacin; cefotaxime; clindamycin and streptomycin. It can be concluded that *M. bovis* can be the causative agent of mastitis in Egyptian Buffaloes and could be a potential risk for zoonotic transmission to man as well as economic losses. So, strict hygienic regulations and novel diagnostic tools should be used for prevention and detection of tuberculous mastitis in buffaloes' farms. The general public health should also be intensely warned from consuming raw or unpasteurized milk. All these prophylactic measures will eventually lead to a positive impact on public health.

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Authors' Contribution

EK presented the concept of the study. EK and RK planned methodology and wrote the manuscript. HE and EK reviewed the final manuscript and managed funds for the study.

Key words

Mycobacterium bovis, Buffaloes, Mastitis, Isolation, PCR, Antimicrobials

Mastitis signifies an inflammatory disorder of the udder regardless of the cause. It is a worldwide problem, characterized by physical, chemical and microbiological deviations in the milk and pathological changes in the glandular tissue of the udder (Khan and Muhammad, 2005). Clinical mastitis does not pose any problem in its detection, because of the grossly visible changes in the affected gland and its secretion. Clinical mastitis was determined using visual inspection of udders and detection of macroscopic clots and flakes in milk (Linda *et al.*, 2010). In clinical mastitis there are physical, chemical and bacteriological changes in the milk along with it the udder may turn hard, red and hot to the touch. The animals feel pain on touching

the udder. The shape of udder changes grossly, the uneven sizes of teats along with hardness in the inflamed teats is the indication of intra-mammary infection in buffaloes. The strip cup method can be used for detection of clinical mastitis in buffaloes. The clinical mastitis is classified based on severity, rapidity of onset and duration into peracute, acute, sub-acute and chronic forms (Du Preez and Giesecke, 1994). Buffalo milk has great commercial potential due to high nutritional value in relation to the high levels of fat, protein, solids, and minerals (especially calcium and phosphorus), being used more widely as a raw material for preparation of milk products, since it is increasing the demand for these derived by consumers more demanding and who seek a differentiated product, who values the species in dairy farming (Araújo *et al.*, 2012).

Diagnosis of clinical mastitis is based on the abnormal

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appearance of the milk. Milk may be off colour, watery, bloody or have the appearance of serum, pus and clots, flakes and shreds consisting of cellular and fibrin debris. The abnormal colour of milk is the result of changes in vascularity during inflammation and flow of blood from body of animal to the udder.

Mastitis leads to progressive change in the secretory apparatus and ultimately loss of milk production. Somatic cell count (SCC) of 0.6 to 1 million cells/mL is associated with an 8 to 12% reduction in herd milk production (Jones, 1986). In Nili-Ravi buffaloes, mastitis shortens lactation period of each animal by 57 days on an average and reduces 438 kg of milk per lactation (Cadey *et al.*, 1983).

Bovine tuberculosis is a re-emerging zoonosis of socioeconomic reputation (Ali *et al.*, 2014). Bovine tuberculosis is one of the most important chronic contagious diseases in dairy animals and human. It affects a wide host range and represents a major public health threat. It reduces the productive performance of the lactating animal by 25% (Radostits *et al.*, 2000). Bobadilla-del Valle *et al.* (2015) showed that human TB caused by *M. bovis* may be frequent but undetected. Human TB caused by *M. bovis* is transmitted mainly through consumption of unpasteurized dairy products, and it is less frequently attributed to animal to human or human to human contact. Mastitis causes economic losses, and also has serious public health concerns (Chakrabortya *et al.*, 2019).

Materials and methods

A total number of 100 samples of mastitic milk from native breeds lactating buffaloes from four private farms were collected aseptically according to guidelines of National Mastitis Council (1990). After discarding the first three milking streams, approximately 50 ml of milk was collected aseptically from each mastitic milk in sterile test tube. The milk samples were transported on ice to private lab at Alexandria city where they were immediately cultured or stored at 4°C for a maximum of 48h until cultured on standard bacteriological media.

Five mL of milk was centrifuged at 3000 rpm for 20 min inoculated in duplicate in Lowenstein-Jensen media (Sigma-Aldrich), and incubated at 37°C for 3 months. Colonies suggestive of Mycobacteria were stained by Ziehl Neelsen stain (Quinn *et al.*, 1994). Samples identified as acid-fast bacilli (AFB) by Ziehl Neelsen stain were subjected to molecular identification.

The thermal cycler for PCR comprised initial denaturation at 94°C for 4 min 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, Extension at 73°C for 1 min, and final elongation at 74°C for 10 min. A 500bp product was visible on 1.5% agarose gel.

The antimicrobial susceptibilities of *Mycobacterium bovis* were determined according to CLSI, 2006. The antimicrobial agents tested were: Amikacin (AMK) (30 µg); cefotaxime (CEF) (30 µg); ciprofloxacin (CIP) (5 µg); clindamycin (CLI) (2 µg); erythromycin (E) (15 µg); gentamycin (GN) (10 µg); norfloxacin (NOR) (100 µg); streptomycin (SM) (1µg); and sulphamethoxazole-trimethoprim (SXT) (23.7 + 1.25 mg). The results of sensitivity of microorganisms to different antibiotic discs were measured by the diameter of inhibitory zones and compared with antibiotic susceptibility testing sheet to get the result (sensitive, intermediate or resistant) and interpreted in accordance with the recommendations of Clinical and Laboratory Standards Institute (2008).

Results and discussion

Out of 100 buffaloes' mastitic milk sample, the isolation and phenotypic and biochemical characterization revealed 7 samples positive for *Mycobacterium bovis*.

This prevalence is lower than the data published by Jha *et al.* (2007) who isolated 6 (17%) *M. bovis* from 36 buffaloes milk samples in Nepal. Basit *et al.* (2018) reported 4 (6.2%) isolates identified as *Mycobacterium bovis* out of 64 milk samples collected from lactating buffaloes in north east of Pakistan. On the contrary, Shaukat *et al.* (2014) reported that all the mastitis positive animals when tested for TB by SITT (Single intradermal tuberculin test), Z.N staining and PCR were found to be negative. Therefore, they assumed that the incidence of TB in mastitis is not very common in screened population of bovines around Lahore. This is parallel to the studies of Al-Soub and Chako (1996) who reported tuberculous mastitis as a rare disease, which may be due to the hygienic measures, vaccination program and public health education.

The antimicrobial profile of the bacterial isolates from buffaloes' mastitic milk is summarized in Table I. *M. bovis* showed resistance to ciprofloxacin; gentamycin; sulphamethoxazole-trimethoprim. The bacteria showed intermediate sensitivity to erythromycin and norfloxacin; and high sensitivity to amikacin, cefotaxime, clindamycin and streptomycin. The use of the most effective antibiotic is very important for controlling the intramammary infection (Cormican *et al.*, 2008; Yeung *et al.*, 2011; Xiong *et al.*, 2016).

Our results suggested risk of infection to humans through consumption of raw milk, which is similar to data recorded by Basit *et al.* (2018). Also, more efforts are needed in the prevention and control of clinical mastitis in buffaloes (Bhutia *et al.*, 2019).

Table I. The antimicrobial profile of isolated *M. bovis* from buffaloes' mastitic milk samples.

Antimicrobial disc	Zone around discs	Indication of sensitivity
AMK (30 µg)	23 mm	++++
CEF (30 µg)	20 mm	+++
CIP (5 µg)	0 mm	-
CLI (2 µg)	19 mm	+++
GN (10 µg)	0 mm	-
E (15 µg)	9 mm	++
NOR (100 µg)	8 mm	++
SM (1µg)	27 mm	++++
SXT (23.7 + 1.25 mg)	0 mm	-

AMK, Amikacin; CEF, Cefotaxime; CIP, Ciprofloxacin; CLI, Clindamycin; GN, Gentamycin; E, Erythromycin; NOR, Norfloxacin; SM, Streptomycin; SXT, Sulphamethoxazole-Trimethoprim; -, Resistant; +, weakly sensitive; ++, moderately sensitive; +++, quite sensitive; +++++, highly sensitive.

Conclusion

It can be concluded that *Mycobacterium bovis* is the causative agent of mastitis in Egyptian buffaloes, which in return is a great hazard for public health.

Statement of conflict of interest

The authors have declared no conflict of interest.

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