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Prevalence and antimicrobial resistance assessment of some pathogens in bulk tank milk and dairy farm environment in Egypt

Thesis
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Abstract

A total of 775 samples of which 75 were BTM and 700 of farm environmental samples were collected from 5 dairy farms in Kafr-El-sheikh Governorate and Alexandria road, Egypt. Three Governmental farms (A, B and E) and 2 private farms (C and D). The collected samples were examined for the hygienic quality of BTM, the prevalence of some food-borne pathogens and for the antimicrobial resistance of isolated pathogens. The results revealed that the mean values of APC in BTM samples of farms A, B and E were $6.6\times10^5\pm2.4\times10^4$, $2.7\times10^5\pm3.1\times10^4$ & $7.4\times10^4\pm4.7\times10^3$ cfu/ml, respectively, while in private farms C and D were $4.7\times10^4\pm3.7\times10^3$ and $1.2\times10^4\pm1.7\times10^3$ cfu/ml, respectively. The mean values of coliforms in BTM samples of farms A, B and E were $1.6\times10^4\pm1.7\times10^3$, $1.4\times10^4\pm2.9\times10^3$ and $3\times10^3\pm1.4\times10^2$ MPN/ml, respectively, while in farms C and D were $1.3\times10^3\pm3.1\times10^2$ and $3.8\times10^2\pm1.4\times10^2$ MPN/ml, respectively. The mean values of Staphylococci counts in farms A, B and E were $6.4\times10^4\pm1.6\times10^3$, $4.2\times10^4\pm2.2\times10^3$ and $3.5\times10^3\pm4.1\times10^2$ cfu/ml, respectively, while the count in farms C and D were $2.1\times10^3\pm2.5\times10^2$ and $1.7\times10^3\pm3.8\times10^2$ cfu/ml, respectively. The mean counts of SCCs/ml in BTM samples of farms A, B and E were $603\times10^3\pm310\times10^2$, $396\times10^3\pm163\times10^2$ and $169\times10^3\pm140\times10^2$ cells/ml, respectively, while in farms C and D were $174\times10^3\pm146\times10^2$ and $142\times10^3\pm96\times10^2$ cells/ml, respectively. Testing of BTM for quality will provide producers with valuable information concerning the status of health and sanitation in their herds and help to produce high quality BTM.

The examination of BTM and dairy farms environment for prevalence of pathogens using PCR technique indicated that farms A and B were positive for all four concerned pathogens *(S. aureus*, Salmonella spp., *E. coli* and *L. monocytogenes)*, farm E was positive for 3 pathogens *(S. aureus, E. coli* and *L. monocytogenes*), while only *S. aureus* and *E. coli* were isolated from farms C and D. Testing of isolated pathogens against 17 antimicrobial agents revealed resistance and sensitivity of these pathogens in various percentages. The hygienic as well as the public health significance of obtained were discussed.

Key words: BTM, milk quality, pathogens, *Staphylococcus aureus*, Salmonella, *E. coli*, *L. monocytogenes*, dairy farm environment, antimicrobial resistance.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

In recent years, raw milk has become increasingly popular among consumers, who believe it to be more natural and highly nutritious. However, it can be contaminated by a variety of pathogens associated with human illness and disease and can pose severe health risks, including death. That's because raw milk has not undergone a process called pasteurization that kills disease-causing germs (**Oliver et al., 2009** and **Guh et al., 2010**).

The natural raw milk obtained from the mammary gland of healthy animal is usually with low microbial load and the application of all hygienic measures during milking prevents milk from contamination. The bacteria can access to the milk through colonization of the teat canal or gets contaminated from milk utensils or water supply used (**Gruetzmacher** and **Bradley, 1999)**. The levels and types of microorganisms in BTM provide information on the hygienic conditions during various steps of milk production on the farm. Milk contaminated by high levels of bacteria has many undesirable effects on the quality and safety of milk and its products and usually becomes unsuitable for further processing (**Nanu et al., 2007).**

The dairy farm environment and animals on the farm serve as important reservoirs of pathogenic and commensal bacteria that could potentially gain access to milk in the bulk tank via several pathways. Enteric pathogens can gain access to bulk tank milk from infected mammary glands, contaminated udders and milking

machines and/or from the dairy farm environment (**Straley et al., 2006**).

Milking machine wash failures are strongly associated with Coliforms Count, which suggests that proper cleaning of milking equipment plays a fundamental role in minimizing BTM contamination with Coliforms (**Pantoja et al., 2009c**).

Contamination of milk by pathogenic microorganisms at farm level can be of endogenous origin, following excretion from the udder of an infected animal and /or exogenous origin, through direct contact with infected herds or through the dairy farm environment such as water and personnel (**Farzana et al., 2009**).

Several studies have identified milk borne pathogens including *Escherichia coli* O_{157}, *Listeria monocytogenes*, and Salmonella spp., which have been recovered with various prevalence rates from dairy farms (**Cobbaut et al., 2009**; **Cummings et al., 2009**; **Fernandez et al., 2010** and **Fox et al., 2011**). Listeria, Salmonella, and pathogenic *Escherichia coli* are frequently isolated from dairy cattle and from various locations within dairy farm environments such as water, feed, manure, and bird droppings. Listeriosis and salmonellosis can have serious health implications in calves and cattle, but asymptomatic shedding in feces also occurs (**Van Kessel et al., 2004**).

Staphylococcus aureus is responsible for approximately 30% to 40% of all mastitis cases **(Asperger** and **Zanger, 2003).** Unhygienic measures, contaminated equipments, mammary gland infected with *S. aureus* and hands of milkers during handling and processing of

raw milk are considered the main cause of milk contamination with *S. aureus* **(Scherrer et al., 2004).** Presence of enterotoxigenic and antimicrobial resistant strains of *S. aureus* has become remarkably widespread in foods (**Normanno et al., 2007**).

Escherichia coli is a normal inhabitant of the intestines of animals and humans but its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to sever gastrointestinal disturbance (**Soomro et al., 2002**). It is considered as the major and reliable indicator of contamination by manure, soil and contaminated water (**Todar, 2008**). Small percentages of *Escherichia coli* are enteropathogenic results in mild illness; however, some serotypes are enterohemorrhagic and can lead to HUS (**O'Brien and Kaper, 1998**). *Escherichia coli* O_{157}:H_7 is the most common entero-hemorrhagic *E. coli* isolated from clinical cases and has been the source of several food-borne outbreaks in recent years. Dairy cattle are considered a reservoir of the enteropathogenic *E. coli* O_{157}:H_7 (**Wallace, 1999**).

Salmonellosis is the most common food-borne bacterial disease worldwide (**Forshell and Wierup, 2006**). Salmonella is the second leading cause of food borne illness in most developed countries causing diarrhea, cramps, vomiting, and often fever. Food-borne salmonellosis has remained a neglected zoonosis in Egypt and other developing countries of the world. It has been recognized due to consumption of raw or improperly pasteurized milk and milk products (**Karshima et al., 2013**).

Listeria monocytogenes has been considered an emerging public health problem because of its pathogenicity and ability to contaminate food. *Listeria monocytogenes* is capable of multiplying at temperatures ≤7°C and surviving in environments with a wide range of pH values and high salt concentrations (**Ryser, 2001**).

Various species of Listeria are commonly found in soil, decaying vegetation, and water, and well as being part of the fecal flora of animals and humans. *Listeria monocytogenes* is present in the dairy farm environment and can survive in the gastrointestinal tract of cows, thus constituting a source of contamination of BTM (**Latorre et al., 2009b**).

The biggest changes in the SCC are found in the case of the presence or absence of a bacterial infection within the mammary gland, other changes in the SCC, to a lesser extent, are caused by such factors as parity, stage of lactation, season, farm size, sampling interval, metabolic or physiological, "stress", etc (**Olechnowicz** and **Jaœkowski**, **2012**). A dramatic increase in milk SCC occurs in the case of IMI, this cell growth is a result of the transfer of white blood cells from the blood to the mammary gland **(Kelly et al., 2000)**.

Bulk Tank Somatic Cell Count (BTSCC) has three broad applications. They have been used to monitor the prevalence of mastitis in dairy herds, as indicators of raw milk quality and as more general indicators of hygienic conditions of milk production on farms (**Olechnowicz** and **Jaœkowski, 2012**). The correlations between BTSCC and TBC indicate a greater number of cells in smaller quantities of supplied bulk milk (**Van Schaik et al., 2005)**.

The reduction in SCC will be associated with reduced bulk tank TBC (**Berry et al., 2006**).

Caring about food safety for Europeans, the ECC directive 92/46 (1992) stated that milk with SCC over 400,000 cells per ml cannot be used for human consumption, while limits are 750,000 in the U.S.A. and 500,000 in Canada **(Schukken et al., 2003**).

Bacterial antimicrobial resistance represents an important current and future problem in infectious disease public health (**Cliver, 2009**). The growth of animals in conditions of crowding often favors the appearance of infectious disease that requires antimicrobial treatment (**Miranda et al., 2009**). There is controversy regarding the use of antibiotics in growth promotion and prophylaxis in agriculture. Some researchers believe that the use of antibiotics is implicated as a contributing source of resistant bacterial strains that can be transmitted to humans through the food chain (**DeFrancesco et al., 2004** and **Edrington et al., 2004**).

The emergence of multiple antibiotic-resistant strains of some pathogens should be of great concern to the public, especially dairy producers, their families, and employees, because some of those organisms are resistant to antibiotics that are commonly used in medical and veterinary practices and to some chemical sanitizer used in the cleaning and sanitization **et al.,** of dairy equipment and utensils.

Antimicrobial resistance of mastitis pathogens to multiple drugs has been reported worldwide (**Waller 2011 and Oliver** & **Muranda, 2012**). This is because of indiscriminate use of the

antibiotics by farmers, thereby rendering them ineffective and leading to permanent loss of the mammary tissues. The pathogens can transfer the resistance to a sensitive bacterium by conjugation known as R-plasmid mediated antibiotic resistance (**Ahmad et al., 2001**). The prevalence of antibiotic resistance usually varies between isolates from different samples and even between herds in the same farm (**Chaudhary & Payasi, 2013** and **Rall et al., 2013**). It is currently not possible to effectively and consistently exclude such multi antibiotic resistant strains from the human food chain, which means that they continue to pose a significant clinical threat to consumers and concomitant economic threats to the food production and processing industry (**Walsh et al., 2005**).

The main **objectives** of this study were to provide an objective interpretation of available scientific data on the prevalence, antimicrobial resistance of some pathogens and the public health risks associated with the consumption of raw milk containing those pathogens and to provide advice on strategies which may be employed to impact on any identified risk.

The **purposes** of this study were to:

- Determine the hygienic quality of BTM by determination of SCC, TBC, Coliforms Count, and Staphylococcal Count.
- Ascertain the prevalence of some food-borne pathogens in BTM and in the dairy environment.
- The identification of isolated pathogens using conventional methods and PCR techniques.