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Staphylococci in cattle and buffaloes with mastitis in Dakahlia Governorate, Egypt

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ABSTRACT

The aim of this study was to provide the first detailed insight into the population structure of Staphylococcus aureus in one modern dairy farm (Gamasa) and several household cows and buffaloes in Dakahlia Governorate, Egypt. Eight hundred seventy-two quarter milk samples of 218 dairy cattle and buffaloes with clinical and subclinical mastitis were investigated. Bacteria were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and staphylococci were further characterized by DNA sequencing of 16S rRNA genes and microarray analysis. Staphylococcus aureus was present in 5.6% of all collected samples, whereas methicillin-resistant S. aureus (MRSA) represented 24.5% of all identified S. aureus (12/49). Six clonal complexes (CC) of S. aureus were detected. Staphylococcus aureus CC398 (ST291/813)-MSSA (methicillin-susceptible S. aureus) was identified frequently in the Gamasa farm in addition to a few CC5-MRSA-V isolates. However, a small number of different isolates of S. aureus were found in household cattle and buffaloes harboring different CC. The presence of these genotypes of S. aureus in milk might indicate a public health hazard, because all of these CC have previously been isolated from human patients. Thus, a recommendation was given to the owner of the dairy farm to review the hygiene regimen on the farm. In perspective, further investigation regarding S. aureus screening of all lactating cows and personnel on the farm is warranted.

Key words: bovine, mastitis, microarray, Egypt, *Staphylococcus aureus* CC398 (ST291/813)-MSSA

INTRODUCTION

Staphylococcus aureus is a ubiquitous zoonotic pathogen of particular relevance for both human and veterinary medicine. In humans, studies have shown a prevalence of 30% in healthy carriers (van Belkum et al., 2009). Staphylococcus aureus causes a variety of skin and soft tissue infections, ranging from minor infections to life-threatening conditions such as necrotizing fasciitis, as well as pneumonia and septicemia. Some strains produce toxins, which can lead to toxic shock syndrome or food intoxication. It is estimated that intoxication caused by S. aureus ranks third out of common food poisoning causes, and it is associated with consumption of different categories of food including raw milk and dairy products (Headrick et al., 1998). Methicillin-resistant S. aureus (MRSA) strains are a major cause of nosocomial infections that lead to increased mortality rates and a substantial increase in human health care costs (Rubin et al., 1999).

In veterinary medicine, bovine mastitis is considered one of the most common and economically important diseases affecting dairy herds worldwide. It causes significant economic loss (Seegers et al., 2003). Staphylococcus aureus is one of the major pathogens causing mastitis in dairy animals (Monecke et al., 2007, Haran et al., 2012). The ability of the organism to cause a multitude of infections is probably due to the expression of various toxins, virulence factors, and cell wall adhesion proteins. The bacterium has the ability to survive phagocytosis in the udder and often causes chronic inflammation (von Eiff et al., 2006). These infections frequently do not respond to routine therapy. Hence, the economic impact of S. aureus mastitis is usually due to increased milk SCC, decreased milk production. increased costs of veterinary treatment, and premature culling of infected animals (Vink, 1995; Seegers et al., 2003). The resistance of S. aureus to antibacterial

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agents presents an increasing challenge and can complicate the treatment of infections (Monecke et al., 2007). High prevalence of antibiotic resistance was generally observed in both bovine and human S. aureus isolates from Egypt (Vink, 1995; El-Jakee et al., 2011) due to widespread, uncontrolled, and indiscriminant use of antibiotics. Thus, MRSA are continually gaining clinical significance. These strains show resistance against all types of β -lactam antibiotics that are frequently used for empirical treatment of mastitis. Fortunately, the prevalence of MRSA strains in bovine mastitis seems to be generally low (Monecke et al., 2007; Vanderhaeghen et al., 2010; Schlotter et al., 2012), but in dairy farms, the emergence of antibiotic-resistant microorganisms including staphylococci poses a potential public health hazard.

The identification and characterization of staphylococci can be performed using a multitude of phenotyping and genotyping tools such as biochemical investigations, molecular biological assays (PCR, DNA sequencing), and physical techniques such as matrixassisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). In recent years, DNA microarrays have been developed not only to investigate the expression of multiple genes in tissues, but also to genotype bacteria and for epidemiological studies. It has recently been suggested that a variety of clonal complexes (CC) of S. aureus can play a role in bovine mastitis. Bovine udder infections were shown to be associated with the carriage of staphylococci that show completely different spectra of virulence factors (Herron-Olson et al., 2007; Schlotter et al., 2012). Vaccination against S. aureus infections would be a promising approach to prevent mastitis in dairy cattle; however, the reported efficacy of S. aureus vaccines remains unsatisfactory (Lee et al., 2005).

In Egypt, the population of cattle and buffaloes was 3.8 million in 2011, with buffaloes comprising 55% of the population; a total of 7,900 kt of milk was produced (Morelon, 2011). Reliable information about the prevalence of S. aureus and other staphylococci among dairy cattle and buffaloes in Egypt is limited. Vink (1995) reported a mastitis prevalence of <10% on an individual cow basis on a private farm in which S. aureus was the predominant pathogen (76.2% of identified pathogens). In contrast, on a government farm that kept Holstein-Friesian cattle, the mastitis prevalence was as high as >60% (Vink, 1995). Interestingly, buffaloes seem to be more resistant to mastitis than cows are (Vink, 1995). More recently, multidrug resistance was detected in both buffaloes and cows infected with coagulase-positive staphylococci and CNS (Dorgham et al., 2013). To our knowledge, no detailed genetic analyses are performed routinely on MRSA and methicillinsusceptible *S. aureus* (MSSA) strains isolated from Egyptian cattle herds and household cows or buffaloes with mastitis. This study was therefore conducted to obtain detailed data on the presence of staphylococci in milk of cattle and buffaloes with clinical and subclinical mastitis in the Egyptian Nile Delta region, and to characterize *S. aureus* strains using DNA microarrays.

MATERIALS AND METHODS

Animal Population

The present study was conducted in 2011 and 2012 on 218 dairy animals (200 cattle and 18 buffaloes) with clinical (n = 85) and subclinical mastitis (n = 133) from different localities in Dakahlia Governorate in the eastern Nile Delta region of Egypt. Their ages ranged between 4 and 7 yr. Sixty-seven dairy cattle and 18 buffalo cows (Bubalus bubalis) were kept by smallholders at different localities at Aga district, whereas 133 dairy cattle belonged to a well-organized commercial farm located in Gamasa on the coast of the Mediterranean Sea. The Gamasa farm is located 80 km from the Aga district. The household cows were mostly Holstein-Friesian and only a few of them were local Egyptian breed; all dairy cattle in the Gamasa farm were Holstein-Friesian. On the farm in Gamasa, modern management techniques and good hygiene standards were applied. An automatic milking machine was used regularly at Gamasa farm.

All animals investigated were subjected to thorough clinical examination. After milking diseased animals, the size and conformation of the udder as well as the relative sizes of all quarters were inspected. The udder tissue and supramammary lymph nodes were also thoroughly examined. Animals with clinical mastitis were identified when one or more of the following signs were observed: cardinal signs of inflammation in one or more of an udder's quarters, signs of systemic reaction such as fever, depression, disturbed appetite, and abnormal physical character of milk such as clot formation, discoloration, alterations in viscosity, aberrant smell, or presence of blood. Due to the absence of observable clinical signs in animals with subclinical mastitis, the presumptive diagnosis was done based on laboratory diagnostic tests of milk samples including California Mastitis Test (CMT) and SCC. Cows with positive CMT or those having SCC >200,000 cells/mL but lacking clinical signs were considered to be affected with subclinical mastitis.

An informed consent for staphylococci investigation was given by the owners. All procedures were performed in accordance with the principles and specific guidelines presented in the *Guidelines for the Care and*

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