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UDK 616.98:636.2
Professional paper
Received: 18 July 2011
Accepted: 14 December 2011

CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF COW MASTITIS CAUSED BY *STAPHYLOCOCCUS AUREUS* AND ITS METHICILLIN-RESISTANT STRAINS

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Summary

Mastitis represents one of the most important problems in modern dairy production from the economic, diagnostic and public-health related point of view. Economic significance of the mastitis is related to the decreased milk production, lower quality of the milk, veterinary expenses and withdrawing of the milk due to the antibiotic treatment. Detection of mastitis is often complicated due to the subclinical nature of the mammary infection in which the increase of the somatic cells is only sign of the infection. Public health importance rises from the possibility of the transmission of zoonoses as well as due to the residue of the antibiotics secreted by milk.

Staphylococcus aureus is the most commonly isolated udder pathogen around the world. Since it is contagious, critical time for spreading among the cows in a herd is the milking time. Observed prevalence of *S. aureus* mammary gland infection varies from 2% to 50% even above and depends on the milking hygiene. Clinical manifestations of the *S. aureus* mastitis may vary from mild cases with elevated somatic cells only to the gangrenous cases with lethal exit.

Methicillin-resistant staphylococci as mastitis pathogens were first identified in the 1972. During the last decade methicillin-resistant *Staphylococcus aureus* (MRSA) was identified as a mastitis pathogen in few occasions in Belgium, Poland, Hungary and Germany. Spreading of the MRSA between animals and humans is documented. Although the prevalence of mammary gland infection caused by MRSA is low, there is thread of spreading of the MRSA among herds by animal trade. Spreading within the herd is enabled with large number of possible contacts between cows by milkers and milking equipment.

Keywords: cow; mastitis; *Staphylococcus aureus*

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INTRODUCTION

Mastitis is cause of the direct and indirect losses on dairy farms. About 150 species of microorganism, mostly bacteria is able to cause mastitis [1] They are divided in two groups: contagious and environmental udder pathogens. Members of the contagious udder pathogens are *S. aureus, Streptococcus agalactiae* and *Mycoplasma* sp. [2]. Although the group of contagious pathogens is small regarding the number of species included, in many countries this group is more often isolated from udder secretions. Contagious pathogens are spread between cows during the milking time by milking equipment such as teat cups, towels and milkers hands [2].

S. aureus is one of the most commonly isolated mammary gland pathogen in Croatia and worldwide. Cow with infected mammary gland is difficult or even impossible to cure successfully due to [3]: the ability of the bacteria to produce different enzymes and toxins which cause damage in the udder tissue and enable the bacteria to penetrate the tissue; the ability of the bacteria to survive in the keratin layer of the teat canal which in normal circumstances acts inhibitory; the ability to avoid phagocytosis due to the presence of the protein A in some strains. Protein A binds Fc portion of the antibody hence bacteria remain unrecognisable to the neutrophylic granulocytes; the ability to survive, even multiplication in phagocytes; the ability to produce beta-lactamase. Nearly 50% of *S. aureus* strains isolated from bovine mastitis produce beta-lactamase; the ability to induce formation of abscesses and fibrosis around the infection site. Antibiotics cannot penetrate the fibrous membranes which surround infection site hence *S. aures* is protected against the antibiotics; the placement of *S. aureus* inside the cells in which antibiotic concentration cannot reach enough concentration; the existence of bacteria in the L-shape which is missing membrane structure. Membrane structures are the target place for certain antibiotics.

ECONOMIC IMPLICATIONS OF THE *S. AUREUS* MASTITIS

According to the recent analyses of the economic impacts of *S. aureus* mastitis the losses per cow due to the intramammary infection in standardised 305-day lactation reach 160 dollars [4]. Primiparous cows infected by *S. aureus* after one clinical episode produce in average 8.4 L of less milk per day compared to the production before the onset of infection. In older cows average drop in milk production after the clinical episode is 5.5 L per day [5]. Another study covering 1431 dairy herds carried out in Lombardia, Italy, showed that absolute annual loss of milk production due to the *S. aureus* udder infection was 5778 tonnes. Calculated money loss per cow was between 55 and 113 euro per cow in an infected herd [6,7].

SOURCES OF THE INFECTION

S. aureus can be isolated from different body parts of an animal as well as from the environment. However an infected udder quarter remains the main reservoir of the bacteria [8,9,10]. In many occasions *S. aureus* was isolated from swabs taken from the cows head, skin swabs, legs and nasal mucosa. Furthermore *S. aureus* was found on the milkers hands as well as on the nasal mucous membrane of the humans working at the dairy farms, in bedding and the drinkers [11]. Although *S. aureus* is transmitted mostly during the milking time, heifers are often carriers in spite of the fact they never milked [3,10]. Recent researches show that many biotypes and genotypes exist on the dairy farms [12,13,14]. Variability of the genotypes could be explanation for different success in controlling *S. aureus* mastitis in dairy farms.

Generally the prevalence of intramammary infection (IMI) caused by *S. aureus* is lower in herds with applied basic preventive measures. It seems that effect of preventive measures is lower if the strains able to survive in environment dominate within a herd [3].

The teat cup is considered as the main way of transmission and the teat skin as the main reservoir of the *S. aureus* even though some strains isolated from the teat skin never cause mastitis (8,9,10,15). Transmission of human strains to cows, although possible, is not important factor for intramammary infection caused by staphylococci [16].

The fact that the staphylococci can be isolated from bedding, air and from the flies led to conclusion of some authors that sources of *S. aureus* are in the cow's environment. In spite of the successful isolation of staphylococci from environment, an infected cows or heifers remain as the main reservoir of pathogen for healthy animals [10].

CLINICAL FEATURES OF INTRAMAMMARY INFECTION

Mastitis caused by *S. aureus* can be expressed by wide spectrum of clinical signs, from mild cases without clinical signs to extreme cases with lethal exit. Per acute form of infection is often seen as a gangrenous mastitis with lethal exit. Acute and sub acute cases resemble mastitis caused by other pathogens. These cases often lead to chronic forms of infections [3]. Chronic and subacute cases are the most common forms and from the herd health point of view they are the most important. In many occasions their clinical feature is characterised by elevation of somatic cells only. However the presence of infection in a herd can be observed by clinical episodes and unsuccessful repeating treatment attempts in a same animal.

MONITORING OF RAW MILK QUALITY IN THE REPUBLIC OF CROATIA AND DIAGNOSIS OF MASTITIS

Monitoring of raw milk quality intended for public consumption regarding somatic cell and total bacteria count on monthly basis is in charge in the Republic of Croatia. Milk producers are under permanent control of the Central laboratory for milk control Križevci which provides analyses. Somatic cell count is checked monthly in a farm sample. Producers are informed about individual monthly result as well as geometric mean is calculated for period over last three months. If the three-month geometric mean exceeds proposed value (over 400.000/ml) milk producer has to call the field veterinarian to identify problematic cows. In problematic herds all the cows must be checked by the California mastitis test. Samples from positive quarters must be sent to the Croatian veterinary institute for bacteriological examination. Results of bacteriological examination are sent to the field veterinarians and to the county and state veterinary inspection. Therapy of mastitis should be carried out according to the susceptibility testing which is obligatory part of the laboratory examination.

Diagnosis of mastitis caused by staphylococci is based on the microbiological examination of a quarter milk sample. Sample is spread over ¼ of Petri dish on aesculyn agar with 5% ovine blood. After 24-hours incubation at 37 °C inoculated media is checked for bacterial growth. Suspected colonies are sub cultured on Baird Parker agar and checked for coagulase production using rabbit plasma.

PREVALENCE OF INTRAMAMMARY INFECTIONS CAUSED BY STAPHYLOCOCCI

S. aureus is the most commonly isolated udder pathogen in Croatia and worldwide [16,17,18]. Prevalence of infection varies between countries even within a country. According to the literature data prevalence varies from 2% in Denmark to the 63% in Finland. Two studies carried out in Germany reported 16% and 42%, a study in Finland reported 38%, in Estonia 58% while reported prevalence in Switzerland was 34.5% [19].

Reported prevalence of udder infection caused by *S. aureus* in Croatia was 11% and 12%, respectively, in two studies carried out with an 8 year time gap. Same studies revealed 34% and 28% of cows with mastitis regardless of aetiology [20]. Hence it can be concluded that prevalence of staphylococcal mastitis is even lower than in some European countries.

PUBLIC-HEALTH IMPLICATIONS OF INTRAMAMMARY INFECTIONS

Staphylococcal enterotoxin

Intoxication caused by staphylococci is the most commonly observed food-borne intoxication in the world. Large scale four-year surveillance carried out in 17 countries reported the highest incidence reaching 58.5 outbreaks per 10^7 inhabitants caused by staphylococci in Cuba, Hungary and Finland [21]. In larger outbreaks milk and dairy products were involved regardless of whether produced from pasteurised or unpasteurised milk [22].

The scientific evaluation of the role of staphylococcal enterotoxin in food-borne intoxications started in the 1914 while in the 1929 the association of some strains of staphylococci with food-borne intoxication was confirmed. A several types of enteroxins have been revealed since then. Hence there are at least eleven types of enterotoxins classified so far. The frequency of occurrence of staphylococcal strains bearing one or more genes coding enterotoxins varies between 20-100%, depending of the source of bacteria and geographic origin [23,24,25]. Possibility of confirmation of genes responsible for enteroxin production in strains isolated from milk grows by improving the methods of gene detection confirming that potential of enterotoxin production in milk is high. Published reports warn that intramammary infections caused by *S. aureus* has a public-health implications hence they are the risk factors for occurrence of enterotoxin in milk and dairy products [16].

ANTIBIOTIC RESISTANCE

Although there are a many reasons which compromise antibiotic treatment of *S. aureus* mastitis, resistance of bacteria toward antibiotics is one of the most important. Special attention is paid to the resistance against methicillin because it is coded by the gen *mecA* which is coding resistance against almost all beta-lactam antibitocs. It should be emphasised that beta-lactam antibiotics are widely used in mastitis treatment.

METHICILLIN-RESISTANT *S. AUREUS*

The first case of cow mastitis caused by MRSA was reported in the 1972 [26]. Since then scientific and general public have been occasionally informed about the isolation of MRSA from mastitic cows. So far the cases of cow mastitis caused by MRSA were observed in several European countries (Hungary, Belgium, Switzerland, Germany) [27,28,29,30].

Epidemiology

In the Belgian study eleven out of 118 examined strains (9.3%) of *S. aureus* isolated from udder secretion beard the gen *mecA* [28]. Two isolates was originated from clinical cases while nine was originated from subclinical mastitis. Percentage of infected cows varied between 0-7.4%, while percentage of infected quarters varied between 0 to 1.98 %. the rear quarters were affected more frequently than hind quarters. All isolates belonged to the type Livestock associated-MRSA (LA-MRSA) CC 398 clone. Authors concluded that about 10% of Belgian dairy herds are affected by udder infection caused by MRSA. The observed prevalence of MRSA associated cow mastitis in the Southwest German during the study carried out on three herds varied between 5.1 – 16.7% of cows [30]. In the same study MRSA was detected in nose swabs of the farm personnel (7/9), nose swabs of cows (7/15), calves (4/7), in milk samples from all three farms and even from swine housing stalls on the farms (4/5). In both studies the *spa*-type t011 was observed.

Virulence factors important for the infection of a mammary gland such as the toxin responsible for toxic shock syndrome, haemolysin and enteroxin are not usually seen in LA-MRSA strains. However the Belgian study revealed that MRSA showed the potential to colonize the cow's mammary gland.

The Hungarian study reported on several cases of subclinical mastitis in cows caused by MRSA. Isolated strains could not be genetically distinguished from strains isolated from the farm personnel in close contact with animals [27].

MASTITIS CONTROL

Dairy cows are exposed to many mastitis pathogens. It is impossible to achieve mastitis free status in a dairy herd. However some pathogens can be eradicated (for example *Streptococcus agalactiae*) or the infection rate can be maintained at acceptable level (*S. aureus*). Hence an effective mastitis control comprises several practices which prevent spreading of a pathogen from cow to cow.

Overall milking hygiene prevents the occurrence of new mastitis cases in a herd and includes wide spectrum of procedures. Milkers should use disposable gloves during udder preparation in milking parlour. Only clean and dry udder can be milked, first few streams of milk should be discarded. Individual disposable towels soaked with disinfectant for udder wiping are recommended. Teat dipping at the end of milking is advisable since it is effective in prevention of contagious pathogens spreading. Cows should be milked by order: first young and healthy then older healthy cows and cows experiencing mastitis at the end.

Antibiotic should be used at drying off in all cows in a herd regardless of the infection status. Decision regarding the choice of antibiotic depends on susceptibility pattern of the dominating pathogen in the herd.

Antibiotic treatment of subclinical cases in lactating cows is usually reserved for younger cows (up to third lactation) and genetically valuable animals with special attention to withdrawal period. Efficacy of the treatment should be checked by the repeated microbiological examination of the quarter sample: seven and 14 days after withdrawal.

Cows infected by *S. aureus* showing the clinical signs of mastitis should be cured and efficacy of treatment is checked twice as mentioned above.

Appropriate hygiene must be maintained in stalls including clean and dry bedding.

Finally milking machines must be maintained in a functionally and technically acceptable state which ensures secure milking. Parts of milking machine which is in contact with milk should be carefully cleaned and disinfected after every milking.

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Sažetak

Kliničke i epizootske značajke mastitisa krava uzrokovanih bakterijom *S. aureus* s osvrtom na meticilin-rezistentne sojeve

U suvremenom mliječnom govedarstvu mastitis je jedan od najvažnijih problema u ekonomskom, dijagnostičkom i javnozdravstvenom smislu. Ekonomsko značenje mastitisa ogleda se u smanjenoj proizvodnji mlijeka, lošijoj kakvoći, troškovima liječenja i bacanju mlijeka. Otkrivanje mastitisa često je otežano zbog supkliničke naravi procesa, pri čemu je povećanje broja somatskih stanica jedini znak upale. Mlijekom se mogu prenositi i uzročnici bolesti ljudi, a opasnost za ljudsko zdravlje predstavljaju i rezidue antibiotika i lijekova koji se izlučuju mlijekom.

Bakterija *S. aureus* najčešći je uzročnik mastitisa krava kod nas i u svijetu. Uzročnik je svrstan u skupinu kontagioznih uzročnika koji se u stadu širi uglavnom za vrijeme mužnje. Prevalencija infekcije uzrokovane tom bakterijom kreće se od 2 do više od 50% i u izravnoj je vezi s razinom higijene pri mužnji. Kliničko očitovanje u širokom je rasponu od sasvim blagih, s povećanim brojem somatskih stanica kao jedinim pokazateljem infekcije, do gangrenoznih oblika sa smrtnim ishodom. Jednom inficirana životinja može izlučivati uzročnika tijekom više laktacija ako infekcija prijeđe u kronični oblik, pri čemu povremeno mogu nastupiti i kliničke epizode praćene lokalnim znacima upale.

Meticilin-rezistentni stafilokoki kao uzročnici mastitisa krava prvi su put potvrđeni 1972. godine. U stadima mliječnih krava potvrđeni su tijekom nekoliko posljednjih godina u više navrata u Belgiji, Poljskoj, Mađarskoj i Njemačkoj, a dokazan je i slučaj prijenosa na ljude. Premda se radi o niskoj prevalenciji među stadima, postoji bojazan da bi se trgovinom životinjama mogao proširiti. Širenje unutar stada omogućeno je velikim brojem kontakata između krava preko muzača i opreme za mužnju.

Ključne riječi: krava; mastitis; *Staphylococcus aureus*