IMPACT OF *STAPHYLOCOCCUS AUREUS* AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ON UTERINE DISEASE IN DAIRY CATTLE AFTER PARTURITION

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Abstract
The aim of this study was to identify vaginal flora in Black and White Romanian cows diagnosed with puerperal endometritis. The cows studied came from two dairy farms, were in the first 4 weeks post-partum and had various puerperal diseases with variable pathological evolution. The dinamics of isolation of different bacterial species from lochia or vaginal discharges was made in four periods of the puerperium. Bacteriological examination was performed in accordance with routine laboratory techniques, including typical colony morphology on usuale culture media, chromogenic agar, Gram stain, type of hemolysis, characteristic growth on Baird-Parker (BP) agar and biochemical tests. In the course of puerperium, approximately 22,2% of microorganisms detected in the 18 cases were represented by the Staphylococcus aureus, what means that may be a significant pathogen of puerperal infection. Isolation in 11,1% of cases of methicillin-resistant stains of Staphylococcus aureus (MRSA) is important in terms of direct impact on human and animal health.
Key words*: Cows, Puerperal endometritis, Staphylococcus aureus, MRSA*

Introduction

A large number of microorganisms have been reported to contaminate the uterus of dairy cows after calving, and these microorganisms are recognised as a major etiological factor of uterine disease (Földi and others 2006). After parturition, bacteria from the animal's environment contaminate the uterine lumen of most cattle.

Uterine infection can be categorized into puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis (Sheldon et al., 2006).

Endometritis is one of the most common diseases that occurs in dairy cow after several weeks postpartum period (Sheldon et al., 2006).

In the context of clinical endometritis, staphylococci are generally considered to be potential pathogens or opportunist contaminants (Williams E. et al., 2005).

Staphylococcus aureus (*S. aureus*) is a microorganism that is present as a commensal on the skin, the nose and mucous membranes of healthy humans and animals (Turner N.A. et al., 2019).

In recent years, studies into livestock-associated *S. aureus* including methicillin-resistant (MRSA) strains have provided new information regarding their origin and host adaptation, and their capacity to cause zoonotic infections of humans (Ross Fitzgerald J., 2012).

MRSA in animals

The presence of methicillin-resistant Staphylococcus aureus (MRSA) in animals such dairy cattle involve a probable human origin adapted to cattle, but also was found distinct bovine types which are clearly different from human isolates (Cuny C. et al, 2010).

MRSA was first detected in the early 1970s from the milk of dairy cows with mastitis in Belgium, and these samples were most likely contaminated by humans (Devreiese, 1975).

After Belgium, other geographic areas had reports of MRSA frequently in livestock animals, dairy cattle and milk (Vanderhaeghen W, 2010).

Since the raport from 1972, MRSA was found in other domestic species, like dogs (Pak et al., 1999, Loeffler et al., 2005), cats (Bender et al., 2005), horses (Anzai et al., 1996, Hartmann et al.,1997), sheep (Goni et al., 2004, Gharsa et al., 2012) and pigs (Voss et al., 2005).

MRSA strains from genital samples have been identified in lemurs, chimpanzees and gorillas in Africa (Lozano C. et al., 2015).

Anzai T. et all, (1996) isolated methicillin-resistant *Staphylococcus aureus* strains from mares with metritis and the infection was from a stallion with skin lesions of the hind leg.

The majority of the studies suggested that the samples with MRSA found in dairy cattle were contaminated by humans, but some reports indicated that the MRSA present in cows is bovine-specific (Lee J.H., 2003).

Material and method

Criteria for inclusion in the study:

- the bacteriological examination was performed on samples taken from cows in the first four week after parturition;
- 18 Black and White Romanian dairy cows from 2 farms (Iași County) with clinical signs of clinical endometritis that persist more than 21 days after calving;
- the diagnosis of endometritis on clinical examination and the presence of purulent vaginal discharge within 21 days or more after parturition (Sheldon and others, 2006);
- purulent secretions were recovered from cows without antibiotic treatment in history.

To perform this examination, lochia and genital secretions were collected in sterile conditions, from the vulvar, vaginal or uterine level.

Isolation of Staphylococcus aureus strains

The samples were collected in sterile swabs, and were trasported in a short time and in refrigeration conditions. After collecting the discharges were immediately inoculated into liquid culture medium and incubated at 37ºC for 24 h aerobically.

The isolation and direct identification of *Staphylococcus aureus* and *Methicilin Resistant Staphylococcus aureus* was carried out on chromogenic agar plate, Sa Select and MRSA Select II Medium from Bio-Rad Laboratories (fig.1 and fig.2).

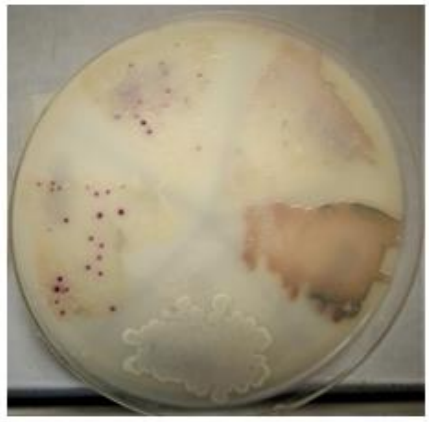


Fig.1 Pink colonies of *Staphylococcus aureus* on SaSelect Agar

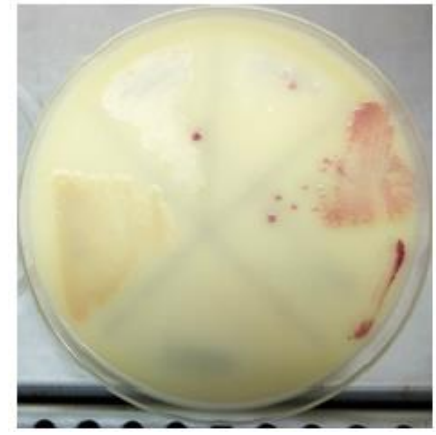


Fig. 2 Pink colonies of MRSA on MRSA Select Agar

To confirm the identification of these bacteria have beed made additional testing.

For each sample were isolated and selected typical *S. aureus* and MRSA colonies for purification (24-48h at 35ºC) and further characterization on non-selective medium (blood agar and Muller Hinton Agar, Bio-Rad Laboratories).

Staphylococcal colonies (orange to pink colonies of *S. aureus* from SaSelect Agar and pink colonies from MRSA Select Agar) were inoculated on blood agar (that contains 5% sheep red blood cells) to observ beta-hemolysis produced by *Staphylococcus aureus* (fig.3).

Fig.3 *Staphylococcus aureus*. Beta hemolysis on blood agar.

Staphylococcus aureus is characterized by a round shape (coccus or spheroid shaped), Gram-positive (purple), and found as either single cells, in pairs, or more frequently, in clusters that resemble a bunch of grapes (fig.4).

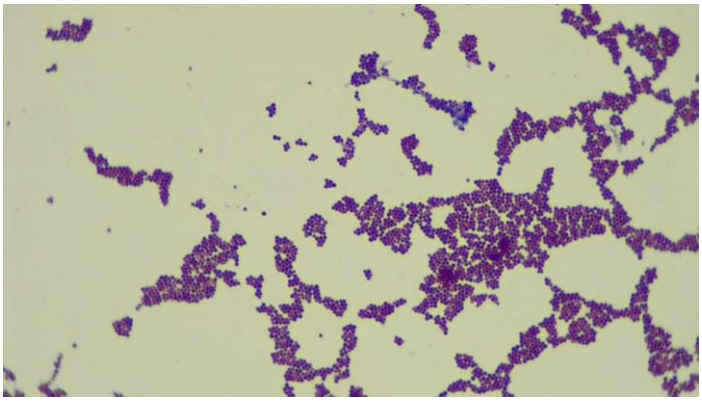


Fig.4. Methicilin resistant *Staphylococcus aureus* cells (Gram positive coci), Gram staining, x1000 MO

The presence of methicillin resistant *Staphylococccus aureus* (MRSA) in clinical samples were evaluated in two chromogenic agar plates, MRSA Select II and Brilliance.

On Brilliance agar the typical colonies of MRSA were denim-blue (fig.5). Both culture media are sensitive for screnning MRSA. The detection of MRSA in chromogenic agar media was reported in recent studies performed by van Loo IHM et al. (2007), Haitske Graveland et al. (2009), Riedel Stefan et al.(2010).

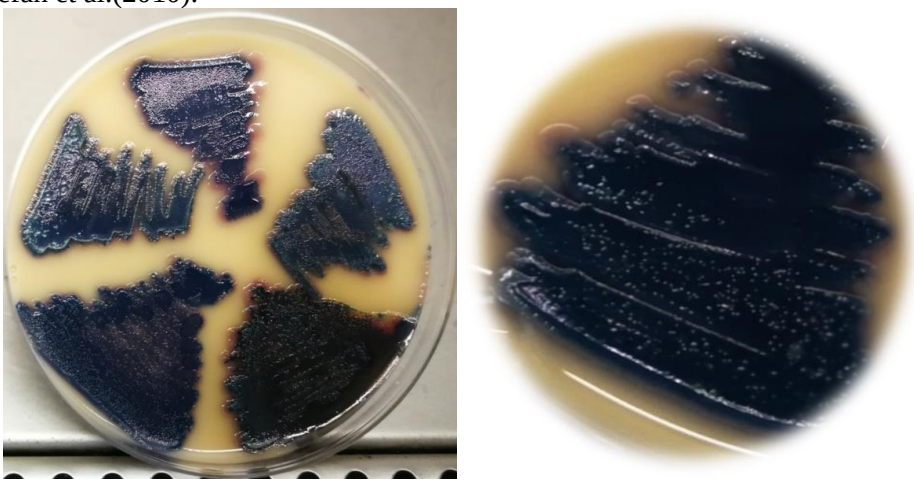


Fig. 5. Denim-blue colonies of MRSA. Brilliance agar

The Baird Parker Agar (Oxoid) allows the growth of *Staphylococcus aureus* and selectively inhibits the growth of other bacteria (fig.6).

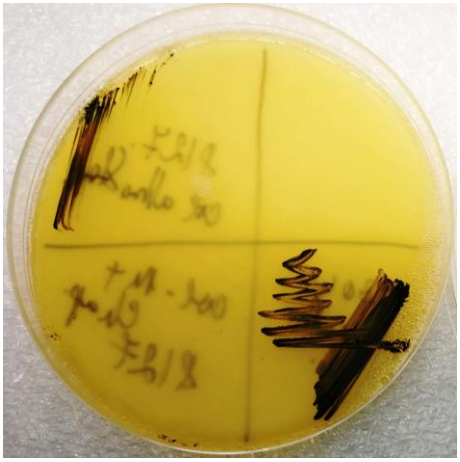


Fig. 6. *Staphylococcus aureus* on Baird-Parker Agar

Further, the biochemical test were performed for confirmation. The identity of *Staphylococccus aureus* isolated on Baird-Parker Agar was confirmed with a coagulase reaction.

Mannitol Salt Agar (Chapman medium) was used for the selective isolation and differentiation of *Staphylococcus aureus* from mixed culture (fig.7).

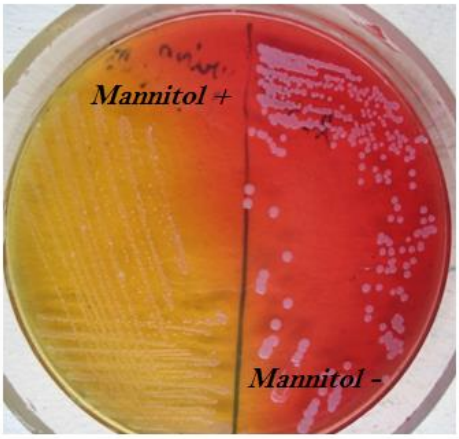
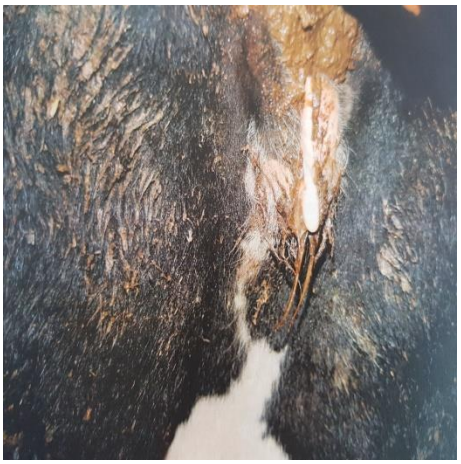


Fig. 7. *Staphylococcus aureus* (Mannitol +), yellow colonies with yellow zones on Chapman medium

Results and Discussion

Clinical signs on puerperal endometritis

Confirmation of clinical endometritis was made based on vaginal discharge during trans-rectal examination. The observed leaks had a sero-mucous, yellowish-brown appearance, sometimes with fibrin deposits.



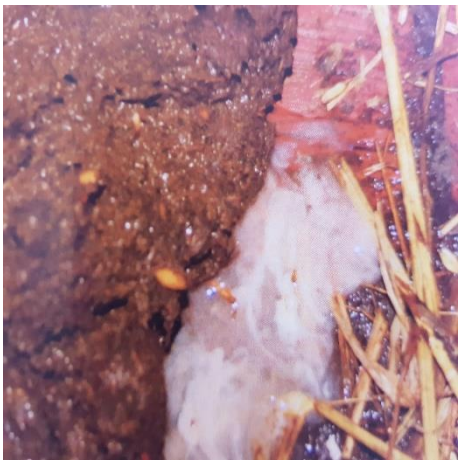


Fig. 8. Clinical appearance of vaginal discharge in purulent endometritis

Bacteriological examination

Out of 18 cows were sampled at 28-55 days postpartum and all contained different strains of bacteria. *Staphylococcus aureus* and *Methicilin resistant Staphylococcus aureus* were detected in 4 (22,2%) and 2 (11,1%) samples in pure culture. MRSA was detected in 2 samples in pure culture from 18 cows.

The 2 tipes of bacteria were identified based on cultural characters from chromogenic agar, Gram stained, selective media and confirmed by standard biochemical tests like -Chapman medium (mannitol +), Baird Parker medium, coagulase test, API STAPH test.

Previous results have suggested that Staphylococcus aureus was a predominant species isolated from cows with puerperal endometritis (Salah N. et al., 2017).

Staphylococcus aureus and *Methicillin Resistant Staphylococcus aureus* have a major implication in healthcare infections worldwide, because of the cattle contact as a risk factor for MRSA colonization in humans (Cunny C. et al., 2010).

Acording to an aetiological investigation of China's dairy cow, *Staphylococcus aureus* (SA) is one of the most common pathogen of endometritis (Meng Dan et al, 2019).

Éva Juhász-Kaszanyitzky et al., (2007) reports an infection rate of Staphylococcus aureus at aprox. 20-50%.

The massive use of antibiotics on farms has led to the adaptation and evolution of pathogens so the cattle can represent ecological niches for the development of multidrug resistance in MRSA strains (Gill S.R., et al., 2005).

Cows can carry S. aureus in their skin, udder, nasal cavity and rectum. S. aureus multiplies and survives in animal organisms, but can live in the environment for a long time, and then the most important route of transmission is by the hands of the farmer, veterinarian or caretaker (Marsilio F., et al., 2018).

Humans are the natural hosts for *S. aureus* and MRSA and the appearance of MRSA in dairy farms is a serios threat to public health because of cross-infection between animals and humans (Stefani S. et al., 2012).

The detection of MRSA isolates cultured from blood and infected wound sites in humans is strong circumstantial evidence that these organisms are capable of causing clinical disease (L.Garcia-Alvarez et al., 2011).

Conclusions

The results suggested that *S. aureus* might play a major role in cow endometritis infection. The discovery of MRSA in dairy cows suggests that these animals might provide a reservoir of infection and close links with farms or contact with dairy cattle could be risk factors that increase the likelihood of MRSA carriage or infection in patients.

Due to the high capacity of *Staphylococcus aureus* to acquire, maintain and mobilize antimicrobial resistance genes, further molecular surveillance is essential to monitor their progress over time.

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