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Letter to the Editor

Retrospective multicenter study reveals absence of MRSA-associated bovine mastitis in Brazil (1994 to 2016)

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Dear Editor,

The rapid dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) among different hosts has posed a major challenge to public health [1,2]. Worryingly, MRSA have been associated with life-threatening nosocomial infections and, more recently, they also became globally recognized as important veterinary pathogens [1-3]. In this regard, there are increasing reports of MRSA in food-producing animals, with special concern to dairy cows suffering from mastitis [3,4]. In this study, we investigated the presence of methicillin resistance in one of the largest collection of *S. aureus* recovered from bovine mastitis in Brazil.

Between 1994 and 2016, a total of 412 *S. aureus* isolates were recovered from bovine mastitis samples (clinical and subclinical mastitis) from 372 herds in rural properties of four Brazilian states (Minas Gerais, São Paulo, Rio de Janeiro and Goias). Bacterial analysis was conducted by culturing 0.01 mL of each sample on 5% ovine blood agar plates. The plates were incubated for 24-48 hours at 37 ºC. Then, colony morphology was observed and Gram staining and identification tests were conducted [5]. The microorganisms were identified from subcultures on plates containing Brain Heart Infusion agar. The Gram-positive, catalase-positive cocci were identified as coagulase-negative staphylococci and coagulase-positive staphylococci by means of tube coagulase tests. Among the coagulase-positive staphylococci, *S. aureus* was identified based on a positive acetoin test (Voges-Proskauer test) [5].

The identification of *S. aureus* isolates was further confirmed by PCR, targeting the portion of conserved thermonuclease gene (*nuc*). Antimicrobial susceptibility was determined by disc diffusion method, using breakpoints approved by the Clinical and Laboratory Standards Institute [6]. All bacterial isolates were tested against amoxicillin/clavulanic acid (20/10 µg), ampicillin (10 µg), cefoxitin (30 µg), ceftiofur (30 µg), cephalothin (30 µg), ciprofloxacin (5µg), enrofloxacin (5µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (30 µg), oxacillin (1µg), penicillin G/novobiocin (10 UI/30 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg). Additionally, the presence of the methicillin resistance genes (*mec*A and *mec*C) was investigated according to the methodology described by Mehrotra *et al*. [7] and Paterson *et al*. [8], respectively.

Strains were considered as multidrug-resistant (MDR) when they were resistant to 1 ≥ antimicrobial agents in 3 ≥ classes of antimicrobial [9]. In this respect, 15/412 (3.64%) of *S. aureus* strains were defined as MDR. However, if we adopt the classification suggested by Magiorakos *et al*. [10], in which ampicillin resistance is not considered, MDR profile was evidenced in only 4/412 of the investigated strains (0.97%).

Intermediate resistance or resistance to at least one of the antimicrobials tested was observed in 58.74% (242/412) of the strains. Strains were more often resistant to ampicillin (221/412, 53.64%), followed by tetracycline (66/412, 16.02%) and erythromycin (28/412, 6.80%). On the other hand, resistance to neomycin (12/412, 2.91%), gentamicin (6/412, 1.46%), enrofloxacin (5/412, 1.21%), ciprofloxacin (3/ 412, 0.73%), trimethoprim/sulfamethoxazole (2/412, 0.49%) and amoxicillin/ clavulanic acid, ceftiofur and penicillin G/novobiocin (1/412, 0.24%) were less frequently observed.

None of the strains was resistant to cephalothin, cefoxitin or oxacillin. The absence of resistance to cefoxitin and oxacillin, used to predict resistance to methicillin, means absence of phenotypic resistance to methicillin and other anti-staphylococcal penicillins or penicillin-stable penicillins. Moreover, all 412 *S. aureus* strains presented negative amplification for *mec*A and *mec*C genes, supporting the phenotypic results.

Despite the clinical and epidemiological importance of *S. aureus* in Brazilian dairy farms, there is scant information regarding phenotypic and genotypic resistance profiles of such isolates. In Brazil, the occurrence of MRSA isolated from bovine mastitis has been reported on rare occasions [4,11]. Our results suggest that MRSA does not disseminate in Brazilian dairy herds. In this regard, there are two hypotheses for the absence of MRSA observed in our study: the competition with other circulating methicillin-susceptible *S. aureus* strains [12]; and late establishment of important livestock-associated MRSA lineages in the Americas (*e.g.*, ST398) [13].

In summary, none of the 412 *S. aureus* strains investigated displayed phenotypic and genotypic resistance to methicillin. A low prevalence of MDR profiles was observed. The prevalence of MRSA in Brazilian dairy cattle does not appear to be concerning at present time. However, since MRSA has serious public health implications, continuous surveillance is urgently required for monitoring the dissemination of these clinically important bacteria throughout the food chain.

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Ethical approval

This study was approved by the Ethics Committee for the Use of Animals (CEUA) of the Faculty of Veterinary Medicine of University de São Paulo (protocol number 6323200215).

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