



Characteristics of *Streptococcus agalactiae* belonging to CC103 clone circulating among dairy herds and pregnant women in Brazil

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Streptococcus agalactiae or group B *Streptococcus* (GBS) is a highlighted pathogen in the veterinary setting and is among the major agents of contagious mastitis in dairy cattle, leading to remarkable economic losses in the dairy industry every year [1]. The quality and quantity of milk produced are directly affected by GBS mastitis and milk disposal is usually required. In most serious cases, infection culminates in animals' culling [2]. This condition is of special concern in low- and middle-income countries that stand out as top milk producers [3]. GBS is the third most common pathogen associated with clinical bovine mastitis in Brazil, one of the five largest milk producers worldwide [4].

In addition, GBS is a major cause of neonatal infection [5]. Over the years, GBS has been the focus of epidemiological studies that are aimed at elucidating its potential transmission between distinct hosts. Analyses of GBS population structure have revealed the circulation of host-specialist and host-generalist lineages. The clonal complex (CC) 17, for example, is considered a specialist lineage associated with human neonatal meningitis. Conversely, CC61 and CC67 are mainly found in bovine hosts [1]. CC1, CC19, and CC23, on the other hand, represent generalist lineages, found in both human and bovine isolates [6].

Nevertheless, spillover events have been reported in the literature, characterized as sporadic findings of specialist

lineages in different types of hosts, such as the detection of a ST17 GBS isolate in bovine milk in Brazil [7]. Indeed, the transmission of GBS between humans and other animals has been addressed by evolutionary and epidemiological studies, suggesting that interspecies transmission can occur by multiple routes and in a bilateral manner [1, 6, 8].

In this context, a more recently reported lineage has gained attention. CC103, mainly represented by ST103 and ST314, has become one of the dominant bovine GBS clones in Europe and Asia, being associated with the reemergence of GBS mastitis outbreaks after a long time of successful control of the disease. Although it was barely detected in cattle until the early 2010s, CC103 has been detected in other sources since then, including humans and the farm environment [6, 9].

The recent and increasing interest in CC103 GBS isolates led us to reevaluate and further characterize a subset of our GBS collection recovered before 2010, when description of CC103 was still a rare event worldwide. This subset of our collection was assembled by analyzing 1589 milk samples collected from 401 cows belonging to 20 different dairy herds in the state of Rio de Janeiro/Brazil, from May 2006 to November 2008. A total of 529 (33.3%) samples were positive in California mastitis test (CMT) [10], and GBS was isolated from 92 (17.4%) of these mastitic samples, representing 16 different herds (Fig. 1).

From these 92 GBS isolates, 69 (75% of total) were serotyped by the immunodiffusion method [11], and nearly half of them (32; 46.4%) belonged to serotype Ia. In addition, 89 GBS isolates (96.7% of total) were analyzed by PFGE following previous recommendations [12], and a major clonal complex (namely, CC-A) was detected, comprising 33

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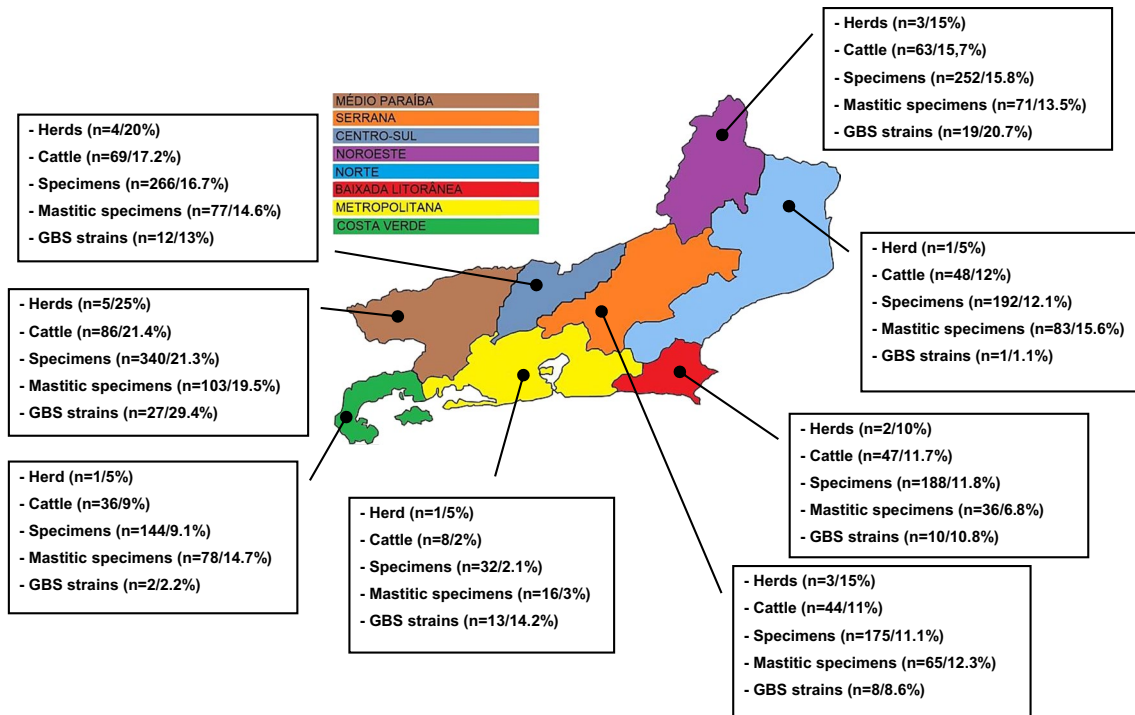


Fig. 1 Map of the State of Rio de Janeiro indicating the location and number of herds included in the present study, number of cattle analyzed, number of total clinical specimens and mastitic specimens, and number of *Streptococcus agalactiae* (GBS) isolates by region

(37.1%) isolates associated with serotype Ia and representing 6 different herds. The large predominance of serotype Ia and CC-A within this collection of bovine GBS isolates sparked our interest because serotype Ia is the serotype most associated with CC103 and had not been frequently reported among bovine GBS strains in Brazil. In contrast, serotypes III and V have been found as the most frequent in the country according to other studies [13, 14], even though recent data indicate a trend towards a more frequent detection of serotype Ia [4].

We were able to recover 12 GBS isolates belonging to serotype Ia and CC-A, representing all the 6 herds comprised by CC-A, and submitted to further characterization. Identification of GBS isolates was confirmed by CAMP factor production and hippurate hydrolysis tests [15], as well as by MALDI-TOF MS according to the manufacturer's instructions (Bruker Daltonics). Capsular type was confirmed by latex agglutination using the ImmuLex Strep B latex kit (SSI Diagnostica, Denmark). Multilocus sequence typing [16] showed that serotype Ia CC-A isolates belong to either ST103 (1) or ST314 (11), a single-locus variant (SLV) of ST103, confirming that CC-A, the most prevalent PFGE cluster among bovine isolates recovered in 2006–2008 in Rio de Janeiro/Brazil, represent the CC103 lineage. CC103 was first described in Brazilian bovine isolates recovered in 2010–2011, and it only represented nearly 5% of the isolates analyzed in this previous study [13]. Here, not only CC103

was associated with the predominant PFGE cluster found among isolates from bovine mastitis (representing more than 30% of the GBS strains analyzed) but also represented isolates recovered before 2010, when reports of CC103 were still rare worldwide [6].

Genes encoding pilus variants (PI-1, PI-2a, and PI-2b) were detected by PCR according to Otaguiri et al. [17], and all 12 serotype Ia CC-A isolates harbored the PI-2b variant only. A correlation between pilus variant and clinical source has been reported in GBS, being PI-2a (with or without PI-1) the most common variant in human isolates and PI-2b (with or without PI-1) the most frequent among bovine isolates [18], suggesting that pilus may have a role in host specificity. Evaluation of biofilm production was performed according to Alvim et al. [19]. All ST314 strains were classified as strong biofilm producers, while the single ST103 strain was classified as weak biofilm producer.

Production of extracellular deoxyribonucleases (DNases) and lactose fermentation tests were performed as described elsewhere [15, 20]. All 12 GBS isolates belonging to serotype Ia and CC-A were positive for DNase production and lactose fermentation tests. Both characteristics may confer advantages to microorganisms present at certain sites of infection. The production of extracellular deoxyribonucleases or DNases has been associated with dissemination through the host by evasion of the immune system, while lactose fermentation confers an evolutionary advantage to

bovine strains due to the role of lactose metabolism in the adaptation to the bovine mammary gland environment [21].

Antimicrobial susceptibility was determined according to the CLSI guidelines by the disk-diffusion technique [22], using penicillin G (10 µg), tetracycline (30 µg), levofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), and vancomycin (30 µg). Tetracycline resistance genes (*tetM* and *tetO*) were detected by PCR as previously described [23]. All 12 GBS isolates were resistant only to tetracycline, either mediated by *tetM* (in the eleven ST314 isolates) or *tetO* gene (in the single ST103 isolate). Interestingly, previous studies have shown that tetracycline resistance, specially the *tetM* gene, is more prevalent and commonly found in GBS clones circulating in human populations, which may be a result of positive selection after the widespread use of tetracycline in human medicine [6, 24]. Moreover, although only one ST103 strain was detected among bovine CC103 isolates, this single strain showed differences when compared to the other eleven ST314 strains, such as being classified as weak biofilm producer and harboring *tetO* as the tetracycline resistance determinant.

As CC103 has been also reported among human GBS strains from other places across the world [6, 9] and serotype Ia is the most common capsular type among GBS isolates recovered from pregnant women in our setting [6], we performed a search in our collection of human GBS isolates for strains presenting serotype Ia and the PI-2b variant, which is, as stated before, an unusual pilus variant in human GBS isolates. Among 325 GBS isolates recovered from anovaginal specimens of pregnant women between 2008 and 2021 and for which we had information available, 13 (4%) met the expected profile (serotype Ia and PI-2b positive) and were submitted to MLST. All 13 isolates belonged to ST103. For comparative purposes, we further characterized these 13 human ST103 strains regarding DNase production (3 isolates were positive), lactose fermentation (2 isolates were positive), biofilm production (all were classified as strong biofilm producers), and antimicrobial susceptibility profile (only 1 isolate was tetracycline-resistant and harbored the *tetO* gene). Curiously, most human CC103 strains were susceptible to tetracycline, in contrast to bovine CC103 strains. These results show that those ST103 isolates recovered from human sources present characteristics that are more usually associated with bovine-adapted GBS lineages, such as presence of PI-2b variant, susceptibility to tetracycline, and ability to ferment lactose, suggesting a bovine ancestry for these isolates.

Our results suggest that CC103 circulates among different host species in Rio de Janeiro/Brazil since before 2010, with a prevalence of at least 30% among bovine and 4% among human GBS investigated, reinforcing the concept that CC103, although showing primarily characteristics of a bovine-adapted GBS clone, still keeps some degree of host

generalism and, as such, may be able to jump between hosts. Among GBS from human sources, the finding of serotype Ia along with the unusual PI-2b variant may serve as predictive characteristics for CC103 detection. Moreover, a clear difference in tetracycline susceptibility profile was detected in bovine versus human CC103 isolates. This is interesting since tetracycline is one of the most used antimicrobial agents for contagious mastitis treatment in Brazilian dairy herds, and it may impose a positive pressure towards the selection of resistance mechanisms [25]. Our results may help to inform on public health risks and opportunities to improve surveillance and control of multihost GBS in Brazil. Collected data indicate that continuous surveillance and extensive characterization of CC103 isolates are essential for a better understanding of GBS epidemiology in the One Health context.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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