Antimicrobial resistance profile of non-aureus Staphylococci isolates from buffalo, goat and sheep mastitis in the Northeast region of Brazil

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Supplementary file

Bacterial isolates

A total of 190 non-aureus Staphylococci (NAS) strains, isolated from milk samples collected from buffalo (n=52), goats (n=66), and sheep's (n=72) mastitis cases between March 2014 and July 2017 in different states in Brazilian Northeast Region as pointed localizeds in Alagoas: São Luiz do Quitunde (43 buffalo); Viçosa (10 goats) / Bahia: Valente (14 goats) / Paraíba: Bananeiras (20 sheep) / Pernambuco: Custódia (3 goats; 11 sheep); Floresta (9 sheep); Limoeiro (8 sheep); Petrolina (9 sheep); Ribeirão (9 buffalo); Santa Maria (18 goats); Serra Talhada (7 sheep); Sertânia (21 goats; 8 sheep). The isolates were stored under freezing in glycerinated BHI (Brain Heart Infusion) broth at - 20ºC at the Laboratory of Infectious Diseases (LDIC) – UFRPE. 52 strains were isolated from buffalo, 66 from goats, and 72 from sheep's mastitis. Biochemical identification of the isolates was performed by testing the coagulase and acetoin as recommended by the National Mastitis Council (NMC, 2017).

Genomic DNA Extraction and Polymerase Chain Reaction (PCR)

Bacterial genomic DNA was extracted from 1 mL of culture grown in BHI (Brain Heart Infusion) broth using the Wizard Kit SV Genomic DNA Purification System (Promega®-Madison, Wisconsin, USA) according to manufacturer's instructions. Polymerase chain reaction (PCR) was performed for amplification of *blaZ* gene (Figure 1), which encodes beta-lactamases, in addition to *mecA* and *mecC* genes detection, which are inducers of the beta-lactam site of action modification.

Reactions were assembled separately for each gene in a final volume of 15µL per well, containing 100ng of DNA template, 10pmol of each oligonucleotide (Table 1), Taq buffer (10mM Tris, 50mM KCl, 2.5mM MgCl2), 200mM dNTPs and 1U Taq DNA

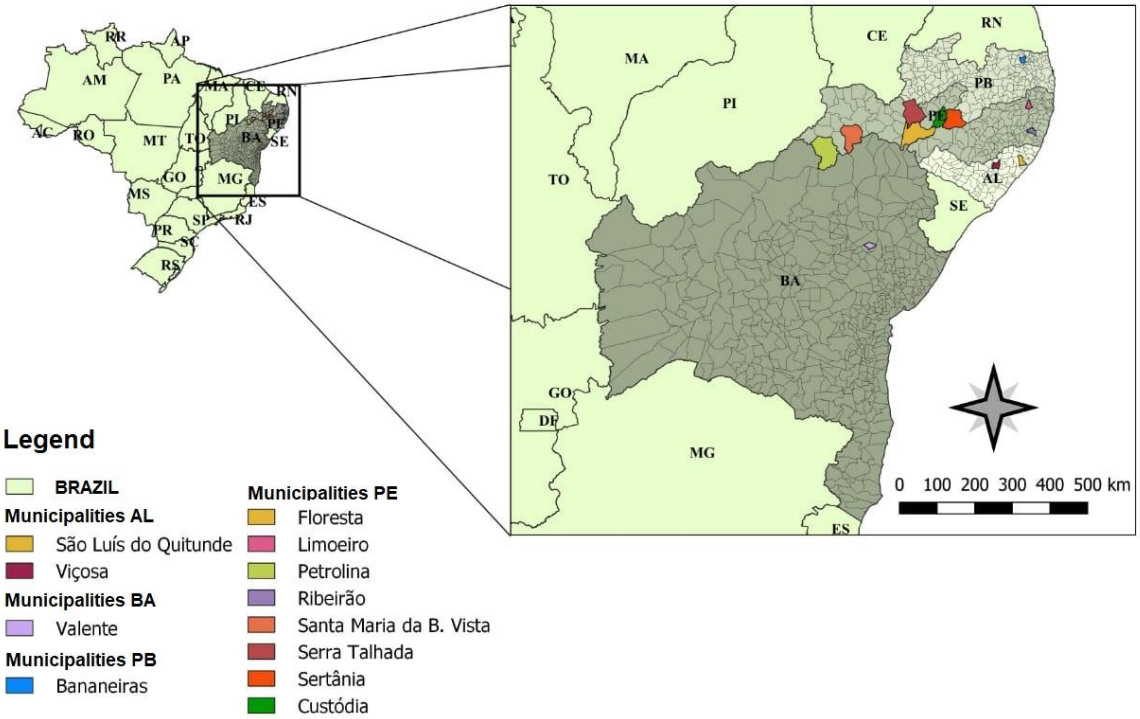
polymerase (Cenbiot, Taq DNA polymerase, Ludwig Biotec, Porto Alegre, RS, Brazil). Thermal profiles of amplifications were 4 min. at 94 °C, followed by 32 cycles of denaturation at 94 °C for 30 sec., annealing at 50.5 °C for 30 sec. (*blaZ* gene) or 55 °C (*mecA* and *mecC* genes) and extended at 72 °C for 30 sec., with final extension at 72 °C for 5 min. Then 10μL of each reaction was electrophoresed for 40 minutes at 100V in 1.5% agarose gel stained with BlueGreen, visualized and photodocumented under ultraviolet light.

Table S1. Sequences of the oligonucleotides used in this study and sizes of the amplified fragments, in base pairs (bp)

Oligonucleotide	Sequence (5'-3')	Amplicon (bp)	Reference
blaZ	F-AAGAGATTTGCCTATGCTTC R-GCTTGACCACTTTTATCAGC	517	Sawant *et al.* 2009
mecA	F-TGGTATGTGGAAGTTAGATTGGGAT R-CTAATCTCATATGTGTTCCTGTATTGGC	155	Nakagawa *et al.* 2005
mecC	F-CATTAAAATCAGAGCGAGGC R-TGGCTGAACCCATTTTTGAT	188	Paterson *et al.* 2012

Figure S1:

Distribution map of the municipalities sampled, located in the Northeast region, Brazil.



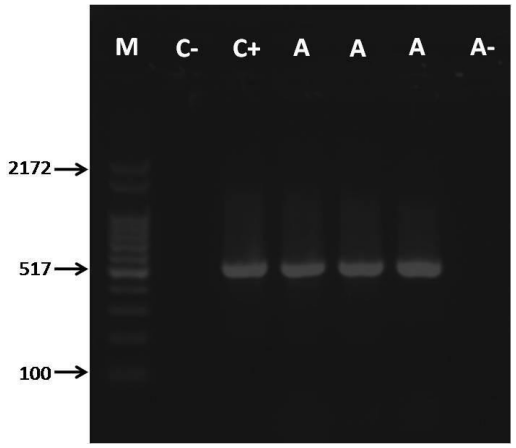


Figure S2. Amplification of *blaZ* gene fragment in NAS isolated from buffalo, goat and sheep with mastitis. Column M: 100bp molecular weight marker (Invitrogen); Column C-: negative control; C+ column: positive control; Column A1: Positive bubaline sample; Column A2: Positive goat sample; Column A3: Positive sheep sample; Column A-: Negative sample.

Antimicrobial susceptibility test

In vitro antimicrobial resistance was determined by disk-diffusion method for the following drugs: amoxicillin (30μg), ampicillin (10μg), cefotaxime (30μg), cefoxitin (30μg), ceftriaxone (30μg), gentamicin (10μg), norfloxacin (10μg), oxacillin (1μg), penicillin G (10U), sulfazotrim (23.75/1.25μg), tetracycline (30μg) and vancomycin (30μg). Multiple antimicrobial resistance (MAR) index was calculated according to Krumperman (1983). The minimum inhibitory concentration (MIC) for antimicrobials (amoxicillin, cephalexin, cefotaxime, ceftriaxone and oxacillin) was also detected according to CLSI (2015).

Statistical analysis

Epi Info™ software (version 7.2) was used to statistical analyzes. Chi-square test was used to verify the statistical significance in the antimicrobial resistance frequencies and MIC values for different species studied. Less than 0.05 p-value was considered statistically significant (Sampaio, 2002). A logistic regression analysis was performed, considering as a dependent variable the *blaZ* gene (presence or absence) (Hosmer & Lemeshow, 1989).