Occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) in "coalho" cheeses produced in Brazil

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SUPPLEMENTARY FILE

Material & Methods

Isolation of Staphylococcus aureus

In the laboratory, with the aid of sterile scalpels, the samples were cut and weighed. 25 g of random portions of the food were collected, including surface and depth. Subsequently, 225 ml of 0.1% peptone salt solution were added, obtaining a 1:10 dilution; the samples were subjected to the process of homogenization in the "stomacher" for 60 seconds, and from this dilution, serial dilutions up to 1 × 10-5 were performed.

After all dilutions were prepared, a 0.1 ml aliquot of each was transferred to the surface of a petri dish containing Baird-Parker agar (LABORCLIN, BRAZIL), enriched with egg yolk emulsion and potassium tellurite, and spread with the aid of a Drigalski loop, always in duplicate.

All inoculated plates were taken to the bacteriological incubator and incubated for 48 hours at 35 °C - 37 °C. After the incubation period, the plates that had between 20 and 200 colonies were selected and the colonies were categorized, being divided into typical and atypical. From each plate, which the two types of colonies existed, 3 typical and 2 atypical colonies were removed with the aid of a sterile platinum handle; from the plates which there were only typical colonies, 5 colonies were removed; and the plates which only atypical colonies were observed, there was no removal of colonies for molecular tests, only for counting the CFU/g of the sample.

Multiplex PCR

For the reaction, 30 ng of genomic DNA were used, 20 pmol of specific primers for each gene, 160 µg of each dNTP, 3 mM magnesium chloride (MgCl2), 50 mM potassium chloride (KCl), 10 mM Tris-HCl (pH 9.0), and 1.2U of Taq DNA Polymerase (Invitrogen, Brazil), generating a reaction product with a total volume of 25 µl.

The amplification reactions took place in a thermocycler (Byocycler) programmed for 30 complete thermal cycles; and the S. aureus strains standard ATCC 43300, ATCC 13565, ATCC 14458, ATCC 19095 and ATCC 13566, possessing the mecA and nuc genes, were used as positive controls of sea, seb, sec, and tst1, respectively.

After the reactions, the amplification products (amplicons) were analyzed by electrophoresis in 1.5% (w/v) agarose gel, marked with ethidium bromide (1.5 mg/ml), and observed in a UV transilluminator. The primers used in the reactions are described in Table S1.

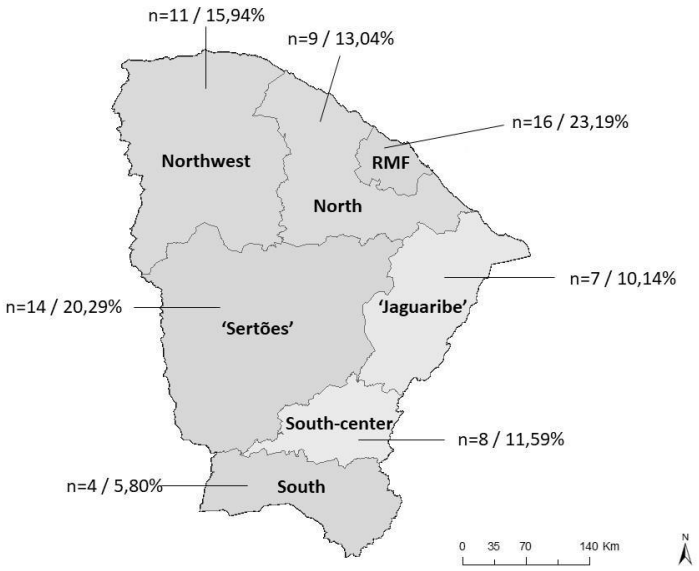
Table S1:

Sequence of primers used and sizes of amplicons in number of base pairs.

Gene	Primer	Sequence 5' – 3'	Amplicon (pb)	Reference
nuc	*nuc*-F	GCGATTGATGGTGATACGGTT	279	KATEETE *et al*., 2010
	nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
mecA	*MecA*-F	GTAGAAATGACTGAACGTCCGATAA	310	MCCLURE *et al*., 2006
	MecA-R	CCAATTCCACATTGTTTCGGTCTAA		
blaZ	*blaZ*-F	AAGAGATTTGCCTATGCTTC	517	SAWANT; GILLESPIE; OLIVER, 2009
	blaZ-R	GCTTGACCACTTTTATCAGC		
sea	*sea*-F	TAAGGAGGTGGTGCCTATGG	180	CREMONESI *et al*., 2005
	sea-R	CATCGAAACCAGCCAAAGTT		
seb	*seb*-F	CATCGAAACCAGCCAAAGTT	478	JARRAUD *et al*., 2002
	seb-R	TCGCATCAAACTGACAAACG		
sec	*sec*-F	ACCAGACCCTATGCCAGATG	371	CREMONESI *et al*., 2005
	sec-R	TCCCATTATCAAAGTGGTTTCC		
tst1	*tst1*-F	ATGGCAGCATCAGCTTGATA	300	AKINEDEN et al 2001
	tst1-R	TTTCCAATAACCACCCGTTT		

Figure S1:

Map of Ceará with the distribution of the number of *S. aureus* strains in 'coalho' cheese samples and the percentage by mesoregion of the state of Ceará - Brazil.



Adapted from Map of Mesoregions of Ceará, available at: http://www.baixarmapas.com.br/mapa-de-mesorregioes-do-ceara/. Accessed on July 27, 2020

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