

Susceptibility profile and antimicrobial multiresistance of *Staphylococcus aureus* strains from Brazilian “coalho” cheese

D.M. Santos¹, R.O.J. Silva¹, S.T. Ortiz¹, M.R.O. Silva¹, S.A.C. Andrade² and E.C.L. Machado¹

¹Laboratory of Food Microbiology, Academic Center of Vitória, Federal University of Pernambuco, Alto do Reservatório, s/n, Bela Vista, 55608-680, Vitória de Santo Antão, Pernambuco, Brazil

²Laboratory of Fruits and Vegetables, Department of Chemical Engineering, Federal Rural University of Pernambuco, Manoel de Medeiros, s/n, Dois Irmãos, 52171-900, Recife, Pernambuco, Brazil

This study aimed to determinate the susceptibility profile and antimicrobial multiresistance of *Staphylococcus aureus* strains from “coalho” cheese commercialized on Vitória de Santo Antão, Pernambuco, Brazil. The *S. aureus* strains were obtained from 06 samples of “coalho” cheese, being 03 pasteurized (Type A) and 03 unpasteurized (Type B) samples. The susceptibility antimicrobial test was performed by disk diffusion method using Erythromycin 15µg, Clindamycin 2µg, Ciprofloxacin 5µg, Gentamicin 10µg, Tetracycline 30µg, Amikacin 30µg, Penicillin G 10 UI, Chloramphenicol 30µg, Sulfazotrin 25µg and Cefoxitin 30µg as antimicrobials reference. A total of 48 strains of *S. aureus* were obtained and submitted to the antimicrobial susceptibility test. All the strains were susceptible to Ciprofloxacin, Gentamicin and Amikacin, while 24 (50%) *S. aureus* strains presented resistance to Penicillin and thus considered as penicillinase producers and and 5 (10.42%) *S. aureus* strains were resistant to cefoxitin, being considered as MRSA strains. For the Multiple Antibiotic Resistance (MAR) indexes, were observed values of 0.2, 0.3 and 0.4 correlated with the resistance to two or more antimicrobials class and 0.2 index was the most frequent. As conclusion, there was an occurrence of *S. aureus* strains with antimicrobial multiresistance in both type A and B “coalho” cheese samples, emphasizing the importance of the control of the abusive use of antibiotics by supervisory agencies.

Keywords: Antimicrobial resistance; “Coalho” cheese; *Staphylococci*; Milk derivatives

1. Introduction

The “coalho” cheese is a typical artisanal product from Brazilian Northeast that presents a great local production and consumption. Characterized by a medium to high humidity, this artisanal cheese could be easily contaminated by pathogenic microorganisms. Its trade is very widespread, being found in open-air markets, supermarkets and can be consumed in different forms: raw, roasted, fried or cooked [1].

Recently, Brazil has been showed great advances in relation to the microbiological quality of the milk produced, mainly after the implementation of the new legislation for the dairy sector [2]. However, there are still problems related to the contamination of the milk [3] and different factors are associated with the sanitary quality of milk, milk production, handling and their cooling. The contaminations that can occur in milk result in a series of changes in its physical-chemical composition, and consequently, it has a repercussion on the reduction of the quality of its derivatives, implying commercial and financial losses [4]. In order to avoid possible intoxications or food-borne diseases through consumption, the Ministry of Livestock and Food Supply determines that “coalho” cheese must be obtained from the coagulation of pasteurized milk and must be marketed with up to 10 days of manufacture [5].

Staphylococcus aureus is one of the most important human and animal pathogenic organism, being widely related with a diverse spectrum of diseases ranging from minor skin infections to life threatening diseases, such as pneumonia and meningitis, as well as, food-borne diseases, production of toxins and antimicrobial resistance [6, 7]. Antimicrobial resistance is an important health problem worldwide because the development of resistance has been associated with the extensive therapeutic use of antimicrobials or with their use as growth promoters in animal feed production. The antimicrobial multiresistant *S. aureus* strains may have an increased ability to spread, especially if they are enhanced with virulence genes. Artisanal products such as “coalho” cheese have been implicated as potential source for the transmission of the pathogen to humans. Furthermore, milk derivative products contaminated with antibiotic resistant bacteria represent ideal vehicles for the transmission of pathogenic strains [8].

In face of this perspective, the objective of this study was to evaluate the susceptibility profile and antimicrobial multiresistance of *Staphylococcus aureus* strains obtained from “coalho” cheese commercialized on Vitória de Santo Antão, Pernambuco, Brazil.

2. Material and Methods

2.1 Obtention and preparation of the “Coalho” cheese samples

For this study, the “coalho” cheese samples were collected from local and open-air markets in Vitória de Santo Antão, Pernambuco, Brazil, totaling 06 samples, being 03 pasteurized (Type A) and 03 unpasteurized (Type B). All samples

were identified, stored into thermal boxes with ice, conducted to the Laboratory of Food Microbiology of the Federal University of Pernambuco and maintained under refrigeration until microbiological analysis.

Posterly, $25 \pm 0,2$ g of each “coalho” cheese sample was taken and combined with 225 ml of sterile peptonated water 0.1% (w/v) in a sterile polyethylene bag and pummeled with a Stomacher during 5 min. This mixture (10^{-1}) was shaken and subsequent serial dilutions were prepared in accord with Normative Instruction N° 62/2003 of the Ministry of Livestock and Food Supply [9]

2.2 Determination of the coagulase-positive *Staphylococci* and confirmation of *Staphylococcus aureus*

The determination of coagulase-positive *Staphylococci* was performed by *spread plate* method as described in the Normative Instruction N° 62/2003 of the Ministry of Livestock and Food Supply [9]. A volume of 100 µl of each dilution was spreaded onto Petri plates containing Baird Parker (BP) agar supplemented with 20% egg yolk tellurite emulsion. The plates were incubated at 37°C for 24 – 48 hours. Typical and atypical colonies as circular, smooth, black or not and surrounded by an opaque zone with an outer clear zone were selected, counted and the results expressed in colony forming units (CFU/g) for “coalho” cheese sample. Further, typical and atypical colonies were transferred into tubes containing Brain Heart Infusion (BHI) broth, incubated at 37°C for 24 hours and used for the coagulase test in order to confirm coagulase-positive *Staphylococci*. The colonies that showed to be coagulase-positive were taken for *Staphylococcus aureus* confirmatory testing such as Gram stain, catalase, mannitol fermentation and DNase.

2.3 Antimicrobial susceptibility test and multiple antibiotic resistance (MAR) index

All *S. aureus* strains were used for the antimicrobial susceptibility test by disk diffusion method as CLSI M100-S25 protocol [10]. Erythromycin 15µg, Clindamycin 2µg, Ciprofloxacin 5µg, Gentamicin 10µg, Tetracycline 30µg, Amikacin 30µg, Penicillin G 10 UI, Chloramphenicol 30µg, Sulfazotrin 25µg and Cefoxitin 30µg were used as antimicrobials reference. The antimicrobial susceptibility profile was obtained by measuring the sizes of the inhibition zones and expressed in millimeters, being classified as resistant, intermediate and susceptible according to the reference table established by CLSI. The multiple antibiotic resistance (MAR) index was defined as a/b , where a represents the number of antibiotics to which the isolate was resistant, and b represents the number of antibiotics to which the isolate was exposed [11].

3. Results and Discussion

A total of 48 *Staphylococcus aureus* strains were obtained from “coalho cheese” samples and submitted to the antimicrobial susceptibility test, being 15 (31.25%) from pasteurized cheese (Type A) and 33 (68.75%) from unpasteurized cheese (Type B). The antimicrobial susceptibility profile showed that 14 (29.16%) *S. aureus* strains were susceptible to all antimicrobials tested and 34 (70.84%) were resistant to at least one antimicrobial. The Table 1 contains the results for antimicrobial susceptibility profile of each antimicrobial related to 48 *S. aureus* strains. Gentamicin, amikacin and ciprofloxacin were effective towards all the *S. aureus* strains (100% susceptible).

A study carried out with 45 *S. aureus* strains from 10 rennet cheese samples showed a high percentage of resistance to penicillin (100%), tetracycline (75.5%), gentamicin (66.7%), erythromycin (48.9%) and sulfazotrin (26.7%) [12].

Table 1 Antimicrobial resistance percentage for the 48 *Staphylococcus aureus* strains obtained from “coalho” cheese commercialized on Vitória de Santo Antão, PE.

Antimicrobial Agents	Resistant	Susceptible
Erythromycin	11 (22.91%)	37 (77.08%)
Penicillin	24 (50%)	24 (50%)
Clindamycin	11 (22.91%)	37 (77.08%)
Ciprofloxacin	0	48 (100%)
Gentamicin	0	48 (100%)
Cefoxitin	5 (10.42%)	43 (89.58%)
Tetracycline	12 (25%)	36 (75%)
Amikacin	0	48 (100%)
Sulfazotrin	1 (2.08%)	47 (97.91%)
Chloramphenicol	3 (6.25%)	45 (93.75%)

Considering the main resistance phenotypes, our results pointed that 24 (50%) *S. aureus* strains were resistant to penicillin and thus considered as penicillinase producers and 5 (10.42%) *S. aureus* strains, isolated from unpasteurized cheese (Type B), were resistant to cefoxitin and considered as MRSA strains. The CLSI has suggested the usefulness of the cefoxitin disk instead of the oxacillin disk when used as a surrogate marker for the detection of methicillin resistance. In recent years, an increase in the number of methicillin resistant (MR) *Staphylococcus aureus* strains has

become a serious clinical and epidemiological problem, as resistance to this antibiotic implies resistance to all β -lactam antibiotics, including carbapenems. Methicillin resistance in *S. aureus* is mediated through an protein called low-affinity penicillin binding protein (PBP2a) and encoded by *mecA* gene [13].

MLS (Macrolide-Lincosamide-Streptogramin) resistance phenotype was also observed between *Staphylococcus aureus* strains. Resistance to erythromycin as well as to clindamycin was observed in 7 (14.6%) *S. aureus* strains indicated a constitutive type of MLS resistance (cMLS) while 4 (8.33%) *S. aureus* strains showed resistance to erythromycin and being susceptible to clindamycin, were classified only as MS resistance phenotype.

MAR index is a very useful tool to understand the risks associated with to antimicrobial multiresistance. The Table 2 shows the values for the total of 48 *S. aureus* strains. As observed, the most of the *S. aureus* strains presented index of 0.2, which mean resistance towards two antimicrobials of different classes.

Table 2 Percentage of 48 *Staphylococcus aureus* strains in according to the multiple antibiotic resistance (MAR) index.

MAR Index	<i>S. aureus</i> strains
0.1	12 (25%)
0.2	13 (27.1%)
0.3	7 (14.6%)
0.4	2 (4.1%)

Special attention should be given to the MAR index 0.4, since what 2 (4.1%) *S. aureus* strains showed resistance to 4 antimicrobial agents of different classes as macrolide, lincosamide, tetracycline and β -lactams. These strains were isolated from unpasteurized cheese (Type B), showing the great importance of the pasteurization process in elimination of pathogens and the risk of food contamination by resistant bacteria.

It was observed the occurrence of distinct antimicrobial resistance profile between the *S. aureus* strains of “coalho” cheese produced by pasteurized milk (type A) and unpasteurized (Type B), a percentage of 80% corresponding to 12 *S. aureus* strains and a percentage of 66% corresponding to 22 *S. aureus* strains as shown in Table 3, respectively.

Table 3 Percentage of antimicrobial resistance for *Staphylococcus aureus* strains related with the “coalho” cheese type.

“Coalho” Cheese	<i>S. aureus</i> Strains Resistance (%)
Type A (pasteurized)	12/15 (80%)
Type B (unpasteurized)	22/33 (66%)

Staphylococcus aureus is one of the most common microorganisms associated with food poisoning in the world, being a pathogen frequently isolated in artisanal cheeses [14, 15] and used as an indicator of post-process contamination production or hygienic-sanitary conditions of the commercial places [16].

Due to the high antimicrobial multiresistance presented by *S. aureus* strains isolated from “coalho” cheese (Type A and B) and mainly because some of these strains showing interesting resistance phenotypes such as MRSA and cMLS, this study emphasizes the importance of control in the indiscriminate use of antibiotics to avoid problems of resistance acquired by *S. aureus* compromising the quality of milk and derived products contaminated by these strains.

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