

Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Raw Hamburgers and Ready-to-Eat Sandwiches Commercialized in Supermarkets and Fast Food Outlets in Brazil

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Abstract

Although well known, *Staphylococcus aureus* is a bacterium that remains widely studied because of its high pathogenic potential and its ability to develop resistance to antibiotics routinely used in clinical practice. The present study investigated the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hamburgers and sandwiches sold in supermarkets and fast food outlets in Salvador, BA, Brazil. Fifty samples of frozen raw hamburgers (25: beef and 25: chicken) and 50 samples of ready-to-eat sandwiches (25: beef and 25: chicken) were collected and investigated for the presence of MRSA. MRSA was present in 32% of the hamburgers and 8% of the sandwiches. The frequency of MRSA was higher in the samples containing chicken meat. However, the statistical analysis showed no association between MRSA presence and the type of meat investigated ($P > 0.05$). The high prevalence of MRSA in hamburgers and the presence of the microorganism in ready-to-eat sandwiches are worrying and indicate the need for better control during food preparation to prevent the spread of bacteria.

Keywords

Methicillin-Resistant *Staphylococcus aureus*, Hamburgers, Sandwiches, Food Safety

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1. Introduction

With the reduced time available for food preparation and/or consumption, in recent years, there have been changes in individuals' eating habits, motivated especially by urbanization processes and the professionalization of women, substantially favoring the consumption of processed products and food prepared away from home. Thus, the search for ready-to-eat meals produced on a large scale and with reduced cost has culminated in an increased number of fast-foods outlets and individuals eating in cafeterias and trailers [1]-[3].

Staphylococcus aureus is considered the third-most important cause of food-borne disease in the world [4] and has two aggravating characteristics, toxin production and antimicrobial resistance. This pathogen is considered an excellent indicator of thermal processing inefficiency, inadequate hygienic conditions during food production/preparation, or inadequate cooling after food preparation [5].

Regarding antimicrobial resistance, *S. aureus* is one of the most microorganisms implicated in cases of nosocomial infection worldwide. The methicillin-resistant *Staphylococcus aureus* (MRSA) was considered a nearly exclusively responsible pathogen in hospital-acquired infections between 1930 and 1980; however, at the end of 1990, MRSA infections among healthy individuals began to emerge [6] [7].

S. aureus resistance to antibiotics is due to the production of a penicillinase, an enzyme that inactivates the beta-lactâmico ring of the penicillin molecule, making the antibiotic ineffective against *S. aureus*. Methicillin resistance presented by *S. aureus* is mediated by the presence of the *mecA* gene, which encodes the 2a protein (PBP2a) found in the cell wall of the microorganism [7]-[10].

In particular, strains of MRSA harboring genes encoding the Pantone-Valentine leukocidin (PVL) toxin are of concern from a public health perspective because they are usually capable of causing severe skin and soft tissue infections and occasionally necrotizing pneumonia, which is associated with high mortality [11].

This study aimed to investigate the occurrence of methicillin-resistant *S. aureus* (MRSA) in sandwiches and hamburgers marketed in Salvador, BA, and evaluate the association between MRSA isolates and investigated meat.

2. Material and Methods

2.1. Detection of MRSA

Sampling and Laboratory Procedures

Fifty samples of frozen raw hamburgers (25 beef and 25 chicken) and 50 samples of ready-to-eat sandwiches (25 beef and 25 chicken) were collected from supermarkets and fast food outlets, respectively, in the city of Salvador, Bahia, in northeastern Brazil, from September 2013 through December 2013.

To detect MRSA, approximately 500 g of frozen hamburger and 200 g of ready-to-eat sandwiches were aseptically collected in supermarkets and fast food outlets, respectively, and transported to the laboratory for analysis. Prior to analysis, hamburger samples were defrosted in the original package under refrigeration for 24 hours. For sandwiches, the meat (beef or chicken) and bread portions were separated, and the bread was discarded. Approximately 25 g of each food was added to 225 ml of Tryptic Soy Broth (TSB, Difco, Detroit, MI, USA) supplemented with 6.5% NaCl and homogenized in a Stomacher (240 bpm; ITR model 1204, series 126, Esteio, RS, Brazil) for 2 min in a class II biosafety cabinet (Labconco Corporation, Labconco Purifier Class IIb, Total Exhaust, model 36210-04, certified ISO 9002, Kansas City, MO, USA). This homogenate was then incubated at 37°C for 16 - 20 h. Next, a 1-ml aliquot was added to 9 ml of Phenol Red Mannitol Broth (PHMB, HiMedia, São Paulo, SP, Brazil) containing ceftizoxime (5 µg/ml) and aztreonam (75 µg/ml) (Sigma-Aldrich Brazil Ltda, São Paulo, SP, Brazil). From the culture obtained in PHMB, the surface of the hypertonic mannitol agar (HMA, HiMedia, São Paulo, SP, Brazil) was inoculated with a sterile loop. The plates were incubated for 24 h at 37°C and examined for typical colonies, Gram, catalase and coagulase. For confirmation, a maximum of five typical colonies per plate were purified on Tryptic Soy Agar (TSA, Difco, Detroit, MI, USA) and subjected to antibiotic susceptibility testing using the disc diffusion method [12].

2.2. In Vitro Susceptibility to Antibiotics Testing

Cells were grown overnight at 35°C in TSB, suspended in saline solution and adjusted to 0.5 on the McFarland scale (c.a. 10⁸ CFU). The cell suspensions were inoculated with a swab in Mueller-Hinton agar (MHA, HiMedia, São Paulo, SP, Brazil). Bacterial growth was recorded after 18 - 24 h of incubation at 35°C. One *Staphylococcus*

aureus (ATCC 25923) negative control and one methicillin-resistant *Staphylococcus aureus* (ATCC 33591) positive control were used for each set of analyzed samples. Antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion on Mueller-Hinton medium according to the recommendations of the Clinical Laboratory Standards Institute [13]. The isolates were tested against a panel of seven antimicrobial agents: penicillin G (PEN) (10 UI), erythromycin (ERI) (15 µg), tetracycline (TET) (30 µg), oxacillin (OXA) (1 µg), cefoxitin (CEF) (30 µg), vancomycin (VCM) (30 µg) and ciprofloxacin (CIP) (5 µg). The antibiotic disks were purchased from Interlab (Laborclin, Interlab, São Paulo, SP, Brazil).

2.3. Statistical Analysis

The Pearson's chi-square (X^2) (SPSS 17.0 for Windows) was used to determine significant differences ($P < 0.05$) in MRSA contamination between the investigated meats.

3. Results and Discussion

A total of 34 hamburger samples were positive for coagulase-positive *Staphylococci*, 16 (64%) hamburgers made with beef and 18 (72%) made with chicken meat. For the sandwiches, 7 samples were contaminated, 5 (20%) sandwiches made with beef and 2 (8%) made with chicken meat (Table 1).

The MRSA prevalence results in the samples are summarized in Table 2. MRSA strains were isolated from 32% of the hamburger samples and 8% of the sandwiches.

Although the hamburger samples made with chicken meat had the highest prevalence of MRSA, no association between MRSA contamination and type of meat investigated was observed ($P > 0.05$).

The resistant/susceptibility profile of the coagulase-positive *Staphylococci* isolated from the samples is demonstrated in Table 3 and Table 4. In chicken meat samples from sandwiches, resistance equal or higher than 50% was observed for penicillin G (66.7%), erythromycin (50.0%) and oxacillin (66.7%) (Table 3). For isolates from hamburgers made with beef or chicken meat, resistance was always lower than 50% (Table 4).

The present results indicated a high prevalence of MRSA in raw hamburger compared with Kitai *et al.* [14], Van Loo *et al.* [15], De Boer *et al.* [12], Weese [16], Fessler *et al.* [17], Hanson *et al.* [18], and Ageroso *et al.* [19].

In Japan, only two strains of *S. aureus*, both isolated from chicken thigh muscle parts, have been identified as MRSA, suggesting that food handlers have accidentally transmitted the pathogen [14]. Improper handling is currently the primary source of contamination of foodstuffs, and proper hand hygiene, with warm water and soap and antiseptic, would prevent such contamination [20].

Here, the presence of MRSA in ready-to-eat sandwiches indicates that the beef and chicken meat were

Table 1. Frequency of isolation of coagulase-positive *Staphylococci* in hamburger and sandwich samples.

Meat/food	Hamburgers		Sandwiches	
	Samples (n)	Coagulase-positive <i>Staphylococci</i> positive samples (%)	Samples (n)	Coagulase-positive <i>Staphylococci</i> positive samples (%)
Beef	25	16 (64.0)	25	5 (20.0)
Chicken	25	18 (72.0)	25	2 (8.0)
Total	50	34 (68.0)	50	7 (14.0)

Table 2. Frequency of MRSA isolation in hamburger and sandwich samples.

Meat/food	Hamburgers		Sandwiches	
	Samples (n)	MRSA positive samples (%)	Samples (n)	MRSA positive samples (%)
Beef	25	4 (16.0)	25	2 (8.0)
Chicken	25	12 (48.0)	25	2 (8.0)
Total	50	16 (32.0)	50	4 (8.0)

Table 3. Antimicrobial activity of antibiotics against positive-coagulase *Staphylococci* isolated from sandwiches.

Profile	Sandwiches				P-value
	Chicken		Beef		
Penicillin G	N	%	N	%	
Resistant	2	66.7	3	23.1	0.265
Intermediary	0	0.0	5	38.5	
Susceptible	1	33.3	5	38.5	
Total	3	100.0	13	100.0	
Erythromycin					
Resistant	2	50.0	2	15.4	0.154
Intermediary	0	0.0	0	0.0	
Susceptible	2	50.0	11	84.6	
Total	4	100.0	13	100.0	
Tetracycline					
Resistant	0	0.0	1	6.7	0.182
Intermediary	1	20.0	0	0.0	
Susceptible	4	80.0	14	93.3	
Total	5	100.0	15	100.0	
Oxacillin					
Resistant	2	66.7	3	21.0	0.241
Intermediary	0	0.0	5	35.7	
Susceptible	1	33.3	6	42.9	
Total	3	100.0	14	100.0	
Cefoxitin					
Resistant	0	0.0	0	0.0	-
Intermediary	0	0.0	0	0.0	
Susceptible	5	100.0	15	100.0	
Total	5	100.0	15	100.0	
Vancomycin					
Resistant	0	0.0	0	0.0	0.054
Intermediary	1	25.0	0	0.0	
Susceptible	3	75.0	14	100.0	
Total	4	100.0	14	100.0	
Ciprofloxacin					
Resistant	0	0.0	0	0.0	-
Intermediary	0	0.0	0	0.0	
Susceptible	4	100.0	15	100.0	
Total	4	100.0	15	100.0	

Table 4. Antimicrobial activity of antibiotics against coagulase-positive *Staphylococci* isolated from hamburgers.

Penicillin G	Hamburgers				P-value
	Chicken		Beef		
	N	%	N	%	
Resistant	19	43.2	10	33.3	0.391
Intermediary	15	34.1	15	50.0	
Susceptible	10	22.7	5	16.7	
Total	44	100.0	30	100.0	
Erythromycin					
Resistant	11	35.5	6	21.4	0.234
Intermediary	0	0.0	0	0.0	
Susceptible	20	64.5	22	78.6	
Total	31	100.0	28	100.0	
Tetracycline					
Resistant	12	25.0	7	21.9	0.703
Intermediary	7	14.6	3	9.4	
Susceptible	29	60.4	22	68.8	
Total	48	100.0	32	100.0	
Oxacillin					
Resistant	11	39.3	9	31.0	0.480
Intermediary	2	7.1	5	17.2	
Susceptible	15	53.6	15	51.7	
Total	28	100.0	29	100.0	
Cefoxitin					
Resistant	1	2.3	0	0.0	0.455
Intermediary	1	2.3	0	0.0	
Susceptible	41	95.3	33	100.0	
Total	43	100.0	33	100.0	
Vancomycin					
Resistant	2	5.0	0	0.0	0.200
Intermediary	0	0.0	0	0.0	
Susceptible	38	95.0	32	100.0	
Total	40	100.0	32	100.0	
Ciprofloxacin					
Resistant	0	0.0	0	0.0	0.228
Intermediary	2	4.2	0	0.0	
Susceptible	46	95.8	34	100.0	
Total	48	100.0	34	100.0	

insufficiently cooked or that cross-contamination occurred after cooking in sandwich preparation by an asymptomatic food handler or utensils.

In Brazil, Costa [21] reported that some preparations of meats ready for consumption were contaminated with MRSA. The author considered the occurrence of contamination after the preparation of the meat dishes by asymptomatic carriers or contaminated utensils used in the distribution of meals, once the thermal processing was sufficient to eliminate the microorganism.

According to the results of the present study, 66.7% of the coagulase-positive *Staphylococci* isolated from samples of chicken sandwiches were resistant to penicillin, a drug used in human and veterinary medicine. For beef sandwiches, this resistance was observed in only 23.1% of the isolates.

In Brazil, the resistance of the *S. aureus* to penicillin varies from 20% to 100%, whereas the percentage of resistance to other antimicrobials is lower [22]. In several countries, however, large-scale studies have shown that resistance of the microorganism to penicillin is approximately 60%, and studies from 11 different countries have demonstrated that the prevalence of samples resistant to various antibacterials routinely used to treat mastitis was generally low [23].

Susceptibility to vancomycin, the drug of choice for MRSA treatment in humans, was observed in all isolates from samples of beef sandwiches and beef hamburgers, whereas 75% of the isolates from chicken sandwiches were susceptible (Table 3). These results indicate that selecting vancomycin for MRSA infection treatment is suitable.

Cefoxitin was recently shown to be a marker of resistance to MRSA. However, the results demonstrated that 100% of the isolates of sandwiches samples were susceptible to this marker (Table 3). For hamburger samples, similar results were found (Table 4).

Fessler *et al.* [17] demonstrated that all MRSA isolates were resistant to oxacillin, and 62.5% exhibited multidrug resistance. Hanson *et al.* [18] showed that MRSA isolated from samples of pork, beef, chicken, and turkey were resistant to oxacillin and were multidrug resistant. Van Loo *et al.* [15] found similar results to those of Hanson *et al.* [18]; *i.e.*, samples of pork and beef were contaminated with MRSA, and all isolates were resistant to oxacillin and cefoxitin. Recently, Lin *et al.* [24] found that *S. aureus* ST398 isolated from retail foods were most frequently resistant to penicillin (100%), followed by trimethoprim, erythromycin, ampicillin (each 86.7%), clindamycin (80.0%), and tetracycline (26.7%). All ST398 isolates were susceptible to amikacin, chloramphenicol, cefoxitin, gentamicin, oxacillin, and vancomycin.

According to Hososaka *et al.* [25], some clinical isolates of *S. aureus* are *mec A*-positive and susceptible to oxacillin. The presence of this gene was verified in strains of *S. aureus* identified as methicillin-susceptible (MSSA) that were isolated from hamburgers [26].

Due to the growing problem of microbial resistance, the outlook for the use of antimicrobial drugs is uncertain. Consequently, research intending to increase antibacterial activity and characterize the interaction mechanism with antibiotics is needed.

4. Conclusion

The high prevalence of MRSA among the tested foods, primarily in raw hamburger made from chicken meat, and the presence of the microorganism in prepared ready-to-eat sandwiches highlights the necessity of enforcing hygienic practices within the food industry and fast food outlets. Better knowledge of the transmission routes of MRSA in the food chain, “from the farm to the fork”, is necessary to provide tools for preventing the spread of MRSA, clarify the possible health hazards for consumers related to the presence of MRSA in foods, and determine adequate measures for MRSA control.

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