Methicillin-Resistant *Staphylococcus aureus* in Raw Meats and Prepared Foods in Public Hospitals in Salvador, Bahia, Brazil

Wellington Luis Reis Costa, Jeane dos Santos Ferreira, Joelza Silva Carvalho, Ellayne Souza Cerqueira, Lucimara Cardoso Oliveira, and Rogeria Comastri de Castro Almeida

Abstract: This study investigated the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meat and fish and foods prepared from them for patient consumption in public hospitals in Salvador, Bahia, in northeastern Brazil. A total of 114 samples of raw meat and fish (chicken, $n = 30$; beef, $n = 30$; pork, $n = 24$; and fish, $n = 30$) and 63 samples of prepared foods (made with chicken, $n = 15$; beef, $n = 15$; pork, $n = 15$; and fish, $n = 18$) were collected from the kitchens of 10 different hospitals. Of the 114 investigated raw meat and fish samples, 28.1% were positive for MRSA, which comprised 23.3% beef, 23.3% chicken, 37.5% pork, and 30% fish samples. Of the prepared foods, 9.5% were positive for MRSA, which comprised 5.6% chicken products, 6.7% pork products, and 22.2% fish products. MRSA contamination was not detected in prepared beef dishes. A statistical analysis showed no association between the presence of MRSA and the type of raw food ($P > 0.05$). The high prevalence of MRSA among the raw foods tested and the presence of the microorganism in prepared foods emphasizes the necessity of enforcing hygienic practices within hospital kitchens.

Keywords: antimicrobial resistance, hospitals' kitchens, MRSA, prepared foods, raw meat

Practical Application: We assessed the presence of MRSA in raw and ready-to-eat meat and fish products in public hospitals. The results of this work may be used to alert the veterinaries and health authorities that the pathogen has entered the food chain.

Introduction

Staphylococcus aureus, a pathogen involved in severe gastrointestinal disorders, is the most common etiological agent of bovine mastitis (Perillo and others 2012). The microorganism is associated with nosocomial and community-acquired staphylococcal infections, primarily related to the emergence of drug-resistant organisms (DeLeo and Chambers 2009).

Methicillin-resistant *S. aureus* (MRSA) strains were 1st identified in 1961, immediately after the introduction of methicillin in clinical settings (Barber 1961). Since then, increased resistance to methicillin among *S. aureus* isolates has been observed globally (Chambers 1997). Approximately 30 to 50 kb of additional chromosomal DNA, *mec*, which is not found in susceptible strains of staphylococci, is present in methicillin-resistant strains. The *mec* cassette contains *mec*A, the structural gene for penicillin binding protein 2a (PBP2a). PBP2a interferes with β-lactam antibiotics by preventing their effective binding to cell wall proteins (Chambers 1997; Kamal and others 2013).

Livestock-associated MRSA strains have been identified in Europe and the 1st colonization was reported in the Netherlands (Voss and others 2005), where pigs were thought to be a source of MRSA infection in humans (Morcillo and others 2012). Antibiotics used to promote animal growth are generally administered orally, at low doses, and for extended duration. These 3 conditions can induce resistance in pathogenic bacteria and have the potential to generate residues in animal tissue and meat products (Lipsitch and others 2002). Thus, these treatments are controversial and have been criticized by health authorities and consumer advocates in Brazil and other countries.

Because *S. aureus* is highly prevalent in food and food environments, MRSA may follow the same transmission pattern, and although MRSA infections have not been associated with consumption of contaminated meats, the pathogen has entered the food chain. Recent studies confirmed MRSA detection with varying incidence in meat, including beef, pork, chicken, fish, and another animals (Huijsdens and others 2006; Normanno and others 2007; Van Loo and others 2007; De Boer and others 2009; Lozano and others 2009).

Strategies to control and prevent the spread of MRSA in the hospitals include the early identification of positive patients, patient isolation, skin and nasal decontamination, hand hygiene, and decontamination of clinical areas (Sexton and others 2006).

Because the complete eradication of MRSA may be impossible, the control of transmission seems to be an appropriate goal. The identification of MRSA sources, such as food, is essential for monitoring and preventing infections. In addition, interest in hospital-associated MRSA infections and in community-acquired MRSA infections has been increasing in recent years (Gelatti and others 2009). Thus, meals prepared in hospitals should receive special attention because this food is intended for consumption by

MS 20141342 Submitted 8/4/2014, Accepted 10/28/2014. *Authors Costa is with Pharmacy Faculty, Federal Univ. of Bahia. Rua Barão de Geremoabo, s/n, Ondina, Cep: 40.170-290, Salvador, BA, Brazil. Authors Ferreira, Carvalho, Cerqueira, Oliveira and Almeida are with Food Science Dept, Nutrition School, Federal Univ. of Bahia. Av. Araújo Pinho, n° 32, Canela, Cep: 40.110-160, Salvador, BA, Brazil. Direct inquiries to author Almeida (E-mail: rogerianut@gmail.com).*

doi: 10.1111/1750-3841.12723

Journal of Food Science A Publication of the Institute of Food Technologists

patients, a population at high risk for acquiring various diseases. This study investigated the presence of MRSA in raw ingredients and prepared foods destined for patient meals in public hospitals in Salvador, Bahia, Brazil.

Materials and Methods

Detection of MRSA in raw meat

Sampling and laboratory procedures. A total of 114 samples of raw meat were collected in 10 of the 14 public hospitals in the city of Salvador, Bahia, in northeastern Brazil, from July 2011 to May 2012. The samples were from different lots and were collected during 3 different visits to each hospital, which comprised one raw meat type in each hospital during each visit. In total, 30 samples of beef, 30 samples of chicken, 24 samples of pork, and 30 samples of fish were collected. Pork was not processed in hospitals B and C.

For the detection of MRSA, approximately 250 g of food was aseptically collected in the hospitals' kitchens and transported to the laboratory for analysis. Approximately 25 g of food was added to 225 mL of Mueller–Hinton broth (MHB) supplemented with 6.5% NaCl and homogenized in a Stomacher (240 bpm; ITR model 1204, series 126, São Paulo, SP, Brazil) for 2 min in a class II biosafety cabinet (Labconco Corp., Labconco Purifier Class IIb, Total Exhaust, model 36210-04, certified ISO 9002, Kansas City, Mo., U.S.A.). This homogenate was then incubated at 37 °C for 16 to 20 h. Next, a 1-mL aliquot was added to 9 mL of phenol red mannitol broth containing ceftizoxime (5 μg/mL) and aztreonam (75 μg/mL; PHMB; Sigma-Aldrich Brazil Ltda, São Paulo, SP, Brazil), followed by direct plating on the surface of MRSA-ID medium (BioMérieux Brazil S/A, Rio de Janeiro, RJ, Brazil; De Boer and others 2009). From the culture obtained in PHMB, the surface of the selective MRSA-ID medium was inoculated with a sterile loop. The plates were incubated for 24 h at 37 °C, and if the colonies were difficult to identify, the incubation was extended for another 24 h. The plates were examined for typical green colonies. For confirmation, a maximum of 5 selected typical colonies per plate were subcultured on trypticase soy agar (TSA, DIFCO, Detroit, MI, USA).

Detection of PBP2a test. The SlidexR MRSA Detection (Biomerieux, Brazil S/A, Rio de Janeiro, RJ, Brazil), which is based on the agglutination of latex particles coated with monoclonal antibodies against PBP2a, was performed and interpreted according to the manufacturer's instructions. One *S. aureus* (ATCC 25923) negative control and one MRSA (ATCC 33591) positive control were used for each set of analyzed samples.

Detection of MRSA in prepared foods

The hospital kitchens that harbored MRSA in the samples of raw meat were investigated for the presence of MRSA in prepared foods. A total of 63 samples of foods prepared with chicken (n = 15, for example, roasted chicken; chicken stew; grilled chicken; Cuban style chicken; chicken Silveirinha (chicken pieces with carrots and seasoning); stroganoff (chicken breast cooked in sauce containing evaporated milk, champignon mushrooms, and seasonings), and seasoned chicken strips (chicken fingers)), beef (n = 15, for example, seasoned beef strips; beef stew [beef stew cooked with sauce, corn, and seasonings]; roasted beef; steak; steak wrapped in carrots, bacon, and seasonings; fried breaded steak; and beef stew with vegetables [small pieces of beef cooked with vegetables]), pork (n = 15, for example, pork hind legs with mint [pork leg cooked in mint sauce]; sweet and sour sauce pork hind legs; grilled brined pork [ribs]; pork shank in sauce; roasted pork shank; roasted and shredded pork shank; pork shank with Brazilian vinaigrette sauce; pork hind legs in wine; and lemon-grilled brined pork), and fish (n = 18, for example, fish stew [fish, coconut milk, tomato, and seasoning]; Escabeche of fish [regional food made with fish fillet, coconut milk, potatoes and seasoning]; Merluza [*Merluccius*] grilled fish fillet; Tilapia [*Oreochromis niloticus*] fish fillet in sauce; Gomes de Sá style fish; fish mixture; Bráz style fish; and Merluza fish fillet in sauce) were collected and subjected to the same analysis described in section.

The fish investigated were grown in aquaculture systems and were purchased in frozen fillet (86.7%) form. All dishes were cooked in the oven (180 to 220 °C) or on the stove (87 to 99.5 °C) and distributed to patients after 30 to 60 min.

Data analysis

We used simple logistic regression analyses and 2 tests of association, Pearson's chi-square (χ^2) and Fisher's tests (SPSS 17.0 for Windows), to determine whether there were significant differences ($P < 0.05$) in MRSA contamination between the investigated raw meats.

Results

A total of 451 isolates were obtained from the 114 samples of raw meat, and 98 were identified as positive for MRSA.

The results of the prevalence testing for the raw meat are summarized in Table 1. MRSA strains were isolated from 32 (28.1%) of the 114 samples of raw meat. All of the investigated hospitals' kitchens contained at least one type of raw meat or fish contaminated by MRSA. The lowest prevalence was found in hospital G, which had only one sample of contaminated fish. The highest prevalence was observed in hospitals E and I, with 5 (41.7%) positive samples each. Considering each food separately, the percentages of MRSA-positive samples of beef, chicken, pork, and fish were 23.3% (7/30), 23.3% (7/30), 37.5% (9/24), and 30.0% (9/30), respectively.

From 63 samples of prepared foods, 92 isolates were obtained, and 13 were positive for MRSA. In total, 9.5% of the samples tested positive for MRSA, with the following breakdown by meat: dishes made with chicken, 5.6%; pork, 6.7%; and fish, 22.2%. The dishes made with beef were not contaminated by MRSA (Table 2). The specific dishes contaminated by MRSA were pork hind legs with mint, Tilapia fish fillet in sauce, fish mixture, stroganoff, Merluza grilled fish fillet, and Tilapia fish fillet in sauce.

Although the pork samples presented the highest prevalence of MRSA, the study showed no association between MRSA contamination and the type of raw meat investigated ($P = 0.619$).

A logistic regression analysis model was used with the pathogen as the response variable and raw meat as the independent variable. The model indicates that the probability for raw fish to be contaminated with MRSA was 1.40-fold higher than for raw beef, and pork was 1.97 times more likely to be contaminated than beef (data not shown).

Discussion

Results from this study indicated high prevalence of MRSA in raw meats tested as compared to recent findings by De Boer and other (2009), Normanno and other (2007), and Van Loo and others (2007).

The frequency of the MRSA contamination of foods is expected to be low, and there is often a great variety of background flora; thus, direct isolation on selective plating media is rarely successful

Table 1–Prevalence of MRSA in samples of raw meats in the kitchens of public hospitals in Salvador, BA, Brazil.

		MRSA-positive samples				
Hospital	Total samples (*n*)	Beef	Chicken	Pork	Fish	Total positive samples
A	12	(3) 0	(3) 0	(3) 0	(3) 2	2
B	9	(3) 1	(3) 1	(0) Na	(3) 0	2
C	9	(3) 3	(3) 0	(0) Na	(3) 0	3
D	12	(3) 1	(3) 0	(3) 2	(3) 0	3
E	12	(3) 1	(3) 2	(3) 2	(3) 0	5
F	12	(3) 0	(3) 0	(3) 3	(3) 1	4
G	12	(3) 0	(3) 0	(3) 0	(3) 1	1
H	12	(3) 1	(3) 1	(3) 0	(3) 1	3
I	12	(3) 0	(3) 1	(3) 1	(3) 3	5
J	12	(3) 0	(3) 2	(3) 1	(3) 1	4
Total positive samples	114	(30) 7	(30) 7	(24) 9	(30) 9	32

Na, not applicable (pork was not processed in hospitals B and C). Number of collected samples is given in parenthesis.

Table 2–Prevalence of MRSA in samples of prepared foods in the kitchens of public hospitals in Salvador, BA, Brazil.

		MRSA-positive samples				
Hospital	Total samples (*n*)	Beef	Chicken	Pork	Fish	Total positive samples
A	3	(0) Na	(0) Na	(0) Na	(3) 0	0
B	6	(3) 0	(3) 0	(0) Na	(0) Na	0
C	3	(3) 0	(0) Na	(0) Na	(0) Na	0
D	6	(3) 0	(0) Na	(3) 1	(0) Na	1
E	9	(3) 0	(3) 0	(3) 0	(0) Na	0
F	6	(0) Na	(0) Na	(3) 0	(3) 0	0
G	3	(0) Na	(0) Na	(0) Na	(3) 1	1
H	9	(3) 0	(3) 0	(0) Na	(3) 0	0
I	9	(0) Na	(3) 0	(3) 0	(3) 1	1
J	9	(0) 0	(3) 1	(3) 0	(3) 2	3
Total positive samples	63	(15) 0	(15) 1	(15) 1	(18) 4	6

Na, not applicable (the samples were not analyzed in the hospitals' kitchens because the raw food was negative for MRSA). Number of collected samples is given in parenthesis.

(De Boer and others 2009). According to Nahimana and others (2006), the use of an enrichment broth, as in this study, increases the detection rate of MRSA in samples.

Our results demonstrated no correlation between specific types of raw meat and the prevalence of MRSA. These results are in agreement with the findings reported by De Boer and others (2009).

Although recent studies of MRSA in animals in the Netherlands have focused on pigs, the results of the present and other recent studies (Huijsdens and others 2006; Normanno and others 2007; Walther and others 2008; Hammad and others 2012; Alzohairy 2011; Crago and others 2012; Shahraz and others 2012) show that other food-producing animals are also important reservoirs of MRSA.

Contamination of the raw meats and fish could be traced back to abattoirs and aquaculture systems, respectively, due cross-contamination of the meat at some point during processing. Also, antibiotics used to promote animal growth can induce resistance in bacteria and have the potential to generate residues in animal tissue and meat products (Lipsitch and others 2002). This finding demonstrates the importance of selecting suppliers of meat products and of warning these suppliers about the indiscriminate use of antibiotics in animal health.

Unfortunately, we did not successfully investigate the use of antibiotics in animal husbandry. Thus, further studies are necessary to clarify differences in the MRSA contamination of meats from various countries or regions and the potential relationship with the use of antibiotics in animal husbandry.

In the preparede food, the presence of MRSA indicates that cross-contamination occurred after cooking by an asymptomatic food handler or by utensils used in the distribution of the meals because the thermal processing was sufficient to kill the microorganism.

Dishes prepared with fish presented a higher frequency of contamination by MRSA in this study. The cage-cultured tilapia (*Oreochromis niloticus*) investigated were purchased in frozen fillet (86.7%) form. All of the fish samples contaminated by MRSA were fish fillets.

Certainly, many factors are responsible for variations in prevalence of MRSA, and mostly, the neglected hygienic practices were the main factors. Various studies have demonstrated that the hygienic-sanitary profile of food handlers is often unacceptable in terms of health status, personal hygiene practices, and habits, raising the risk of cross contamination in the handled food (Campos and others 2009; Hammad and others 2012; Kamal and others 2013; Ferreira and others 2014).

In Brazil, recent study performed by Ferreira and others (2014) in the same hospitals where was conducted the current study demonstrated that 28.6% of food handlers were colonized with MRSA.

Given the recent emergence of livestock-associated MRSA strains, such as ST398 (Fitzgerald 2012), it is likely that no single explanation will suffice and that MRSA represents a continuously emergent phenomenon driven by multifactorial interactions between the classic triad of host, pathogen, and environment (Mediavilla and others 2012).

Conclusions

The high prevalence of MRSA among the tested meats, mainly in pork and fish, and the presence of the microorganism in prepared foods highlighted the necessity of enforcing hygienic practices within hospital kitchens. In the future, the molecular

and ecological characterization of isolated MRSA strains must be performed to determine the origin of MRSA contamination. 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 in the hospital environment, to clarify the possible health hazards for consumers related to the presence of MRSA in foods, and to determine adequate measures for MRSA control. Furthermore, restrictive policies on antibiotic use in animal health are necessary for the control of MRSA.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado da Bahia—FAPESB (Government of Bahia, Brazil). The authors also thank the chairmen and nutritionists of the public hospitals of Salvador, BA, Brazil, who approved the investigation.

Conflicts of Interest

The content of this report solely reflects the opinions of the authors, and we report no conflicts of interest. FAPESB did not play a role in the research or writing of the paper.

Authors' Contributions

Wellington L.R. Costa participated in the execution of the analysis and collaborated in the interpretation of the results and the preparation and writing of the manuscript. Jeane S. Ferreira was responsible for the recruitment of participants and the collection and analysis of data. Ellayne S. Cerqueira collaborated in the sample collection and in the execution of the analysis. Joelza S. Carvalho collaborated in the sample collection and in the execution of the analysis. Lucimara C. Oliveira collaborated in the sample collection and in the execution of the analysis. Rogeria C.C. Almeida supervised the entire study and was responsible for the financial support offered by Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and for the preparation, writing, and editing of the research paper. All authors critically reviewed the manuscript and approved the final version.

References

Alzohairy MA. 2011. Colonization and antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia. J Bacteriol Res, Victoria Island 3:63–8.

Barber M. 1961. Methicillin-resistant staphylococci. J Clin Pathol 14:385–93.

Campos AKC, Cardonha MAS, Pinheiro LBG, Ferreira NR, Azevedo PRM, Stamford TLM. 2009. Assessment of personal hygiene and practices of food handlers in municipal public schools of Natal, Brazil. Food Contl 20:807–10.

Chambers HF. 1997. Methicillin-resistance in staphylococci molecular and biochemical basis and clinical implications. Clin Microbiol Rev 10:781–91.

Crago B, Ferrato C, Drews SJ, Svenson LW, Tyrrell G, Louie M. 2012. Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in food samples associated with foodborne illness in Alberta, Canada from 2007 to 2010. Food Microbiol, Lond 32:202–5.

De Boer E, Zwartkruis-Nahuis JTM, Wit B, Huijsdens XW, de Neeling AJ, Bosch T, van Oosterom RAA, Vila A, Heuvelink AE. 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Intl J Food Microbiol 134:52–6.

DeLeo FR, Chambers HF. 2009. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. J Clin Invest 119:2464–74.

Ferreira JS, Costa WLR, Cerqueira ES, Carvalho JS, Oliveira LC, Almeida RCC. 2014. Food handler-associated methicillin-resistant *Staphylococcus aureus* in public hospitals in Salvador, Brazil. Food Contl 37:395–400.

Fitzgerald JR. 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends Microbiol 20:192–98.

Gelatti LC, Bonamigo RR, Becker AP, d'Azevedo PA. 2009. Staphylococcus aureus resistentes à meticilina: disseminação emergente na comunidade. Anais Brasileiros de Dermatologia 84:501–16.

Hammad AM, Watanabe W, Fujii T, Shimamoto T. 2012. Occurrence and characteristics of methicillin-resistant and—susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish. Intl J Food Microbiol 156:286–9.

Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck MEOC, Pluister GN, Voss A, Wannet WJB, de Neeling AJ. 2006. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob 5:26–29.

Kamal RM, Bayoumi MA, Abd El Aal SFA. 2013. MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. Food Contl 33:49–53.

Lipsitch M, Singer RS, Levin BR. 2002. Antibiotics in agriculture: when is it time to close the barn door? Proc Natl Acad Sci 99:5752–4.

Lozano C, López M, Gómez-Sanz E, Ruiz-Larrea F, Torres C, Zarazaga M. 2009. Detection of methicillin-resistant *Staphylococcus aureus* ST398 in food samples of animal origin in Spain. J Antimicrob Chemother 64:1325–6.

Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. 2012. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Curr Opin Microbiol 15:588–95.

Morcillo A, Castro B, Rodríguez-Álvarez C, González JC, Sierra A, Montesinos MI, Abreu R, Arias A. 2012. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in pigs and pig workers in Tenerife, Spain. Foodborne Pathogens Dis 9:207–10.

Nahimana I, Francioli P, Blanc DS. 2006. Evaluation of three chromogenic media (MRSA ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12:1168–74.

Normanno G, Corrente M, La Salandra G, Dambrosio A, Quaglia NC, Parisi A, Greco G, Bellacicco AL, Virgilio S, Celano GV. 2007. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. Intl J Food Microbiol 117:219–22.

Perillo J, Ceccarelli D, Spagnoletti M, Lollai S, Cappuccinelli P, Colombo MM. 2012. Molecular characterization of enterotoxigenic and borderline oxacillin resistant *Staphylococcus* strains from ovine milk. Food Microbiol 32:265–73.

Sexton T, Clarke P, O'Neill E, Dillane T, Humphreys H. 2006. Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene. J Hosp Infect 62:187–94.

Shahraz F, Dadkhah H, Khaksar R, Mahmoudzadeh M, Hosseini H, Kamran M, Bourke P. 2012. Analysis of antibiotic resistance patterns and detection of *mec*A gene in *Staphylococcus aureus* isolated from packaged hamburger. Meat Sci 90:759–63.

Van Loo I, Huijsdens X, Tiemersma E, De Neeling A, Van de Sande-Bruinsma N. 2007. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis 13:1834–9.

Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965–6.

Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, Brunnberg L. 2008. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. Vet Microbiol, Amsterdã 127: 171–8.