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Antimicrobial Susceptibility and Enterotoxin-Encoding Genes in *Staphylococcus* spp. Recovered from Kitchen Equipment from a University Hospital in Rio de Janeiro, Brazil

Roberta Fontanive Miyahira, Emanoella Araújo Santos, Robson Souza Leão, Angela Corrêa de Freitas-Almeida, and Mara Lucia Queiroz

This study was conducted to determine the occurrence of antimicrobial resistance and enterotoxin-encoding genes (EEGs) in *Staphylococcus* spp. recovered from equipment used to prepare hospital meals, in a university hospital in Rio de Janeiro, Brazil. Sixty samples were collected from semi-industrial equipment (one blender and one mixer) in the hospital's kitchen. Resistance genes and SCC*mec* types were detected by PCR. From the 40 isolates of *Staphylococcus* spp. identified, 8 were *Staphylococcus aureus*. Thirty-two (80%) *Staphylococcus* spp. isolates were resistant to at least one antimicrobial agent. Resistance genetic determinants were detected: *erm* gene (*Staphylococcus epidermidis* [$n=2$]; *Staphylococcus hominis* [$n=1$]), *mecA* gene (*S. epidermidis* [$n=2$]), and *aa(6′)-aph(2″)* gene (*Staphylococcus caprae* [$n=1$], *S. epidermidis* [$n=2$], *S. hominis* [$n=1$], *Staphylococcus pausteri* [$n=1$], *Staphylococcus simulans* [$n=1$], and *Staphylococcus warneri* [$n=1$]). The presence of at least one EEG in 83% ($n=33$) of the isolates was identified. Two strains of *S. epidermidis* were methicillin-resistant *S. epidermidis* (MRSE) and harboring SCC*mec* type IV. *Staphylococcus* spp. contaminated some hospital kitchen's equipment, indicating that hygiene procedures should be improved. Results also indicate that meals can be a vehicle to disseminate multiresistant *Staphylococcus* spp., including MRSE, and *Staphylococcus* with EEGs.

Keywords: hospital meals, *Staphylococcus* spp., kitchen equipment, antimicrobial resistance, enterotoxin

Introduction

The importance of providing wholesome food to hospitalized patients and the deleterious effect that contaminated food may cause to the patient's recovery has been extensively studied.[1–3] Foodborne outbreaks in hospitals may occur due to poor kitchen hygienic condition and lack of training of food handlers.[3]

Food handlers play an important role in food safety, since they may introduce pathogens in the food product during processing, distribution, and transport.[4] According to Köck *et al.*,[5] 41% of nonhospitalized adults harbor *Staphylococcus aureus* in their noses. Borges *et al.*[4] showed that in Brazil, *S. aureus* isolated from meals that were related to a single strain isolated from food handlers suggested that the reason for diet contamination may be a result of food handling.

Hospital equipment has been shown to be contaminated with nosocomial pathogens with most of them being capable of surviving for weeks on healthcare surfaces and may be transmitted directly or indirectly to patients. In some hospitals, contamination surveillance is not implemented, therefore their contamination levels are still unknown.[6,7]

Gram-positive cocci are still a huge threat for public health, especially with the increasing incidence of methicillin-resistant *S. aureus* (MRSA). Methicillin resistance is mainly conferred by the *mecA* gene carried in a family of mobile genetic elements called staphylococcal cassette chromosomes (SCCs). Besides promoting SCC*mec* acquisition, the hospital environment appears to contribute to the genetic diversity generation in the SCC*mec* elements.[8,9]

Given the rapid increase in antimicrobial resistance and the important impact of these multidrug-resistant (MDR) organisms on morbidity and mortality rates, it is paramount to set up regular and precise microbial surveillance programs to offer extensive information on antimicrobial susceptibility patterns in different geographical regions and in different time periods.[10]

Besides antimicrobial resistance, the pathogenicity of *Staphylococcus* spp. is directly related to the capacity of production of staphylococcal enterotoxin and staphylococcal

Departamento de Microbiologia, Imunologia e Parasitologia, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro–UERJ, Rio de Janeiro, Brasil.

enterotoxin-like. The staphylococcal enterotoxins may cause toxic-shock syndrome and food poisoning, in addition to allergic reactions and autoimmune responses. In addition, the staphylococcal enterotoxins may act as potent gastrointestinal toxins and as super-antigens that stimulate proliferation of nonspecific T cells. The staphylococcal enterotoxins are resistant to gastrointestinal proteases and conditions such as freezing, drying, heat treatment, and harsh environment.[11]

Considering the high number of immunocompromised patients inside hospitals, studies on antimicrobial resistance and the presence of enterotoxin-encoding genes (EEGs) in *Staphylococcus* spp. recovered from these environments are very important. Therefore, the aim of this study was to verify the occurrence of *Staphylococcus* spp. isolated from a state university hospital kitchen's equipment, to determine their antimicrobial resistance profiles and to identify the presence of EEGs.

Materials and Methods

Sample analysis

During a 15-week period, a total of 60 samples were collected from semi-industrial equipment (1 blender and 1 mixer) from the kitchen of a tertiary care teaching hospital with 525 beds located in the city of Rio de Janeiro. The kitchen is located inside the hospital and has about 80 m^2. It presents 20 employees, aged between 28 and 60 years, working on alternate days. Samples were collected from surfaces of the equipment, after decontamination processes, with sterile swabs twice a week (one collection per shift) as previously described.[12] Equipment is used to prepare soups, mashed potatoes, paste diets, and juices. The equipment microbiological analysis included isolation of *Staphylococcus* spp. in Baird-Parker Agar (BP-OXOID, Ltd., Basingstoke, Hampshire, England).

Identification of Staphylococcus *spp.*

After storage, strains were inoculated into 3 ml of Brain Heart Infusion (Difco, Becton Dickinson, MD) and incubated at 35°C ± 2°C for 18/24 hr. Growth was plated on Nutrient Agar (BP-OXOID, Ltd.) and incubated at 35°C ± 2°C for 18/24 hr. The bacteria were identified by mass spectrometry analysis (MALDI-TOF MS) according to a standard extraction protocol using formic acid, as recommended by Bruker[13] and by classical methodology.[14]

Antimicrobial resistance

Antimicrobial susceptibility testing (AST) was determined by the disk diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute.[15] The following antimicrobial drugs were tested (BP-OXOID): penicillin (P 10 U), erythromycin (E 15 μg), gentamicin (CN 10 μg), rifampin (RF 5 μg), clindamycin (DA 2 μg), trimethoprim/sulfamethoxazole (SXT 1.25 μg/23.75 μg), tetracycline (TE 30 μg), cefoxitin (FOX 30 μg), and ciprofloxacin (CIP 5 μg). Reference strain *S. aureus* ATCC 25923 was used as control.

Minimum inhibitory concentration (MIC) was determined by broth microdilution method using cation-adjusted Mueller–Hinton broth (Difco Laboratories) for oxacillin (OX), vancomycin (VA), gentamicin (CN), and erythromycin (E) (Sigma-Aldrich, Inc., St. Louis) for all isolates and according to CLSI guidelines.[15] Reference strain *S. aureus* ATCC 29213 was used as control.

Detection of resistance genes

The detection of genes encoding resistance mechanisms was performed by PCR for genes, *ermA, ermB*, *aac(6′)-aph(2″)*, and *mecA*, as previously described.[16–18] Strains isolated by Carneiro *et al.*[19] were used as positive control.

Molecular typing

Characterization of SCC*mec* types I, II, III, IV, and V was carried out, for all methicillin-resistant *Staphylococcus* spp. (MRS) isolates, by multiplex PCR analysis as previously described.[20]

Detection of enterotoxin genes

The enterotoxin genes detection was determined by PCR. Specific primers were used to detect *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu* genes.[21–23] Strains FRI472 (*sed*, *seg*, *sei*, and *sej*), FRI137 (*sec*, *seh*, *sel*, *sem*, *sen*, *seo*, and *seu*), FRI913 (*see*, *sek*, and *seq*), FRI S6 (*seb*), FRI 361 (*ser*), FRI100 (*sea*), and HMPL 280 (*sep*) were used as positive controls, generously provided by D. Doro and T.C.Oliveira at Universidade Federal de Londrina, Brazil.

Results

Out of the 60 samples analyzed, 40 strains of *Staphylococcus* spp. were isolated and identified: 20 from the mixer and 20 from the blender. *S. aureus* was the most frequent species ($n=8$), followed by *S. caprae* ($n=6$), *S. warneri* ($n=6$), *S. epidermidis* ($n=4$), *S. pausteri* ($n=2$), *S. auricularis* ($n=2$), *S. hominis* ($n=2$), *S. kloosii* ($n=2$), *S. haemolyticus* ($n=2$), *S. saprophyticus* ($n=2$), *S. simulans* ($n=2$), *S. cohnii* ($n=1$), and *S. succinus* ($n=1$) (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/mdr).

AST and MIC testing revealed that 80% (32/40) of the isolates were resistant to at least one antimicrobial drug. All *S. aureus* were susceptible to oxacilin. Among the coagulase negative *Staphylococcus* (CoNS), 25% (8/32) were resistant to oxacillin by MIC determination (Fig. 1) and *mecA* gene was detected in two methicillin-resistant *S. epidermidis* (MRSE) isolates, which also presented resistance to cefoxitin in AST (Table 1). MRSE isolates harbored SCC*mec* type IV. All isolates were susceptible to vancomycin on MIC testing.

Gentamicin resistance was observed in 22% (7/32) of CoNS isolates (Fig. 1). *Staphylococcus* spp. resistant to gentamicin, presented this phenotype in both AST and MIC testing and *aac(6′)-aph(2″)* gene was detected in all these isolates (Table 1).

Twenty percent of the isolates (8/40) showed resistance to erythromycin in both tests, however, one *S. aureus* isolate showed resistance to this antimicrobial only in AST and one *S. kloosii* isolate only in MIC testing (Supplementary Table S1).

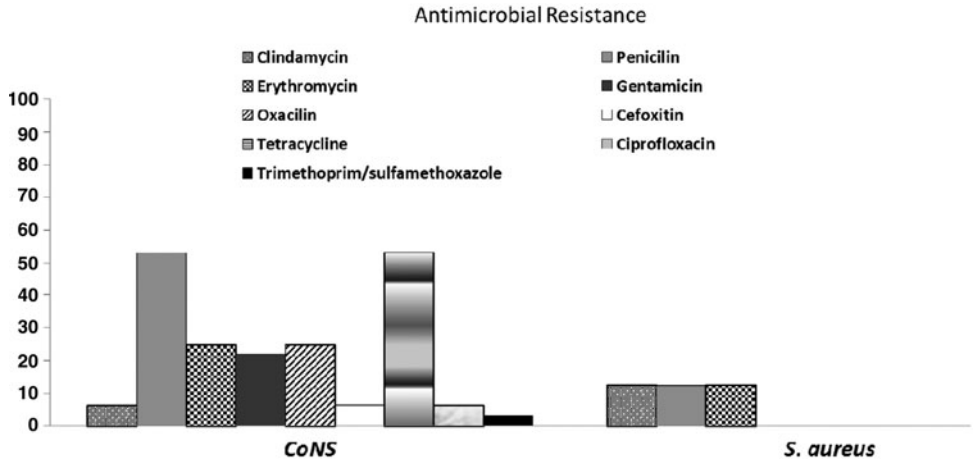

FIG. 1. Percentage of antimicrobials resistance in CoNS and *Staphylococcus aureus* isolated from equipment used in the kitchen of a University Hospital in Rio de Janeiro. CoNS, coagulase negative *Staphylococcus.*

The presence of *ermA* gene was observed in one *S. epidermidis* isolate validated by both tests. The *ermB* gene was observed in one *S. epidermidis* and one *S. hominis* subsp. *hominis* isolate, results confirmed by MIC testing and the AST (Table 1).

There was at least one EEG in 83% (33/40) of the isolates. All *S. aureus* isolates ($n=8$) showed at least one of the EEGs investigated. Two *S. aureus* isolates presented, simultaneously, three enterotoxin genes and five CoNS isolates alike showed more than five enterotoxin genes (Supplementary Table S1). The *seg*, *sei*, *sek*, *sel*, *sem*, and *sen* genes were detected in 9, 14, 2, 7, 6, and 22 CoNS isolates, respectively (Fig. 2). The *seg*, *sei*, and *sen* genes were detected in two, three, and eight *S. aureus* isolates, respectively (Fig. 2).

Discussion

Among the Gram-positive bacteria, staphylococci, streptococci, and enterococci are important causes of both community- and hospital-acquired infections. In Brazil, The SENTRY Antimicrobial Surveillance Program was conducted in four Brazilian cities (São Paulo, Porto Alegre, Brasília, and Florianópolis) and revealed that Gram-positive organisms most frequently isolated from bloodstream infections were *S. aureus*, followed by CoNS.[24,25] The most prevalent species isolated from blood cultures are *S. epidermidis*, *S. haemolyticus*, and *S. hominis*.[25,26] In general, 31.0% of *S. aureus* strains were resistant to oxacillin (MRSA) and the vast majority of MRSA strains were also resistant to clindamycin, ciprofloxacin, and levofloxacin. Almost 80% of CoNS strains were resistant to oxacillin. This organism showed high rates of resistance to most antimicrobial agents.[25]

Another study in Brazil evaluated 160 CoNS isolated between 2002 and 2009 from blood cultures of patients hospitalized in different wards of a teaching hospital in São Paulo. Out of the 160 isolates analyzed, 111 were identified as *S. epidermidis*, followed by *S. haemolyticus* ($n=16$), *S. hominis* subsp. *novobiosepticus* ($n=13$), *S. saprophyticus* ($n=9$), *Staphylococcus capitis* subsp. *urealyticus* ($n=5$), *Staphylococcus schleiferi* subsp. *schleiferi* ($n=3$), and *Staphylococcus xylosus*, *S. warneri*, and *S. caprae* with 1 isolate each. The *mecA* gene was detected in 116 isolates (72.5%).[27]

TABLE 1. MINIMUM INHIBITORY CONCENTRATION, ANTIMICROBIAL SUSCEPTIBILITY, AND PRESENCE OF GENES ENCODING TO ERYTHROMYCIN, GENTAMICIN, AND METHICILLIN RESISTANCE IN STRAINS OF *STAPHYLOCOCCUS* SPP. ISOLATED FROM KITCHEN OF A UNIVERSITY HOSPITAL IN RIO DE JANEIRO

		Antimicrobials					
		Erythromycin		*Cefoxitin*	*Gentamicin*		
Strains	*Species*	*MIC (μg/ml)*	*AST (mm)*	*AST (mm)*	*MIC (μg/ml)*	*AST (mm)*	*Genes*
3A	*S. caprae*	1	30	30	**8***	**12**	*aa(6′)-aph(2″)*
7	*S. epidermidis*	**64**	**6**	**19**	**128**	**12**	*aa(6′)-aph(2″), mecA, erm*A
19	*S. epidermidis*	**64**	**6**	**21**	**16**	**10**	*aa(6′)-aph(2″) mecA, erm*B
2	*S. hominis* subsp. *hominis*	1	30	35	**128**	**6**	*aa(6′)-aph(2″)*
3B	*S. hominis* subsp. *hominis*	**>128**	**6**	25	<0.25	20	*erm*B
16A	*S. pausteri*	0.5	**20**	26	**16**	**8**	*aa(6′)-aph(2″)*
4	*S. simulans*	0.5	30	33	**32**	**13**	*aa(6′)-aph(2″)*
35	*S. warneri*	1	28	30	**16**	**10**	*aa(6′)-aph(2″)*

Bold values represent antimicrobial resistance.
MIC, minimum inhibitory concentration; AST, antimicrobial susceptibility testing.

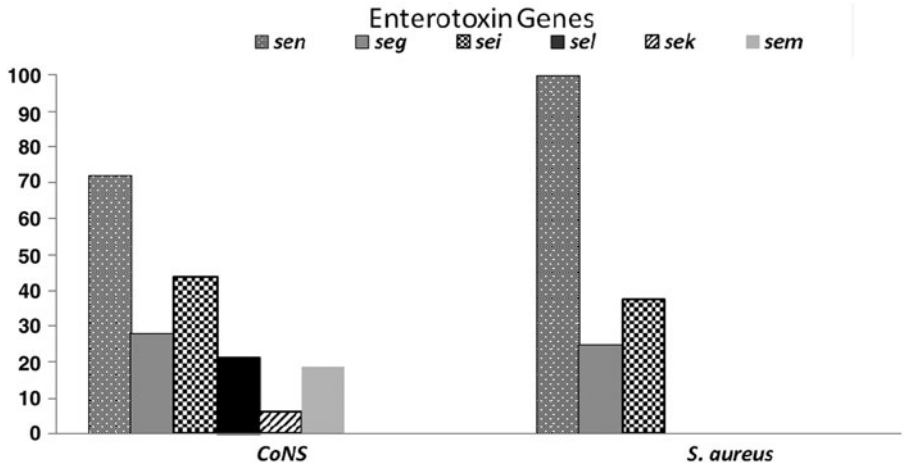

FIG. 2. Percentage of enterotoxin-encoding genes in strains of CoNS and *S. aureus* isolated from equipment used in the kitchen of a University Hospital in Rio de Janeiro.

Evidence has shown that food unsafe handling is a problem for immunocompromised and elderly patients and is not only widespread, but it is also expected to grow.[28] Our results showed the presence of MRSE and MDR *Staphylococcus* spp. in the blender and mixer used in the hospital kitchen. Therefore, the meals prepared with these two pieces of equipment may potentially disseminate bacteria with important resistance mechanisms inside the hospital, especially because some of these foods do not follow a heat treatment afterward. The results of AST indicate high rates of antimicrobial resistance in *S. epidermidis* and *Staphylococcus pasteuri* isolates. Resistance to six antimicrobial agents were found in 50% (2/4) of *S. epidermidis* isolates, including cefoxitin, which is used to define resistance or susceptibility to oxacillin.

MDR is common in *S. epidermidis*, more than 70% of *S. epidermidis* isolates circulating in the hospital environment are resistant to oxacillin, and ~60%, 40–55%, and 55% are resistant to gentamicin, clindamycin, and ciprofloxacin, respectively.[29] Kitchen equipment contamination with *S. epidermidis*, carring the *mecA* gene, is an important epidemiological issue that must be highlighted. As previously mentioned, the food prepared using contaminated equipment by these bacteria can be a vehicle for the dissemination of resistance markers in the hospital environment. As a consequence, we can assume that patients ingesting these contaminated food can become colonized by these bacteria with the subsequent gene transfer to other pathogens or foster the multiresistant isolates emergence, which may lead to unsuccessful therapeutic options.

MRS have acquired and integrated SCC*mec* that carries the *mecA* gene and other antibiotic resistance determinants.[8] Two *S. epidermidis* isolates were found carrying SCC*mec* types IV, both isolated from the mixer. Several studies have shown that SCC*mec* type IV is common among *S. epidermidis*.[9,30] Other data show that SCC*mec* type IV also predominates among community-acquired (CA) MRSE.[31] In another study, carriage of MRSE-SCC*mec* type IV was found to be common in patients at hospital admission, including those with no previous exposure to healthcare environments. MR-CoNS are probably disseminated in the community, notably in people with no previous exposure to healthcare environments. MRSE, the most prevalent species, may act as a reservoir of SCC*mec* type IV for CA-MRSA.[8]

In addition, resistance to five antimicrobials was observed in both strains of *S. epidermidis*, including resistance to erythromycin and gentamicin. In these two strains the erythromycin and aminoglycosides encoding resistance genes were found. MRS has the ability to display high rates of resistance to multiple antimicrobial drugs worldwide.[29,32]

The *erm* genes confer cross-resistance to macrolides, lincosamides, and streptogramin B and can be expressed constitutively or be inducible. According to Lenart-Boron *et al.*[33] the PCR technique allows the detection of antibiotic resistance genes that may not be necessarily expressed. Therefore, the results of the molecular analyses may vary from the phenotypic results. Another important aspect of this study is that genes encoding resistance mechanisms to erythromycin were not found in isolates that were susceptible to AST and MIC techniques.[33] A similar result was observed by Gatermann *et al.*[34] who demonstrated that strains of *Staphylococcus* spp. that were phenotypically susceptible to erythromycin did not show any of the genes for resistance to this antimicrobial. In our study, several isolates were resistant to erythromycin but the gene coding for resistance were absent. The presence of *ermA* and *ermB* genes was evaluated in all strains of *Staphylococcus* spp. However, other genes may be responsible for erythromycin resistance, such as the *ermC* and *msrA* genes.[32] It is believed that the observed phenotype is probably related to the presence of other genes that have not been investigated.

Resistance to aminoglycosides in CoNS isolates is more common than in *S. aureus*.[35] CoNS have been identified as a reservoir for resistance determinants, including genes encoding aminoglycoside-modifying enzymes; moreover, the conjugal transfer of resistance determinants among *S. epidermidis* and *S. aureus* leads to the rapid spread of these determinants in the hospital environment.[36]

Seven strains of CoNS were found resistant in both AST and MIC testing to gentamicin, and all strains showed the *aac(6′)-aph(2″)* gene. *S. caprae* and *S. warneri* were the most frequent species in our study and showed those characteristics. This result is relevant since *S. caprae* is considered a commensal and it becomes a human pathogen in many CA or/and hospital-acquired infections.[37] Although

S. warneri rarely causes disease in healthy people, there are reports of *S warneri* infection in immunocompromised patients with invasive treatments or medical device implants.[38,39]

In this study, the CoNS isolates showed resistance to a higher number of antimicrobial drugs than *S. aureus* isolates. The food prepared using the equipment contaminated by bacteria, showing the resistance profiles found, is a great concern since the food is often not heated after being processed in such equipment. Furthermore, the presence of pathogens or opportunistic bacteria in foods to be served to immunocompromised patients may be harmful.

Antimicrobial resistance aside, food outbreaks by *Staphylococcus* spp. are generally related to food contamination by food handlers or contaminated surfaces after the heat treatment. The importance of food contamination by *Staphylococcus* spp. relates to their ability to produce enterotoxins. In this study the presence of at least one EEG was observed in 83% (33/40) of the isolates studied. The *seg*, *sei*, *sek*, *sel*, *sem*, and *sen* enterotoxin genes were observed in 28%, 43%, 5%, 18%, 18%, and 78% of the isolates, respectively. The eight *S. aureus* isolates found had at least one enterotoxin gene, within the studied ones, whereas the enterotoxin genes studied were found in 25 isolates of the CoNS. Because prepared foods do not undergo heat treatment after use of the contaminated equipment, it may enable the proliferation of *Staphylococcus* spp. isolates in the food and consequent production of enterotoxin, since time between the preparation and distribution can be very long. Furthermore, it is known that staphylococcal enterotoxins have heat tolerance capacity.[40]

The present results confirmed the toxin profiles of *S. aureus* isolates, since all *S. aureus* isolates showed some staphylococcal enterotoxin searched. However, these findings also indicate that CoNS isolates have EEG. For a long time, *S. aureus* was considered the only pathogenic species of the genus, whereas the CoNS were classified only as contaminants. The reason for this is that the potential of enterotoxigenic in CoNS isolates had much controversy in the past. However, many studies have demonstrated that CoNS isolates not only have EEG but also produce clinically significant toxin concentrations.[41,42]

Significant contamination was found (colony counts above 2×10 CFU/cm^2 in 70% of the equipment[12]) on the equipment used to prepare meals to hospitalized patients. These findings showed that good hygiene practices (GHP), especially handling the kitchen equipment, should be improved. A report of these results was sent to the Division of Nutrition of the University Hospital and GHP control measures were reviewed. Moreover, training was given to the food handlers, aiming to prevent cross-contamination within the kitchen environments and equipment.

The detection of *Staphylococcus* spp., isolates with high MIC testing to gentamicin, erythromycin, and oxacillin and the detection of *ermA*, *ermB*, *aac (6′)-aph (2″)*, and *mecA* genes turns meals into a potential vehicle to spread important resistance markers inside hospitals. Additionally, the isolation of coagulase positive *Staphylococcus* (CoPS) and CoNS isolates harboring EEG in equipment used to prepare meals is an additional complication in this scenario. Dissimilation and spread of potential harmful bacteria, such as *Staphylococcus* spp. in hospitals is an important clinical issue, especially hospital-associated bacteria containing genes coding for antimicrobial resistance and toxins, which are a potential threat to successful treatment in imunocompromised patients. Considering that there are few reports in the literature on the role of contamination of hospital diets with MDR staphylococcal strains and with genes for the production of enterotoxins, further studies are suggested to elucidate this subject.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Mara Lucia Queiroz, PhD
Departamento de Microbiologia
Imunologia e Parasitologia, Faculdade de Ciências Médicas
Universidade do Estado do Rio de Janeiro
Avenida 28 de Setembro
87-Fundos
3° andar. Vila Isabel
Rio de Janeiro CEP 20.551-030
Brazil

E-mail: qmara6@gmail.com