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Food handler-associated methicillin-resistant *Staphylococcus aureus* in public hospitals in Salvador, Brazil

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ABSTRACT

The aims of this study were to evaluate the presence of methicillin-resistant *Staphylococcus aureus* on the hands and anterior nares of food handlers in public hospitals in the city of Salvador, Bahia, in north-eastern Brazil and to evaluate the effectiveness of antiseptics for controlling contamination. Swabs from the nose and hands were collected from 140 food handlers in ten public hospitals, and methicillin-resistant *S aureus* (MRSA) isolates were confirmed by assessing their growth on selective media, coagulase testing and evaluating their antibiotic susceptibilities. Seventy (50.0%) food handlers were colonized with coagulase-positive *Staphylococci* on their hands and/or nares, and 40 (28.6%) food handlers were colonized with MRSA. The evaluation of susceptibility to the most commonly used anti-MRSA drugs demonstrated that 72.9% of the isolates from the handlers' hands and 82.5% of the isolates from the anterior nares showed resistance to vancomycin. The presence of MRSA was not correlated with the specific job function of the food handlers ($p > 0.05$). The logistic regression analysis of the antimicrobial activity of antiseptics against MRSA isolates indicated that 2% chlorhexidine had a significantly higher removal rate than those of alcohol gel and 10% PVP-I (iodophor) ($p < 0.05$); only 2.2% of the MRSA strains were resistant to chlorhexidine.

1. Introduction

Staphylococcus aureus is a common member of the natural microbiota of human skin and the nasal passages (Hanson et al., 2011). The main reservoir of *Staphylococci* in humans is the nostrils, although *Staphylococci* can also be found on the hands. As a potential pathogen, it may adversely affect human and animal health by causing severe necrotic lesions, abscesses, endocarditis and bacteremia (Gelatti, Bonamigo, Becker, & d'Azevedo, 2009; Kamal, Bayoumi, & Abd El Aal, 2013; Kumari, Mohapatra, & Singh, 2008).

S. aureus generally produces "coagulase", an enzyme that causes clot formation whereas most other *Staphylococcus* species are coagulase-negative. Although *S. aureus* is usually coagulase-positive, some strains may be atypical in that they do not produce coagulase (Saravanan & Nanda, 2009).

A methicillin-resistant strain of *S. aureus* (MRSA) has recently emerged as a serious life-threatening infective agent that does not respond to many antimicrobial treatments (Klevens et al., 2007). The emergence and rapid spread of this organism has created important new challenges for infection prevention and control services in hospitals and other health care facilities (Kumari et al., 2008; Salmenlinna, Lyytikainen, & Vuopio-Varkila, 2002).

A study conducted in England and Wales (Ellington, Yearwood, Ganner, East, & Kearns, 2008) reported an increase in the number of deaths attributable to MRSA infection. These infections can be expensive in terms of antibiotic therapy, isolation facilities and materials and the length of hospital stays (Kumari et al., 2008).

S. aureus isolated from patients may be classified according to two criteria: by the origin of the isolate and by the level of β-lactam resistance. Hospital-acquired (HA) MRSA generally produces enterotoxin and toxic-shock syndrome toxin, and CA-MRSA produces exfoliative toxin and Panton-Valentine leukocidin (Hososaka et al., 2007).

MRSA synthesizes a penicillin binding protein (PBP2a or PBP2′) encoded by the *mec*A gene on a mobile genetic element (Staphylococcal cassette chromosome *mec*, *SCCmec*) that counteracts the inhibitory effect of β-lactam antibiotics (methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin) by preventing them from binding to cell wall proteins (Hososaka et al., 2007; Kamal et al., 2013; Saravanan & Nanda, 2009).

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Until the early 1980s, MRSA reports consisted of isolated cases; in 1982, epidemic MRSA strains were described as multi-resistant strains with a special capacity to colonize patients and staff and to cause widespread infection outbreaks (Rana & Shrivastava, 2011). According to Hammad, Watanabe, Fujii, and Shimamoto (2012), MRSA and methicillin-resistant coagulase-negative *staphylococci* are acknowledged as zoonotic multidrug-resistant pathogens that cause nosocomial infections and community-acquired diseases in humans, and infections in many animal species.

In the 1990s, reports of community-associated MRSA (CA-MRSA) infections among healthy individuals began to appear, and these infections were shown to be associated with genetically distinct lineages of MRSA (Kumari et al., 2008; Mediavilla, Chen, Mathema, & Kreiswirth, 2012; Salmenlinna et al., 2002).

In contrast to health care-associated (HA)–MRSA strain types, there is evidence that CA-MRSA strain types frequently spread from person to person in households. Although the transmission of HA-MRSA may occur via asymptomatic carriers, less is known about MRSA and methicillin-susceptible *S. aureus* (MSSA) dissemination in community settings (Miller et al., 2012).

Because *S. aureus* is highly prevalent in food and food environments, MRSA may follow the same transmission pattern. Many studies have identified MRSA in retail meat products (De Boer et al., 2009; Hanson et al., 2011; Lim et al., 2010; Lozano et al., 2009; Van Loo et al., 2007), raw milk and dairy products (Kamal et al., 2013), and raw and ready-to-eat fish (Hammad et al., 2012). In addition, a specific MRSA strain (CC398) has been linked with various food animals and people in contact with them (Kamal et al., 2013).

The danger posed by food handlers in the transmission of food poisoning and other gastrointestinal disorders has long been recognized. In hospitals, where many patients may have impaired resistance to infections, the consequences of contaminating food with bacterial pathogens can be particularly serious (Oteri & Ekanem, 1989).

Many hospital food handlers are most likely not aware of the tremendous threat they pose to patients. Thus, the role of food handlers in food preparation is crucial in determining the hygienic status of the final product; poor handling increases the likelihood of contamination by human-borne microbes, including multidrug-resistant and/or enterotoxigenic staphylococci (Kamal et al., 2013).

Strategies to control and prevent the spread of MRSA include the early identification of positive patients through screening, patient isolation, hand hygiene, nasal and skin decontamination, and adequate cleaning and decontamination of clinical areas (Sexton, Clarke, O'Neill, Dillane, & Humphreys, 2006). Hand hygiene (i.e., hand washing with soap and water or using a waterless, alcohol-based hand rub) has long been considered one of the most important infection control measures for preventing health care-associated infections. However, compliance by health care workers' compliance with recommended hand hygiene procedures has remained unacceptable, with rates generally below 50% in terms of the total number of hand hygiene opportunities (Green et al., 2006, 2007).

Biocides (antiseptics, disinfectants and preservatives) and other antimicrobial agents play an important role in limiting the potential sources of infection and have yet to be proven effective in decreasing the infection rates (Poole, 2002). Biocides have been used in various forms for centuries. Arguably the most important antiseptic agents introduced since 1945 are biguanides (chlorhexidine, alexidine and polymeric forms), amphoteric surfactants, bisphenols (triclosan, aldehydes), CRAs (isocyanurates), iodine-releasing agents (iodophors), isothiazolones and peracetic acid.

The objectives of the present study were to evaluate the presence of methicillin-resistant *S. aureus* on the hands and nares of food handlers in public hospitals in the city of Salvador in northeastern Brazil and to evaluate the use of antiseptics in controlling contamination.

2. Materials and methods

2.1. Assessment of the presence of coagulase-positive Staphylococci on hands and nares

2.1.1. Sampling and laboratory procedures

We screened 463 food handlers registered in 10 of the 14 public hospitals in the city of Salvador, Bahia, in northeastern Brazil. Of these, 247 agreed to participate in the study (response rate of 51.2%) and gave subscribing to the free and informed consent; among of those who agreed to participate, 140 (56.68%) were randomly selected for the sample.

The samples were aseptically collected using swabs moistened with Stuart medium. Individual swabs were rubbed onto the hands and anterior nares of the food handlers during food preparation and were transported to the laboratory in an insulated cold box filled with ice. The same swab was inserted into each nostril, rotated for 5 s and immediately placed in the Stuart medium. In the laboratory, the swabs were vortexed for 30 s, transferred to tubes containing trypticase soy broth (TSB, DIFCO, Detroit, MI, USA) in a class II biosafety cabinet (Labconco Corporation, Labconco Purifier Class IIb, Total Exhaust, model 36210-04, certified ISO 9002, Kansas City, MO, USA) and incubated for 24 h at 37 °C. A loop of liquid was removed from the cultures, streaked onto mannitol salt agar (MHA, HIMEDIA, São Paulo, SP, Brazil) plates and incubated at 37 °C for 24 h. Yellow or off-white colonies, indicating mannitol fermentation (i.e., presumptive coagulase-positive *Staphylococci*), were confirmed by Gram staining, catalase testing and coagulase testing using the Staphclin latex test (Laborclin, Interlab, São Paulo, SP, Brazil) (adapted from Souza & Santos, 2009). One *S. aureus*-positive control sample (ATCC 25923) and one uninoculated negative control sample were used for each set of analyzed samples.

2.2. In vitro susceptibility to antibiotics testing

Cells were grown overnight at 35 °C in TSB, suspended in saline solution and adjusted to a 0.5 point in the McFarland scale (c.a. 10^8 CFU). The suspension of cells was inoculated with a swab in Mueller–Hinton agar (HIMEDIA, São Paulo, SP, Brazil). The bacterial growth was recorded after 18–24 h of incubation at 35 °C. One *S. aureus* (ATCC 25923) negative control and one methicillin-resistant *S. 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 (CLSI, 2011). 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. Assessment of the effectiveness of antiseptics against MRSA strains

A lightly modified diffusion agar method was used with antibiotic disks substituted for antiseptic disks. MRSA cells were grown overnight at 35 °C in TSB, suspended in saline solution and adjusted to a 0.5 on the McFarland scale (c.a. 10^8 CFU). An aliquot of 100 μl of the cell suspension was inoculated with a swab in Mueller–Hinton agar, and an aliquot (10 μl) of each antiseptic with 70% alcohol gel (Protex; Provider Indústria e Comércio LTDA, Louveira, SP, Brazil),