Isolation of Methicillin-resistant *Staphylococcus aureus* and *S. epidermidis* from Handles of Shopping Trolleys in Supermarkets In Malaysia

Tong Soon Cheah, Ph D¹, Roseliza, B. Sc.² Khoo Lean Looi² & Nafizah ²

¹ Faculty of Medicine, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh, Malaysia.
² Veterinary Research Institute, Ipoh, Malaysia.

Abstract

A total of 11 isolates of *Staphylococcus aureus* and 70 isolates of *S. epidermidis* were obtained from 340 handles of shopping trolleys of two supermarkets in Ipoh, Malaysia. The methicillin-resistant phenotype of staphylococci was identified with penicillin binding protein (PBP2) latex agglutination test (Oxoid, UK). *Staphylococci* that were positive for PBP2 were used for the detection of *mecA* gene by polymerase chain reaction. Two out of 11 *S. aureus* were positive for *mecA* gene while 2 out of 70 *S. epidermidis* had the *mecA* gene. This the first report on isolation of methicillin-resistant *S. aureus* and *S. epidermidis* from handles of shopping trolleys in Malaysia.

Keywords: Shopping trolley handles, MRSA, MRSE.

Introduction

Interaction between bacteria and humans can lead to infection. Contact can happen in a number of different ways. Humans might directly contact the bacteria, or might contact the organisms via an indirect route involving inanimate objects (fomites). Numerous studies on the contamination of methicillin-resistant *Staphylococcus aureus* (MRSA) on objects in healthcare setting have been conducted and those are tourniquets, doctors and nurses’ pens, mattresses, television sets, faucet handles, over-bed tables, blood pressure cuffs, computers’ keyboards, room door handles, immersion bathtubs, chairs for shower and stretchers for bathroom (Berman et al., 1986; Boyce et al., 1997; French et al., 1998; Blythe et al., 1998; Stacey et al., 1998; Bures et al., 2008; Devine et al., 2001; Oie et al., 2002; Oie et al., 2005). Contamination of methicillin-resistant coagulase-negative staphylococci and MRSA on touch surfaces in public transport have also been reported by other investigators in Belgrade, Portugal and Japan (Stepanovic et al., 2008; Simoes et al 2011; Iwao et al., 2012). In Malaysia, MRSA has been reported in hospitals, among healthy carriers, pigs and pig handlers and doctors’ neckties (Cheong et al., 1996; Mariana et al., 2008; Neela et al., 2009; Koh et al., 2009).

However, there are no reports on contamination of MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) on the handles of shopping trolleys. This paper reports for first time the isolation of MRSA and MRSE from handles of shopping trolleys in supermarkets in Malaysia and this information maybe of public health importance.

Materials and Methods

Isolation of bacteria

A total of 11 isolates of *S. aureus* and 70 isolates of *S. epidermidis* were obtained from 340 handles of shopping trolleys in two supermarkets in Ipoh, Malaysia. The isolates were identified with standard microbiological procedures. All isolates were identified as *S. aureus* and *S. epidermidis* based on morphology, detection of clumping factor and protein A using a rapid slide agglutination test (BactiStaph, remel, USA) and fermentation of mannitol (Kloos and Bannerman, 1999). The methicillin-resistant phenotype of staphylococci was identified with penicillin binding protein (PBP2') latex agglutination test (Oxoid, UK) according the manufacturer’s instructions. Staphylococci that were positive for PBP2’ was used for the detection of *mecA* gene by polymerase chain reaction (PCR).

DNA preparation

A single colony of bacteria was picked using a sterile stab loop and mixed with 30 µL of nuclease-free water in a microcentrifuge tube. The mixture was heated at 95°C for 10 minutes and left at ambient temperature for 2 minutes after which the suspension was centrifuged at 13,000 rpm for 1 minute. The supernatant was used as DNA template in the PCR.

PCR

The final volume of PCR mixture was 50.0 µL containing 27.5 µL nuclease-free water, 10.0 µL 5× MyTaq Polymerase Reaction Buffer, 0.5 µL Taq Polymerase, 1.0 µL of forward primer, MR3 (10 µM), 1.0 µL of reverse primer, MR4 (10 µM) [Taweeporn et al., 2002] and 10.0 µL DNA template. Methicillin-resistant *S. epidermidis* (ATCC 51625) and methicillin-sensitive *S. aureus* (ATCC 31885) were included in the PCR as positive and negative controls. The internal control for the PCR was nuclease-free water without the
DNA template in the PCR mixture. The PCR programme was performed with heating at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds. A total of 30 cycles was performed and at the 30th cycle, final extension was conducted at 72°C for 5 minutes.

The amplification product was separated on 1% agarose gel containing Gel Red stain and was observed using an image capturing machine with ultra-violet light at the wavelength of 365 nm. The bands formed were compared with a 100 bp DNA ladder marker.

Results and Discussion

DNA fragments of the expected size (533bp) were amplified from the the positive control as well as the 4 staphylococcal isolates using the PCR (Fig. 1). The mecA gene was detected in 2 out of 11 Staphylococcus aureus and 2 out of 70 Staphylococcus epidermidis.

Staphylococcus epidermidis isolated from the blood of hospitalized patients were often considered contaminants (Raad et al., 1998). Recently, however, S. epidermidis has emerged together with Staphylococcus aureus as a frequent aetiologic agent of infections associated with catheters and indwelling medical devices (Arciola et al., 2001). The occurrence of methicillin-resistant Staphylococcus aureus infections outside the healthcare setting has also been reported and can pose an important public health problem (Eguia and Chambers, 2003; Weber, 2005; Zetola et al., 2005).

The persistence of clinically relevant bacteria on dry inanimate surfaces was reviewed by Kramer et al., 2006 in which Acinetobacter spp. survived 3 days to 5 months, S. aureus including MRSA persisted from 7 days to 7 months. An investigation on the survival of Enterococci and Staphylococci on hospital fabrics and plastic showed that these bacteria can survive for days to months (Neely and Maley, 2000). In a model on transmissibility of fomites to skin based on contaminated fomites pressed against sterile pigskin for 3 seconds suggests that vinyl and plastic building block toys transmitted MRSA for the longest time, with bacterial recovery lasting more than 2 months after the initial inoculation (Desai et al., 2011). The same authors speculated that nonporous fomites allow bacterial localization only along the surface, allowing increased exposure during contact with the skin. The humid tropical conditions in Malaysia would be conducive for the survival of these bacteria on handles of shopping trolleys which are made of plastic materials. Similarly it was also postulated by Simoes et al., 2011 that the warm temperatures and appropriate humid conditions in Portugal may have contributed to the survival of Staphylococcus aureus on handrails of buses.

It had been shown by data from outbreaks of community-acquired MRSA infection which suggest that skin-skin and skin-fomite contact represent important and common alternative routes of acquisition of the infecting strain (Miller and Diep, 2008). In Malaysia the fomite, such as shopping trolleys handles which are made of plastic materials can become contaminated with pathogenic bacteria and then serve as a reservoir for transmitting these microorganisms to the humans by some form of contact as it was reported that sharing of contaminated sensor wires among fencers has been linked to transmission of MRSA among these individuals (Anon, 2003). Transmission of Staphylococcus aureus, for both susceptible and antimicrobial resistant strains, usually occurs through contact with a person who has a draining lesion or asymptomatic carriage of Staphylococcus aureus (Anon, 2003). Contamination of MRSA and MRSE on the handles of supermarket trolleys could occur as shopping malls are patronized by peoples from all walks of life and from different places such as healthcare centres. Bacterial contaminated trolley handles might be a potential source of infection particularly to very young children who may come in contact with these contaminated objects as some parents place their child in the trolley while shopping in supermarkets. Immunocompromised individuals might also become infected with pathogenic bacteria when they come in contact with contaminated trolley handles in shopping malls. Serious complications can arise from chronic coagulase-negative staphylococci (CoNS) infections, particularly in immuno-compromised, hospitalized, and very young and old patients (Otto, 2013). Staphylococcus epidermidis is the most frequently encountered CoNS species on human skin and by far the most frequent source of CoNS infections (Otto, 2009).

Conclusion and Recommendations

The prevalence of MRSA and MRSE on handles of shopping trolleys might contribute towards the spread of community-acquired MRSA and MRSE. Further studies involving other commonly hand touched fomites in shopping malls are therefore needed so as to elucidate the role of these objects besides humans in the spread of these methicillin-resistant staphylococci.

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References

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Figure 1. DNA from *S. aureus* and *S. epidermidis* isolated from shopping trolley handles in Malaysia was amplified with primers specific for *mecA* gene generating 533 bp fragment. M:100 bp DNA ladder, lane 1 and 2: reaction with DNA from *S. epidermidis*, lane 3 and 4: reaction with DNA from *S. aureus*, lane 5: methicillin-sensitive *S. aureus* (ATCC 31885) corresponding to negative control, lane 6: methicillin-resistant *S. epidermidis* (ATCC 51625) corresponding to positive control, lane 7: nuclease-free water (internal control).