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ScienceDirect
Diagnostic Microbiology and Infectious Disease 57 (2007) 145–151
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Molecular epidemiology and household transmission of community-associated methicillin-resistant *Staphylococcus aureus* in Hong Kong

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Received 26 April 2006; accepted 6 August 2006

Abstract

This study evaluated the clinical and epidemiologic features of individuals with community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Hong Kong from January 2004 through December 2005. Twenty-four episodes of skin and soft tissue infections and 1 episode of meningitis due to CA-MRSA were identified. CA-MRSA infections or carriage was found in 6 (13%) of 46 household contacts. A total of 29 isolates were analyzed by the *Staphylococcus* cassette chromosome *mec* (SCC*mec*) typing, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing. In addition, polymerase chain reaction detection of the genes encoding Panton-Valentine leukocidin was also carried out. It was observed that 24 had SCC*mec* IV/IVA and 5 had SCC*mec* V, and 23 were *pvl* positive. PFGE analysis clustered all except 1 isolate into 3 pulsed-field types (PFTs), HKU100 through HKU300. The HKU100 isolates had genotype ST30-IV identical to the Southwest Pacific clone. The HKU200 isolates belonged to ST59-V and were multiresistant, including an *ermB*-mediated macrolide resistance trait, which is characteristic of the predominant CA-MRSA clone in Taiwan. The HKU300 isolates had unique features (ST8, Panton–Valentine leukocidin negative, and SCC*mec* IVA) typical of CA-MRSA in Japan. In conclusion, CA-MRSA has a propensity to spread within families. Our findings showed that CA-MRSA strains in Hong Kong have diverse genetic backgrounds.

Keywords: *Staphylococcus aureus*; Methicillin; Antimicrobial resistance epidemiology; MRSA; Community

1. Introduction

Staphylococcus aureus is a common cause of community- and healthcare-associated (HA) infections, such as skin and soft tissue infections (SSTIs), pneumonia, and bacteremia. Traditionally, methicillin-resistant *S. aureus* (MRSA) infections are confined to individuals with established risk factors, for examples, nursing home residents, hospitalized individuals, patients submitted to operations, and using indwelling medical devices. Since the 1990s, there were increasing reports of MRSA infections in healthy individuals from the community without established risk factors. These infections occurred in the community and have been called *community-associated MRSA* (CA-MRSA). By means of genotypic studies, it has been shown that most of the CA-MRSAs were genetically distinct from the HA-MRSA (Vandenesch et al., 2003). With few exceptions,

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doi:10.1016/j.diagmicrobio.2006.08.003

CA-MRSA strains possess the Panton–Valentine leukocidin (PVL) genes and one of the novel *Staphylococcus* cassette chromosome *mec* (SCC*mec*) elements (types IV and V) (Boyle-Vavra et al., 2005; Kluytmans-Vandenbergh and Kluytmans, 2006; Takizawa et al., 2005). Moreover, CA-MRSA strains are sensitive to most antibiotics but the β-lactams (Zetola et al., 2005). Nowadays, CA-MRSAs are recognized to cause outbreaks in "closed populations", such as aboriginals, contact sports athletes, inmate of correctional services, military recruits, and children attending day care centers (Kluytmans-Vandenbergh and Kluytmans, 2006; Zetola et al., 2005). Recently, they have even been found to spread inside hospitals and are displacing the traditional HA-MRSA as pathogens in nosocomial infections (Seybold et al., 2006).

In the present study, we describe the epidemiologic features of patients with CA-MRSA infections who were reported to a monitoring system in Hong Kong from January 2004 through December 2005. Because information on household transmission of CA-MRSA is limited (Anonymous, 1999; Faden and Ferguson, 2001; L'Heriteau et al., 1999; Wagenvoort et al., 2005), screening of household members was prospectively conducted upon identification of individuals with CA-MRSA infections.

2. Materials and methods

2.1. Study design and case definition

In January 2004, a monitoring group was formed under the coordination of the Department of Health, Hong Kong SAR, and the Centre of Infection at the University of Hong Kong, Hong Kong, China, to conduct a laboratory-based surveillance for CA-MRSA. The participating microbiology network includes 5 public hospital laboratories, 6 private hospital laboratories, and 6 stand-alone community laboratories. These laboratories were estimated to provide inpatient and outpatient service to half of the 6.5 million populations in Hong Kong. All participating laboratories were requested to screen the clinical information in the request forms and to pay attention to MRSA isolates with a non-multiresistant antibiogram. MRSA isolates suspected to be CA-MRSA were referred to the laboratory in the Centre of Infection for molecular testing. In a prospective manner, all clinical and epidemiologic information were validated by ward visits (for inpatients) and/or by phone calls to the doctors in charge and the patients. In the public hospitals, it is part of the infection control routine for nurses to screen patients with a positive MRSA culture. Since January 2005, all hospital microbiologists in Hong Kong were encouraged to report CA-MRSA cases to this monitoring system. An MRSA case was considered to be community associated if it was isolated from an outpatient or an inpatient within 48 h of hospitalization and in which risk factors for HA infections were absent. Risk factors for HA-MRSA included the following: 1) history of hospitalization, surgery, or dialysis within 1 year of the MRSA culture date; 2) presence of any permanent indwelling catheter or percutaneous device (e.g., tracheostomy tube, gastrostomy tube, or urinary catheter) at the time of culture; and 3) residence in a long-term care facility.

In an attempt to assess the extent of household transmission, household members were contacted through the in-charge doctors or directly by the Department of Health. For household members who agreed to be screened, culture swabs were obtained from the anterior vestibule of the nose, axillary skin, and any cutaneous or wound lesions for isolation of MRSA. Specimens were obtained either in the in-charge doctors' office or during a home visit. Household members were defined as individuals living in the same address regardless of family status.

2.2. Bacterial identification and antimicrobial susceptibility

Bacterial colony morphology, Gram stain, and the following tests were used to identify a bacterial isolate as *S. aureus*: latex agglutination (Slidex Staph Plus; bioMerieux, Marcy-l'Etoile, France), tube coagulase, mannitol, ornithine, and DNase (Brown et al., 2005). Antimicrobial susceptibility testing was performed by the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (formerly National Committee on Clinical Laboratory Standards) recommendations (Clinical and Laboratory Standards Institute, 2005). Inducible clindamycin resistance was tested by placing clindamycin and erythromycin disks at center-to-center distance of 25 mm. Flattening of the clindamycin zone adjacent to the erythromycin disk was used to infer inducible macrolide, lincosamide, and type B streptogramin (MLS_B) resistance (Clinical and Laboratory Standards Institute, 2005). Methicillin resistance was screened by oxacillin and cefoxitin discs and confirmed by *mecA* polymerase chain reaction (PCR) (Clinical and Laboratory Standards Institute, 2005; Ho, 2003).

2.3. MRSA screening

Culture swabs from the household contacts were processed as described previously (Que et al., 2003). A broth enrichment step (mannitol–salt medium, Oxoid; Hampshire, UK) was used, followed by plating onto oxacillin (6 μg/mL) blood and mannitol salt agar.

2.4. Pulsed-field gel electrophoresis

Bacterial DNA was extracted for analysis by a rapid procedure (Chang and Chui, 1998). *Sma*I-restricted fragments were resolved in 1% SeaKem agarose (Bio-Rad Laboratories, Hercules, CA) for 23 h at 6 V/cm, with pulse times ramping from 15 to 60 s in a CHEF Mapper XA system. The pulsed-field gel electrophoresis (PFGE) patterns were analyzed with Gelcompar II software (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were created by means of the Dice coefficient and the unweighted pair-group mean arithmetic (UPGMA) method. Band position and optimization were set at 1.5% and 1%, respectively. A similarity coefficient of 80% was selected to define lineages