Original Article

Genotypic Characterization of Methicillin-resistant *Staphylococcus aureus* Isolated from Pigs and Retail Foods in China*

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Abstract

Objective To investigate the genotypic diversity of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from pigs and retail foods from different geographical areas in China and further to study the routes and rates of transmission of this pathogen from animals to food.

Methods Seventy-one MRSA isolates were obtained from pigs and retail foods and then characterized by multi-locus sequencing typing (MLST), *spa* typing, multiple-locus variable number of tandem repeat analysis (MLVA), pulsed-field gel electrophoresis (PFGE), and antimicrobial susceptibility testing.

Results All isolated MRSA exhibited multi-drug resistance (MDR). Greater diversity was found in food-associated MRSA (7 STs, 8 *spa* types, and 10 MLVA patterns) compared to pig-associated MRSA (3 STs, 1 *spa* type, and 6 MLVA patterns). PFGE patterns were more diverse for pig-associated MRSA than those of food-associated isolates (40 *vs.* 11 pulse types). Among the pig-associated isolates, CC9-ST9-t899-MC2236 was the most prevalent clone (96.4%), and CC9-ST9-t437-MC621 (20.0%) was the predominant clone among the food-associated isolates. The CC9-ST9 isolates showed significantly higher antimicrobial resistance than other clones. Interestingly, CC398-ST398-t034 clone was identified from both pig- and food-associated isolates. Of note, some community- and hospital-associated MRSA strains (t030, t172, t1244, and t4549) were also identified as food-associated isolates.

Conclusion CC9-ST9-t899-MC2236-MDR was the most predominant clone in pigs, but significant genetic diversity was observed in food-associated MRSA. Our results demonstrate the great need for improved surveillance of MRSA in livestock and food and effective prevention strategies to limit MDR-MRSA infections in China.

Key words: Methicillin-resistant *Staphylococcus aureus*; Antimicrobial susceptibility; Genetic diversity; Pig; Retail food

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that has been identified in the community (community-associated MRSA, CA-MRSA), hospital (hospital-associated MRSA, HA-MRSA), and livestock (livestock-associated MRSA, LA-MRSA), particularly in pigs, and is among the most frequent community- and nosocomial- infections in the world[1-2]. The increasing antimicrobial resistance rates of MRSA have accelerated in recent years, and have posed a serious threat to public health[3-4]. In 2011, the total costs due to CA-MRSA was estimated 1.4 billion to 13.8 billion dollars in the United States[5]. The control and prevention of MRSA is considered as a serious public health challenge[6-7]. In recent years, MRSA has been isolated from retail meat and livestock, including pig, poultry, and cattle meat[8-10]. Additionally, pig serves as an important reservoir for the spread of LA-MRSA[11].

Epidemiologically, the relative prevalence of MRSA lineages and their subtypes appears to vary geographically[12-13]. ST398 was the first identified LA-MRSA sequence type (ST) and is the predominant ST identified in Europe. ST9 is the most predominant MRSA clone among pigs in the Asian countries, and both ST398 and ST5 are relatively more frequent in pigs in North America[14-20]. Specifically, significant geographic variation in the ST398 lineage distribution has been reported in Europe, with *spa* types t108 and t011 is common in Netherlands and *spa* type t034 is predominant in Denmark[13,21-22]. In China, t899 and ST9 are the most prevalent *spa* types in pigs[16].

Different molecular typing methods of MRSA have been used for epidemiological studies because different methods may provide diverse discriminatory powers. For this reason, the use of more than one subtyping method, such as pulsed-field gel electrophoresis (PFGE), multi-locus sequencing typing (MLST), *spa* typing, multiple-locus variable number of tandem repeat analysis (MLVA), is essential to more comprehensively assess the genetic diversity of MRSA and to provide the genetic basis for evolutionary and epidemiological studies of MRSA. This study aimed to explore the phylogenetic distribution and population characteristics of MRSA isolated from pigs and retail foods and evaluate the potential risk presented by MRSA to food safety and human public health.

MATERIALS AND METHODS

Sample Collection

In 2011, a total of 56 MRSA strains were isolated from 961 pig nasal swabs obtained from 49 pig farms located in six provinces, and 15 MRSA strains were isolated from 50,316 retail market food samples located in 32 provinces in China. In detail, two isolates were obtained from ready-to-eat (RTE) vegetable salad, five from cooked meat, two from cooked noodles, three from raw pork meat, two from raw mutton and one from raw beef. The prevalence of *S. aureus* in the samples was determined using the qualitative detection method according to National Food Safety Standards of China document GB 4789.10-2010. Briefly, an aliquot 25 g from each sample was transferred to a sterile glass flask containing 225 mL of 10% saline solution (Land Bridge, Beijing, China). Following homogenization, the solutions were incubated at 37 °C for 24 h; while, the nasal swabs were incubated in a 5 mL 10% saline solution (Land Bridge). Loopfuls of the resulting cultures were streaked onto Baird-Parker Agar plate and Blood Agar plate (Land Bridge), respectively, then incubated at 37 °C for 24-48 h. Putative *S. aureus* isolates were tested for coagulase activity, and were further confirmed using API STAPH test strips (bioMerieux, Marcyl'Etoile, France). Finally, all MRSA isolates were then confirmed by detection of 16S rRNA, *nuc*, and *mecA* by PCR[23]. All confirmed MRSA isolates were stored in brain heart infusion broth with 40% glycerol in a -80 °C freezer before further genotypic characterization.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility of all MRSA isolates against 13 antimicrobials was determined by the broth dilution method and interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI)[24]. These antimicrobials were benzyl penicillin (BEN), oxacillin (OXA), gentamicin (GEN), ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MXF), erythromycin (ERY), clindamycin (CLI), vancomycin (VAN), nitrofurantoin (NIT), rifampicin (RIF), tetracycline (TET), and trimethoprim-sulfamethoxazole (SXT). *S. aureus* ATCC 29213 was used as the control for the antimicrobial susceptibility testing.

DNA Extraction

The frozen strains were cultured in brain heart