American Journal of Infection Control xxx (2015) 1-6

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Frequency-risk and duration-risk relations between occupational livestock contact and methicillin-resistant *Staphylococcus aureus* carriage among workers in Guangdong, China

Xiaohua Ye PhD [1], Weidong Liu MSc [1], Yanping Fan MSc, Xiaolin Wang MSc, Junli Zhou MPH, Zhenjiang Yao PhD *, Sidong Chen PhD *

Guangdong Key Laboratory of Molecular Epidemiology, School of Public Health, Guangdong Pharmaceutical University, Guangzhou, China

Key Words:
Community-acquired
Methicillin-resistant *Staphylococcus aureus*
Human
Livestock

Background: Increasing evidence indicates a strong association between occupational livestock contact and methicillin-resistant *Staphylococcus aureus* (MRSA) carriage. However, it remains unclear whether there are frequency-risk and duration-risk relations between occupational livestock contact and human MRSA carriage.

Methods: A cross-sectional survey was conducted in Guangdong, China, using a multistage sampling method. Participants were interviewed and provided a nasal swab for *S aureus* analysis. All MRSA isolates were genotyped by multilocus sequence typing. The dose-response relation was examined using logistic regression models.

Results: Among the 1,860 participants, 1.4% of controls tested positive for MRSA (characterized as sequence type [ST] 59 and ST7), and 7% of workers with livestock contact tested positive for MRSA (characterized as ST9, ST59, and ST7). There was a 5.31 times increased risk of MRSA carriage corresponding to occupational livestock contact (odds ratio = 6.31; 95% confidence interval, 3.44-11.57) using no contact as reference. We found frequency and short-term duration of occupational livestock contact were associated with increased risk of MRSA carriage in a dose-response manner. These significant trends were observed consistently among workers with occupational pig contact. However, no long-term duration-risk increasing trend was observed for occupational livestock or pig contact.

Conclusion: Our findings suggest that there may be dose-response relations between occupational livestock contact and human MRSA carriage. Nasal MRSA clonal complex 9 is not found in controls, but it is found in workers with livestock contact.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial and community-acquired infections in countries worldwide.[1,2] The epidemiologic history of MRSA has been reshaped since the first livestock-associated methicillin-resistant *S aureus* (LA-MRSA) transmission to humans was described in a young daughter of a pig farmer, suggesting human-animal transmission of community-associated MRSA.[3] LA-MRSA is typically community-associated MRSA. Interestingly, multilocus sequence typing (MLST) of LA-MRSA isolates showed that closely related sequence types (STs) within clonal complex (CC) 398 predominate in North America and Europe, whereas most LA-MRSA isolates in Asia belong to CC9 (ST9 and single-locus variants thereof). Recently, hospital outbreaks of LA-MRSA have been reported.[4,5] Meanwhile, serious invasive infections caused by LA-MRSA have been observed in humans.[6-8] Therefore, the emergence of this new LA-MRSA poses a potential public health risk that warrants close monitoring.

It is noteworthy that most studies on LA-MRSA support a positive association between livestock contact and risk of MRSA carriage.[9-12] However, previous research[9-12] has focused on the

* Address correspondence to Sidong Chen or Zhenjiang Yao, PhD, Guangdong Pharmaceutical University, 283# Jianghai Dadao, Haizhu District, Guangzhou, 510310, China.

E-mail addresses: zhjyao2001@yahoo.com (Z. Yao), chensidong1@126.com (S. Chen).

Funding/Support: This work was supported by the Natural Science Foundation of Guangdong Province (no. S2011010002481), the Innovation Fund of the Department of Education of Guangdong Province (no. 2013KJCX0112), and the Joint Natural Sciences Fund of the Department of Science and Technology and the First Affiliated Hospital of Guangdong Pharmaceutical University (no. GYFYLH201331).

Disclaimer: The funders had no role in study design, data collection and analysis, and interpretation of the data.

Conflicts of interest: None to report.

[1] These authors contributed equally to this work.

0196-6553/$36.00 - Copyright © 2015 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ajic.2015.03.026

relative risk for human MRSA carriage comparing exposure with nonexposure to livestock, and little is known about the dose-response relation between occupational livestock contact and MRSA carriage, or the animal-specific associations. The present study aimed to systemically investigate the prevalence of MRSA carriage in workers with and without occupational livestock contact and to confirm the adverse effects of occupational livestock contact. Furthermore, this study builds on previous literature to examine the possible frequency-risk and duration-risk relations between occupational livestock contact and human MRSA carriage.

METHODS

Ethics statement

The study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and this survey qualified as involving no risks to participants. All participants signed an informed consent form.

Study sample and procedures

This cross-sectional study was conducted in Guangdong Province, China, between November 2013 and November 2014. A multistage sample design was used to obtain a representative sample. First, 4 cities were randomly sampled from the 21 cities in Guangdong Province. Second, in each city, we selected a specific number of livestock-related venues (eg, pig farms, slaughterhouses, wet markets) to reach a respondent sample size of 150 workers with occupational livestock contact (including farm workers, veterinarians, slaughterhouse workers, and butchers) and selected 2 factories to reach a sample size of 300 workers without occupational livestock contact. Butchers were included in our study because butchery in China is a special job field. Butchers go to slaughterhouse to kill pigs from 1 AM-5 AM and go to the meat market to sell pork during the day. When butchers work in slaughterhouses they will have contact with live animals, dead animals, and derived meat products in various stages of processing. Finally, all workers in selected venues were sampled to take part in this study.

Quality control

All interviewers in each area were trained to ensure that the survey was carried out according to protocol and that operation procedures were identical across all areas. After obtaining informed consent, eligible participants were asked to complete a face-to-face survey by trained interviewers. All questionnaires were entered by trained data entry personnel. Quality of data was also assured by using double data entry procedures and a system to automatically detect data entry errors.

Detection of S aureus *and MRSA*

Swabs were collected from each nostril of the participants. Swabs were inoculated into 2 mL of enrichment broth containing 1% tryptone, 7.5% NaCl, 1% mannitol, and 0.25% yeast extract and incubated overnight at 35°C ± 1°C. A loopful of the broth was then plated on mannitol salt agar. Suspect *S aureus* colonies were subcultured to 5% sheep blood plates and incubated at 35°C overnight. Initial identification of *S aureus* was based on gram staining, morphology, and traditional biochemical tests, including catalase, DNase, and tube coagulase tests and then confirmed by polymerase chain reaction (PCR) screening for the carriage of 16S ribosomal RNA and *nuc* genes.[13] All isolates identified as *S aureus* were tested for methicillin resistance using the cefoxitin and oxacillin disk diffusion methods outlined by the Clinical and Laboratory Standards Institute guidelines.[14] *S aureus* isolates with zone sizes <21 mm for cefoxitin and 10 mm for oxacillin were identified as suspect MRSA and further tested by PCR for the *mecA* gene.[13]

Molecular typing of MRSA

All MRSA isolates were molecularly characterized by MLST.[15] MLST was performed on de novo, and alleles and STs were assigned using the MLST database (http://www.mlst.net/). CCs were determined using eBURST version 3 (Department of Infectious Disease Epidemiology, Imperial College London, London, UK; http://eburst.mlst.net) and the stringent group definition (6/7 shared alleles).[16]

Study variables

The main outcome variable was nasal carriage of MRSA. The main independent variable was self-reported occupational livestock contact. Frequency of livestock contact (hours per day) was continuous data and was also categorized into 3 groups: no contact with any types of livestock, ≤7 h/d (below median of contact time among persons with occupational livestock contact), and >7 h/d (above median of contact time among persons with occupational livestock contact). Duration of livestock contact was continuous data and was also categorized into 4 groups: no contact with any types of livestock, <1 year, 1-4 years, and ≥5 years. Short-term duration (≤12 months) of livestock contact was categorized into 3 groups: no contact with any types of livestock, <2.5 months (below median of contact time among persons with short-term contact), and 2.5-12 months (below median of contact time among persons with short-term contact). Occupational contact with pigs, poultry, and other animals was asked separately. Covariates in our study were sex, age, education (elementary school, junior high school, senior high school, and above), personal monthly income (≤¥1000, ¥1001-2000, ¥2001-3000, or ≥¥3001), current smoking (smoked daily or occasionally in the last 30 days for at least 6 months, yes or no), antimicrobial use in the last month (yes or no), and hospitalization in the last month (yes or no).

Data analysis

All data were entered in duplicate into the EpiData version 3.1 database (The EpiData Association, Odense Denmark). The frequency-risk and duration-risk relations between occupational livestock contact and MRSA carriage were examined using univariable and multivariable logistic regression models. Linear trends of livestock contact were assessed by modeling contact as continuous variables (arithmetic or logarithmic scale) or categorized variables in logistic regression models. We defined a 2-sided *P* value of ≤.05 as being of statistical significance. All statistical analyses were conducted using Stata version 13.0 (StataCorp, College Station, TX).

RESULTS

Characteristics of the sample

A total of 1,892 participants were interviewed, of whom 1,860 (98.3%) were willing to participate, and they all provided complete data and a nasal swab from both nares. Of those, 682 participants were workers with occupational livestock contact (including 224 farm workers, 20 veterinarian, 194 slaughterhouse workers, and 244 butchers), and 1,178 were control workers without occupational livestock contact. There were statistically significant