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Nasal carriage of methicillin-resistant coagulase-negative staphylococci in healthy humans is associated with occupational pig contact in a dose-response manner

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ABSTRACT

This study aimed to explore the association between occupational pig contact and human methicillin-resistant coagulase-negative staphylococci (MRCoNS) carriage. We conducted a cross-sectional study of pig exposed participants and controls in Guangdong, China, using a multi-stage sampling design. Participants provided a nasal swab for MRCoNS analysis and resulting isolates were tested for antibiotic susceptibility. The dose-response relation was examined using log binomial regression or Poisson regression models. The adjusted prevalence of MRCoNS carriage in pig exposed participants was 1.67 times (95% CI: 1.32–2.11) higher than in controls. The adjusted average number of resistance to different antibiotic classes of MRCoNS isolates from pig exposed participants was 1.67 times (95% CI: 1.46–1.91) higher than those from controls. Notably, we found the frequency and duration of occupational pig contact was associated with increased prevalence and increased number of resistance to different antibiotic classes of MRCoNS in a dose-response manner. When examining these relations by MRCoNS species, there was still evidence of similar exposure-response relations. Additionally, the proportion of tetracycline-resistant and *tet*(M)-containing MRCoNS isolates was significantly higher in pig exposed participants than in controls. These findings suggested a potential transmission of MRCoNS from livestock to humans by occupational livestock contact, and the presence of phenotypic and genotypic tetracycline resistance may aid in the differentiation of animal origins of MRCoNS isolates.

1. Introduction

Coagulase-negative staphylococci (CoNS) are commensal and opportunistic pathogens, which were one of the most common causes of hospital-associated and community-associated infections (Becker et al., 2014; Piette and Verschraegen, 2009; Si et al., 2016). *S. epidermidis* and *S. haemolyticus* are the most important species of CoNS. *S. epidermidis* is the predominant pathogen in bacteremia, endocarditis, urinary tract infection, ophthalmologic infection, peritoneal infection and so on (Christensen and Bruggemann, 2014; Otto, 2009; Piette and Verchraegen, 2009). *S. haemolyticus* has been implicated in septicemia, endocarditis, peritonitis, urinary tract infection, and bone and joint infections (Czekaj et al., 2015; Piette and Verschraegen, 2009). Of particular importance are methicillin-resistant strains since they are resistant to all beta-lactams antibiotics and can increase the morbidity, mortality and treatment cost of staphylococcal infections (Moodley and Guardabassi, 2009; Schmidt et al., 2014). Methicillin-resistance in staphylococci is often mediated by the *mecA* gene, which encodes an alternative penicillin binding protein (PBP2a) that has a lower binding affinity for beta-lactams and confers resistance to these antibiotics. Multiple resistant CoNS species from livestock can serve as reservoirs for *mec* elements (Wendlandt et al., 2013; Tulinski et al., 2012).

Identifying the source, reservoirs and vectors for the spread of antibiotic resistant bacteria poses significant challenges. The hospital environment, the endogenous microflora in patients, and health care workers (HCWs) had been witnessed to play a role (Dalstrom et al., 2008; Vonberg et al., 2006). Livestock may exist as a reservoir of MRCoNS, as demonstrated in Nigeria and Brazil (Chah et al., 2014; Silva et al., 2014). Note that the transmission of livestock-associated methicillin-resistant *S. aureus* (MRSA) ST398 or ST9 from pigs to occupationally exposed humans was reported in Europe, in the USA and in China (Cuny et al., 2009; Smith et al., 2009; Van Cleef et al., 2010; Ye et al., 2016). Although recent studies have revealed the emergence of multiresistance or *cfr*-mediated multiresistance in CoNS from livestock

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and humans with occupational exposure (Cuny et al., 2017; Huber et al., 2011; Sinlapasorn et al., 2015), the potential transmission of MRCoNS from livestock to humans is still unclear. Antibiotics have been used extensively in Chinese livestock production for non-therapeutic purposes such as growth promotion, and there is increasing evidence that routine, non-therapeutic uses of antibiotics in food animals increase the risk of propagation of multidrug-resistant bacteria (Nadimpalli et al., 2015; Thanner et al., 2016). Of particular concern is that the ecology and epidemiological behavior of CoNS is different from *S. aureus*, and these traits also vary strongly among different CoNS species, suggesting intra-species and inter-species diversity in ecology and epidemiological behavior (Souza et al., 2016).

Therefore, we undertook a cross-sectional study of pig exposed participants and controls without occupational livestock contact. The objective of our study is to test the hypothesis that pig exposed participants have a higher prevalence of MRCoNS carriage than controls. We also tested the hypothesis that pig exposed participants were more likely to carry MRCoNS isolates that are resistant to more antibiotics as compared with controls.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and was performed in accordance with the approved guidelines. Before participating, all participants signed an informed consent form.

2.2. Study design and participants

This cross-sectional study was conducted in Guangdong province, China, between November 2013 and November 2014. Briefly, a multistage sampling design was carried out to obtain a representative sample. Firstly, four cities were randomly sampled from 21 cities in Guangdong province. Secondly, in each sampled city, a specific number of pig farms were selected to obtain a respondent sample size of about 60 participants with occupational pig contact (defined as the pig exposed participants, including farm workers and veterinarians) and two nonfarm factories were sampled to enroll about 300 nonfarm controls with no occupational livestock exposure (i.e., workers from the hardware factory or the biscuit factory). Finally, all participants in selected venues were sampled to take part in our study using cluster sampling. After obtaining informed consent, eligible participants were asked to complete a face-to-face survey by trained interviewers. The eligibility criteria for participants included: (1) being able to speak and understand Chinese, (2) being 15 years or older, (3) not working at a health care facility, and (4) having no occupational livestock contacts for controls. The eligibility criteria for nonfarm factories included: (1) having at least 150 workers in the factory, and (2) having no animal or animal food processing in the factory.

2.3. Identification of bacterial strains

After completing the questionnaire, two nasal swabs were taken from each participant. Swabs were soaked in 2 ml of enrichment broth containing 1% tryptone, 1% mannitol, 7.5% NaCl and 0.25% yeast extract, and incubated at 35 ± 1 °C for 24 h. To isolate CoNS, a loopful of the broth was streaked onto mannitol salt agar and incubated at 37 °C for 24 h. From each plate, five colonies morphologically suspicious for staphylococci was sub-cultured to 5% sheep blood agar plates and incubated at 35 °C overnight for further investigation. CoNS isolates were presumptively identified based on colony morphology, positive Gram staining, hemolysis pattern, negative DNase, positive catalase and the negative tube coagulase test. Bacterial DNA was extracted by using the procedure described previously (Su et al., 2011). Species of CoNS (including *S. epidermidis, S. haemolyticus, S. warneri, S. saprophyticus, S. hominis, S. capitis, S. lugdunensis* and *S. caprae*) were identified by multiplex polymerase chain reaction (PCR) of the thermonuclease (*nuc)* gene fragment as described previously (Hirotaki et al., 2011). PCR assays were performed to test the staphylococci 16SrRNA, *nuc* and *mecA* genes (Zhang et al., 2004). If the *16*SrRNA and *mecA* were observed, while *nuc* was absent, the isolates were described as MRCoNS.

2.4. Antibiotic susceptibility testing and resistant genes

The antibiotic susceptibility of all MRCoNS isolates was determined by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2016). According to the CLSI 2016, the following antibiotic disks were used: clindamycin (2 μg), tetracycline (30 μg), erythromycin (15 μg), chloramphenicol (30 μg), ciprofloxacin (30 μg), rifampicin (5 μg), trimethoprim-sulfamethoxazole (23.75 μg), gentamicin (10 μg), quinupristin- dalfopristin (15 μg) and linezolid (30 μg). According to the CLSI 2016, we classified MRCoNS isolates as susceptible or nonsusceptible (including both intermediate and resistant isolates) to each antibiotic. All MRCoNS isolates were also tested through PCR for the carriage of tetracycline-resistant genes [*tet*(M), *tet*(K)] and erythromycin-resistant genes [*erm*(A), *erm*(C)] (Strommenger et al., 2003).

2.5. Study variables

The main outcome variable was nasal carriage of MRCoNS, methicillin-resistant *S. epidermidis* (MRSE) and methicillin-resistant *S. haemolyticus* (MRSH). The main independent variable was self-reported occupational pig contact. Occupational pig contact was binary (yes or no) or continuous variables including frequency of contact (hours per day or days per week) and duration of contact (years or total hours in the life; total hours in the life = h/day * days/week * 52 weeks/year * years). Covariates in this study were gender, age (years), education (elementary school, junior high school, senior high school and above), antibiotic use in the last month (yes or no), and medical facility visit in the last month (yes or no).

2.6. Statistical analysis

All data were entered in duplicate into the EpiData version 3.0 database (The EpiData Association, Odense Denmark). Pearson chi-squared test or Fisher's exact test was used to compare the differences between pig exposed participants and controls. Univariable and multivariable log binomial regression models were used to explore potential frequency-risk and duration-risk relations between occupational pig contact and MRCoNS (including MRSE and MRSH) carriage. Univariable and multivariable Poisson regression models were used to explore potential frequency-risk and duration-risk relations between occupational pig contact and the number of antibiotic classes to which MRCoNS isolates were resistant (based on the CLSI definition). Linear trends of pig contact were assessed by modeling contact as continuous variables (logarithmic scale) in the models. Based on *prior* assumptions, all multivariable models were adjusted for gender, age (in groups), education, antibiotic use in the last month and medical facility visit in the last month. A two-sided p-values < 0.05 was considered as being of statistical significance. All statistical analyses were performed using Stata 14.0 version (StataCorp LP, College Station, Texas, USA).

3. Results

3.1. Demographic information, characteristics of study population and prevalence of MRCoNS

A total of 1422 participants were interviewed in this study (Table 1). Of those, 244 participants were pig exposed participants