Prevalence and relevance analysis of multidrug-resistant *Staphylococcus aureus* of meat, poultry and human origin

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**ABSTRACT**

The aim of this study was to investigate the prevalence, antibiotic susceptibility and genotypes of *S. aureus* of human, meat and poultry origin in Henan Province in China. A total of 327 beef, pork, chicken and human samples collected from local grocery stores and hospital were investigated. The prevalence of *S. aureus* was found to be 26.7% in beef, 37.5% in pork, 30.1% in chicken and 54.8% in human samples, respectively. Isolates were commonly found resistant to ampicillin (66.9%), tetracycline (61.4%), ciprofloxacin (67.5%) and gentamicin (77.1%), however, none of the isolates was resistant to rifampin and vancomycin, and only one strain of human origin was resistant to vancomycin. The multidrug resistance was common among 66.3% of the isolates. Multilocus sequence typing (MLST) analysis showed that two sequence types namely ST-239 and ST-5 were primarily prevalent among chicken and pork isolates while the most prevalent sequence types among beef and human samples were ST-9 and ST-398, respectively. ST-5 was identified among all isolates, suggesting that the source of *S. aureus* contamination among animal food and man has some relevance.

**Key words:** Multidrug-Resistant; MLST, *Staphylococcus aureus.*

**INTRODUCTION**

*Staphylococcus aureus* is an important facultative pathogen in both Human and Veterinary Medicine. In humans it may, as in other animals, belong to the normal skin microflora (Devriese, 1980), but, it can cause a wide range of infections including dermatitis, pneumonia, sepsis, osteomyelitis and meningitis in humans and pigs as well as mastitis in cattle (Hasman et al, 2010). *S. aureus* is commonly isolated from animals like pigs, cattle, poultry, horse and companion animals, as well as from humans (Weese et al, 2010). At the same time, it is also commonly found in various foods (especially of animal origin), and is an important foodborne pathogen worldwide (Normanno et al, 2007). With the extensive use of antibiotics in animal meat production for disease prevention, growth promotion and improved feed conversion efficiency, there is dramatic increase in the prevalence of antimicrobial-resistant bacteria, especially of multidrug resistant ones worldwide. It is speculated that the extensive and often indiscriminate use of antimicrobial agents in animal husbandry sector might constitute a risk factor in creating an animal reservoir of antimicrobial-resistant bacteria (Tenover et al, 1996). From this reservoir, resistant strains or resistance genes might spread to humans via the food chain. Some data have indicated that food-producing animal species, raw poultry and other meat products harbour antimicrobial-resistant, Gram-positive bacteria (Aarestrup 1995, Bates et al, 1994, Borgen et al, 2000, Khanna et al, 2008). Among resistant *S.aureus*, multidrug-resistant and methicillin-resistant *S. aureus* (MRSA) are the most significant ones, which have garnered substantial public attention due to increasing mortality associated with these multidrug resistance strains. Studies have shown that livestock-associated *S. aureus* including MRSA and MDRSA can be exchanged between humans and animals (Harrison et al, 2013, Price et al, 2012). But also can be transmitted from human to human (Van et al, 2014). Several studies have demonstrated the occurrence of high prevalence of multidrug-resistant *S.aureus* among animals, foods of animal origin and contact persons in the European Union, Canada, and United States (Khanna et al, 2008, Smith et al, 2011, Waters et al, 2011), but few studies have been conducted to measure its prevalence and relevance in food products and humans in China. As *S. aureus* from commercially processed poultry and other animals may...
constitute a reservoir for disseminating antibiotic resistance into the community, it is important that resistance among these bacteria should be investigated. The purpose of this study was to determine the prevalence and antibiotic susceptibility profiles of \textit{S. aureus} in retail meat, poultry samples and humans from HeNan province in China, and to analyse the relevance of phenotypic resistance and genotypic characteristics of strains isolated from different origins.

**MATERIALS AND METHODS**

**Sample collection:** From February to October 2009, a total of 265 meat samples including 60 ground beef, 112 ground pork and pork chops and 93 chicken (breasts and thighs) were purchased from 29 retail outlets in five cities of Henan Province, China (XinXiang, ZhengZhou, AnYang, Zhu Madian and XuChang) as well as a total of 62 human samples were collected from five hospitals in these cities. The samples from humans comprised of 24 nasal swabs, 26 blood samples and 12 fester samples. The samples were procured and transported via 24h courier in asepsis packages on ice to reach the investigating laboratory and were processed within 72h of the receipt.

**Sample processing:** All the samples were agitated for 1 minute in 100 mL of 1% peptone broth and then 20 mL of the inoculated broth was mixed with 20 mL of 2XBaird-Parker broth (Beijing Land Bridge Technology Co. Ltd, Beijing, China) and incubated at 37°C for 18–24h. After enrichment, the culture was shaken and a loopful of the culture was inoculated onto Baird-Parker agar plates and incubated for 18–24h for growth. All agar formulations included Egg-Yolk Tellurite Emulsion enrichment.

**Bacterial confirmation:** After a two-step enrichment procedure, putative isolates were identified as \textit{S. aureus} by studying the colony morphology, Gram’s staining, and biochemical tests including fermentation of mannitol and production of coagulase and catalase reaction. Meanwhile it was confirmed as \textit{S. aureus} using a real-time polymerase chain reaction assay targeting the \textit{femA} gene. Following identification, isolates were stored at -80°C in trypticase soya broth (TSB) plus 20% (v/v) glycerol for further use.

**Susceptibility testing to antimicrobials:** Minimum inhibitory concentrations (MICs) were determined by the agar dilution technique according to Clinical and Laboratory Standards Institute (CLSI, 2009) guidelines. The following antimicrobials were used: ampicillin, amikacin, clindamycin, ciprofloxacin, gentamicin, cefoxitin, erythromycin, oxacillin, rifampin, sulfamethoxazole, tetracycline and vancomycin. The MICs were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2009). 

\textit{Escherichia coli} ATCC 25922 and \textit{S. aureus} ATCC 29213 were included as quality control strains in each run. Multidrug resistance was reported as a single isolate resistant (intermediate or complete) to 3 or more unique antimicrobial classes.

**Multilocus sequence typing (MLST):** MLST of \textit{S. aureus} was performed based on seven housekeeping genes namely \textit{arcC, aroE, glpF, gmk, pta, tpi} and \textit{yqiL}, as described elsewhere (Enright, 1999). Sequence types (STs) were determined at the \textit{S. aureus} MLST website (MLST).

**Prevalence calculations:** Samples were reported as positive for \textit{S. aureus} if an isolate was confirmed as \textit{S. aureus} from any of the agar formulations. Positive counts were tallied for each meat and poultry type and divided by the total number of samples tested.

**RESULTS AND DISCUSSION**

**Isolation and identification of \textit{S. aureus}:** A total of 265 meat and poultry samples and 62 human samples were collected and subjected to detection of \textit{S. aureus}. Of the 265 animal products samples, 86 (32.5%) samples were positive for \textit{S. aureus}, including 16 (26.7%) of the 60 ground beef, 42 (37.5%) of the 112 ground pork and pork chops samples, 28 (30.1%) of the chicken samples. Of the 62 human samples, 34 (54.8%) samples were positive for \textit{S. aureus} including nasal swabs (45.8%, 11/24), blood (61.5%, 16/26) and fester (58.3%, 7/12). A total of 166 \textit{S. aureus} isolates were recovered from the 120 \textit{S. aureus} positive samples, including 19 isolates from ground beef, 66 isolates from pork samples, 41 isolates from chicken and 40 \textit{S. aureus} isolates were recovered from human samples (Table 1).

**Antimicrobial susceptibility tests and multidrug resistance:** A total of 166 \textit{S. aureus} isolates were tested for

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**TABLE 1:** Prevalence of \textit{Staphylococcus aureus} from samples.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples</th>
<th>No. (%) of samples positive for \textit{Staphylococcus aureus}</th>
<th>No. of \textit{S. aureus} isolates recovered</th>
<th>No. of \textit{S. aureus} ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>beef</td>
<td>60</td>
<td>16(26.7%)</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Pork</td>
<td>112</td>
<td>42(37.5%)</td>
<td>66</td>
<td>6</td>
</tr>
<tr>
<td>Chicken</td>
<td>93</td>
<td>28(30.1%)</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>human</td>
<td>62</td>
<td>34(54.8%)</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>327</td>
<td>120 (36.7%)</td>
<td>166</td>
<td>23</td>
</tr>
</tbody>
</table>

*a:** One to two \textit{S. aureus} isolates were collected from each positive sample.
susceptibility against 12 antimicrobials. The resistance to gentamicin, tetracycline, ampicillin and ciprofloxacin was highly prevalent, which was 77.1% to gentamicin, 67.5% to ciprofloxacin, 66.9% to ampicillin and 61.4% to tetracycline (Table 2). All S. aureus isolates from animal products samples were susceptible to vancomycin and rifampin and only one isolate from human was resistant to vancomycin. In addition, Multidrug resistance defined as resistance to 3 or more antimicrobials, was observed among the S. aureus isolates and most prevalent among S. aureus isolates from human (80%), followed by those from pork (68.2%), chicken (63.4%) and beef (36.8%) (Table 2).

### MLST sequence types:

A total of seventeen unique MLST sequence types were identified among the S. aureus isolates. The numbers of sequence types were 6 in chicken, 6 in pork, 4 in beef and 7 in human isolates, (Table 1). The most common sequence types were ST-239 (42%) and ST-5 (24%) among chicken, ST-239 (48%) and ST-5 (21%) among pork, ST-9 (52%) and ST-5 (26%) among beef and ST-398 (56%) among human isolates (Figure 1). ST-398 was found only in human and pork isolates, but ST-5 was identified in all four kinds of samples.

In the current study, the prevalence, antibiotic susceptibility profiles and genotypes of S. aureus among meat, poultry and human samples in China was investigated. The results showed the contamination rate of S. aureus among different samples as 26.7% in beef, 37.5% in pork, and 30.1% in chicken, which was similar to other reports from China and other countries. For example, S. aureus was detected in 25.7% of pork samples in China (Wang et al., 2012), 24.2% of pork samples in Korea (Lee, 2003) 39.2% in United States (Kluymans, 2010), and 38.4% in Italy (Normanno et al., 2007). The prevalence of S. aureus in human samples was higher than animal products samples, this may have been due to the fact that the human samples were taken from clinics.

There were differences in prevalence between beef, pork and chicken, which probably was because of testing of different types of meat (i.e. whole meat pieces versus ground meat) and the different contamination sources. Even though there are differences in hygiene control and processing on farms in different countries, the common presence of S. aureus in food indicates it is a potential crisis for human health.

All isolates were screened for susceptibility to antibiotics. The rate of multidrug resistance was 66.3% which was higher than other reports i.e. 52% in the United States, (Waters, 2012). There was a higher prevalence of multidrug resistance in pork and chicken isolates than in beef isolates. These differences may be due to the fact that a substantial amount of antimicrobials is used in pig and poultry farming for growth promotion and for prophylaxis in addition to therapeutic purpose. However, at cattle farm generally, antimicrobials were mainly used for clinical purpose, such as treating of mastitis. All isolates from animal meats samples were susceptible to vancomycin and rifampin and only one vancomycin-resistant isolate was detected from human sample. Vancomycin and rifampin were never approved for food animal production in China, but it been used in clinic for treating infection; therefore, these findings were unexpected and may suggest S. aureus contamination origins was not human (the transfer way from human to animal product). Because ampicillin, gentamicin, ciprofloxacin and tetracycline were cheaper, these antibiotics have been largely used at animal farms and hospitals in China.

The data demonstrated that all isolates had a higher prevalence of
resistance to ampicillin, gentamicin, ciprofloxacin and tetracycline.

Through oxacillin resistance was detected among all the isolates, MRSA from each sample of pork, chicken and human were also detected. Though the sample size was insufficient to accurately estimate prevalence rates, but the data are consistent with another study by Cui et al. (2009), who reported that MRSA are only found at pig farm but not at cattle farm in China. Higher MRSA contamination rates have been estimated among meat and poultry samples in the Netherlands, where ST-398 was the dominant food-borne sequence type (De et al, 2009). In contrast, the MRSA isolates from meat and poultry samples in this study were ST-239 and ST-5, the MRSA from human was ST-398 type.

MLST analysis revealed that three genotypes (ST-239, ST-5 and ST-764) were commonly prevalent among chicken and pork isolates. Which was in disagreement with previous reports showing that the MRSA ST9, ST88 and ST59 clone were predominant in retail animal food in China (Wang et al, 2014). The genotype ST-5 was commonly prevalent among chicken, pork and beef (Fig.1) which suggested that the source of S. aureus contamination has some relevance, but the special relevance is unclear. The longitudinal ‘farm-to-fork’ (farm-to-chicken) study of the origin of S. aureus contamination of food products was conducted to help answer these questions. So additional studies tracing S. aureus genotypes from farm to retail are required to definitively identify the sources of S. aureus contamination. European and North American studies indicate that ST398 can successfully colonize and infect humans (Khanna et al, 2008 and Van et al, 2008). In the present study, ST-398 was the main prevalent genotype among human isolates, but it only appeared in pork isolates, whereas beef and chicken isolates had no ST-398. So there might be a transfer way of “man-to-pork” or “pork-to-man”, which needs to be studied in future.

Data demonstrated that retail meat and poultry are frequently contaminated with multidrug-resistant S. aureus, but the source of contamination is unclear. S. aureus could cause food poisoning (staphylococcal enterotoxin-associated diarrhoea) and all kinds of infection. So there is a need for valid hygienic control and processing on farms to reduce or eliminate this contamination. The proper measures included prevention of cross contamination, adequate cleaning and disinfection and good personal hygiene practices (especially, hand hygiene) and continuing education of the public about safe meat-handling practices.

![Graphs showing sequence types prevalence](image-url)

**FIG. 1:** The sequence types prevalence of the isolates from each sample.
REFERENCES


