Research Note

Prevalence, Antimicrobial Susceptibility, and Enterotoxin Gene Detection of Staphylococcus aureus Isolates in Ready-to-Eat Foods in Shaanxi, People’s Republic of China

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ABSTRACT

Various ready-to-eat (RTE) foods are becoming increasingly popular in the world and could be easily contaminated with various microorganisms including certain pathogens. A total of 342 RTE food samples, including 32 cooked meats, 123 vegetable salads, 26 boiled peanuts, 109 cold noodles, and 52 dried tofu samples, were collected in Shaanxi Province, People’s Republic of China, during the period of July to October 2012 and screened for Staphylococcus aureus. All S. aureus isolates were characterized by antimicrobial susceptibility and PCR for detecting nine enterotoxin genes (sea to sej). Overall, 25.4% of samples were positive for S. aureus, including 10 (31.3%) cooked meats, 34 (27.6%) salad vegetables, 6 (23.1%) boiled peanuts, 20 (18.3%) cold noodles, and 17 (32.7%) dried tofu samples. Of the isolated S. aureus organisms, 98.4% were resistant to at least one antimicrobial agent and 58.6% to three or more antimicrobials. Resistance to erythromycin (78.1%) and tetracycline (40.6%) was most frequently detected, while all isolates were sensitive to vancomycin and amikacin. Moreover, 55.5% of isolates were positive for one or more enterotoxin genes. The genes sed (25.8%) and sea (19.5%) were commonly detected among the isolates; seq, sei, and sej were not found. Our findings indicate that RTE foods in Shaanxi were contaminated with S. aureus isolates that harbored multiple toxin genes and exhibited multiple-drug resistance. Appropriate hygienic measures should be taken by producers, retailers, and consumers to reduce the risk posed by S. aureus in RTE foods.

Staphylococcus aureus is one of the most important foodborne pathogens worldwide. Some strains produce one or more toxins, which include toxic shock syndrome toxin-1, the staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ, and so on), the exfoliative toxins (ETA and ETB), and leukocidin (3). Staphylococcal enterotoxins (SEs) are known to cause food poisoning (11). It was shown that about 95% of staphylococcal food poisoning outbreaks were caused by the classical SEs (SEA through SEE), and the remaining 5% of outbreaks were associated with other identified SEs (7).

The emergence and spread of antimicrobial-resistant strains of S. aureus, especially methicillin-resistant S. aureus in both nosocomial and community settings, have become a major public health concern (9). Several studies have demonstrated the presence of antibiotic-resistant strains of S. aureus in different foods such as raw meat, cheeses, infant foods, and raw cow’s milk (11, 13, 17). Multidrug-resistant S. aureus strains have been emerging, leaving limited choices for their control (13).

Being convenient and affordable, the ready-to-eat (RTE) foods are very popular worldwide, especially in summer and autumn. However, without hygienic measures during preparation, retailing, and consumption, RTE foods can be easily contaminated with microorganisms such as Escherichia coli, Salmonella, Listeria, and S. aureus (8). Moreover, the existence of S. aureus in RTE food has been reported by many countries, such as Korea, Brazil, and Vietnam (5, 6, 14). Few studies about the existence of S. aureus in RTE foods in the People’s Republic of China have been reported. Therefore, this study aimed to determine the prevalence of S. aureus in RTE foods purchased from three cities located in Shaanxi Province, China, and investigate the presence of enterotoxin genes and antibiotic resistance of the isolates.

MATERIALS AND METHODS

Isolation and identification of S. aureus. From July to October 2012, 342 RTE food samples were collected from farmers markets (n = 242) and supermarkets (n = 100) in Xi’an, Baoji, and Yangling of Shaanxi Province, People’s Republic of China. Isolation and identification of S. aureus were performed as described previously (18). Briefly, 25 g of each sample was mixed with 225 ml of buffered peptone water (Beijing Land Bridge Technology Ltd., Beijing, China) in a sterile glass flask. Then, the solution was incubated at 37°C with shaking at 100 rpm for 24 h.
After preenrichment, an aliquot (5 ml) was transferred to 50 ml of Trypticase soy broth (Beijing Land Bridge Technology Ltd.) containing 7.5% NaCl. After 18 to 24 h of incubation at 35°C, a loopful of the culture was spread onto Baird-Parker agar (Beijing Land Bridge Technology Ltd.) plates with 5% egg yolk and tellurite. Following incubation at 35°C for 24 to 48 h, one or two presumptive coagulase-positive colonies per sample (black colonies surrounded by 2- to 5-mm-diameter clear zones) were cultured on Trypticase soy agar (Beijing Land Bridge Technology Ltd.) plates with 0.6% yeast extract for further purification. Colonies were then confirmed as S. aureus by PCR targeting the thermonuclease gene (nuc, S. aureus specific) (1). All isolates were stored at −80°C until use.

**PCR detection of SE genes.** Following the DNA extraction procedures described previously by Wang et al. (17), the amplifications were performed by using a Mycycler thermal cycler (Bio-Rad, Hercules, CA). The presence of nine S. aureus enterotoxin genes (sea, seb, sec, sed, see, seg, seh, sei, and sej) was examined by PCR according to the method of Peles et al. (10), with the primers listed in Table 1. The PCR products were resolved by 1.5% (wt/vol) agarose gel electrophoresis in 1 × Tris-borate EDTA buffer.

**Antimicrobial susceptibility testing.** In accordance with the guidelines of the Clinical and Laboratory Standards Institute (2), antimicrobial susceptibility tests were performed by using the agar dilution method on Mueller-Hinton agar (Beijing Land Bridge Technology Ltd.). The following antimicrobials were tested: chloramphenicol (resistance breakpoint, ≥32 μg/ml), erythromycin (≥8 μg/ml), ciprofloxacin (≥4 μg/ml), tetracycline (≥16 μg/ml), gentamicin (≥16 μg/ml), cefoxitin (≥8 μg/ml), cefoperazone (≥64 μg/ml), vancomycin (≥16 μg/ml), and amikacin (≥64 μg/ml). E. coli ATCC 25922 and S. aureus ATCC 29213 were used as quality control strains.

**Statistical analysis.** Statistical analyses were conducted with Minitab 15 (State College, PA). The chi-square (χ²) or Fisher’s exact tests were applied to evaluate statistically significant differences in the prevalence of S. aureus in different types of foods or markets. P values of ≤0.05 were considered statistically significant.

**RESULTS**

**Isolation and identification of S. aureus.** Of the 342 samples, 87 (25.4%) samples including 10 (31.3%) cooked meats, 34 (27.6%) salad vegetables, 6 (23.1%) boiled peanuts, 20 (18.3%) cold noodles, and 17 (32.7%) dried tofu samples were positive for S. aureus. There was no significant difference in each product category (P = 0.259 (0.05]). A total of 128 S. aureus isolates were recovered from the 87 S. aureus–positive samples (1 or 2 isolates per sample).

Of the 87 positive samples, 24 (24.0%) of 100 and 63 (26.0%) of 242 were from supermarkets and farmers markets, respectively. No significant difference was observed between the two types of market for the frequency of S. aureus isolation (P = 0.695 [0.05]).

**Detection of SE genes.** Of the 128 S. aureus strains tested (Table 2), 71 (55.5%) were positive for at least one SE gene. The most frequently detected gene was sed (25.8%; 33 of 128), followed by sea (19.5%; 25 of 128), see (13.3%; 17 of 128), seb (12.5%; 16 of 128), sec (7.0%; 9 of 128), and seh (0.8%; 1 of 128). None of the isolates harbored seg, sei, or sej. Twenty-four (18.8%) isolates contained at least two of the nine SE genes.

Overall, 13 different SE gene profiles were found among the 128 S. aureus isolates (Table 2). The most common SE gene profile was sed (13.3%; 17 of 128), followed by seh (10.2%; 13 of 128), sea (7.8%; 10 of 128), sea sed and sea sed see (3.9%; 5 of 128 each), see, sec see, and sec sed (3.1%; 4 of 128 each), see and seh see (2.3%; 3 of 128 each), and sed see, see seh, and see sec sed (0.8%; 1 of 128 each).

**Antimicrobial susceptibility testing.** All of the 128 S. aureus isolates were susceptible to chloramphenicol, erythromycin, ciprofloxacin, trimethoprim, tetracycline, gentamicin, and cefoxitin (Table 2). The most common resistance was against erythromycin (78.1%), followed by tetracycline (40.6%), cefoxitin (6.3%), chloramphenicol (3.9%), ciprofloxacin (3.9%), cefoperazone (1.6%), and gentamicin (0.8%). One hundred twenty-six (98.4%) S. aureus isolates were resistant to at least one antimicrobial agent, and 75 (58.6%) to three or more antimicrobials (data not shown).

**DISCUSSION**

In this study, 25.4% of samples were positive for S. aureus, which was higher than the contamination rate of RTE foods (5.98%) reported in Korea (6). There was no significant difference in contamination rate by S. aureus among RTE foods sold in supermarkets and farmers markets. The contamination of RTE foods may originate from more than...
one possible source. One of the possible reasons is that RTE foods are prepared by small-scale local producers without quality control. The second possible source is the reused or improperly washed containers or equipment and primary packaging, especially in farmers markets. Moreover, distribution environments can also affect S. aureus prevalence in food products, particularly the holding temperatures during distribution and retailing. Furthermore, improper handling and cross-contamination during transportation and storage are also possible. The supervision of the food at the factory and circulation should be strengthened to reduce food contamination.

Enterotoxins produced by S. aureus could exist in foods and cause foodborne illness. The enterotoxin genes in S. aureus have been reported by many studies (7, 15, 19), and the prevalence of strains bearing enterotoxin genes varied in these reports. We found that 55.5% of S. aureus isolates were positive for one or more SE genes, which was higher than the rate (49.8%) of the isolates with toxigenic properties in Korea but lower than the 85.0% rate reported in the United States (6, 13). SEA, alone or together with other staphylococcal enterotoxins, is most frequently involved in food poisoning outbreaks worldwide, followed by SEB, SEC, or SED. In this study, we found that sed (accounting for 25.8%) was the most commonly detected, followed by sea (19.5%), see (13.3%), seb (12.5%), and sec (7.0%). This is consistent with a study in which SED (74.5%) was the most frequently detected SEs among six SEs examined (SEA to SEE and SEH) (9). No seg or sei gene was detected, while in another study we found some seg-positive isolates in other types of foods (17). The sed and seg genes are commonly located in the plasmid pIB485, and the detection of one usually indicates the presence of the other. However, 13.3% of isolates contained sed, and none of the isolates contained seg. This is in accordance with Srinivasan et al. (16), who proved that 52.6% of S. aureus strains from cow’s milk with mastitis contained sed, but none contained seg. Both studies (16, 17) confirmed that these genes may be found either alone or together in the same strain. In China, strains carrying the classical SE genes (sea through see) were implicated in most (96.2%) of the staphylococcal food poisoning outbreaks, and fewer outbreaks (3.8%) were associated with strains harboring other identified SEs (19).

Among all isolates from RTE foods, 55.5% were positive for the classical SE genes, which suggests that these S. aureus strains may pose a potential health risk for consumers. In recent years, the emergence of multidrug-resistant (resistant to three or more antimicrobials) strains of S. aureus has gained increasing attention regarding public health in many countries, and it has been demonstrated that resistant strains of S. aureus are present in various foods in different countries (4, 11). Resistances to erythromycin and tetracycline were common in isolates studied. All S. aureus isolates from RTE foods were sensitive to vancomycin and amikacin. This agrees with Wang et al. (18), who reported that no S. aureus isolated from food and food animals in Shaanxi Province was resistant to vancomycin and amikacin. Seventy-five S. aureus isolates showed multidrug resistance. Yan et al. (19) found that 30.8% of S. aureus isolates associated with staphylococcal food poisoning between 2006 and 2009 in Shenzhen, in Southern China, were resistant to at least two antibiotics, and 7.7% of the isolates were resistant to three or more drugs. Podkowik et al. (12) showed in their study that all S. aureus isolates were resistant to at least one antibiotic and 60% of the isolates were resistant to four and more antibiotics. The difference in resistance rates reported in different studies may be caused by the situation of usage of antibiotics in a specific area and the type of samples chosen.

In conclusion, S. aureus existed at a relatively high rate in retail RTE foods in Shaanxi Province in the People’s Republic of China, and many S. aureus isolates are resistant to different antimicrobials. In addition, various enterotoxin genes are found in many S. aureus isolates. The potential of S. aureus in RTE foods to cause food poisoning should not be neglected. Preventative measures to avoid contamination of S. aureus and to inhibit SE production in foods need to be taken. Further research is necessary to understand the origin and transmission route of S. aureus in RTE foods to help reduce S. aureus foodborne poisoning.

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