Short Communication

Relationships between Multidrug-Resistant Salmonella enterica Serovar Schwarzengrund and Both Broiler Chickens and Retail Chicken Meats in Japan

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SUMMARY: We examined 29 isolates of Salmonella enterica subspecies enterica serovar Schwarzengrund from broiler chickens (n = 19) and retail chicken meats (n = 10) in Japan for antimicrobial susceptibility and pulsed-field gel electrophoresis (PFGE) profiling. All isolates exhibited resistance to both bicozamycin and sulfadimethoxine (minimum inhibitory concentration of both antimicrobial agents: ≥512 μg/ml). Nalidixic acid resistance was found in only one broiler chicken isolate. PFGE analysis showed that there were two genotypes among S. Schwarzengrund isolates. Isolates from 11 of 19 broiler chickens and from 6 of 10 retail chicken meats exhibited resistance to dihydrostreptomycin, kanamycin, oxytetracycline, bicozamycin, trimethoprim, and sulfadimethoxine, and had an identical PFGE pattern classified into a predominant genotype. Thus, our results indicate that genetically identical multidrug-resistant S. Schwarzengrund appeared to be disseminated among broiler chickens and retail chicken meats in Japan.

Salmonella enterica, a common cause of human gastroenteritis and bacteremia, is prevalent in a wide variety of animals. Out of many S. enterica serovars, serovars Typhimurium and Enteritidis are the most common causes of human salmonellosis worldwide. In the United States, Denmark, and Southeast Asia, S. Schwarzengrund has been reported as a causative agent of the disease (1-4). In addition, a large-scale outbreak of this serovar caused by contaminated dry dog food has been reported in humans (5).

In the United States, S. Schwarzengrund was the 10th most frequently isolated serovar that caused invasive salmonellosis in humans between 1996 and 1999 (6). In Japan, S. Schwarzengrund was the 10th most frequent cause of food-borne illness in 2007 but was not in the top 10 from 2005 to 2006 (https://hasseidoko.mhlw.go.jp/Byogentai/Pdf/data81e.pdf). Broiler chicken isolates from broiler chickens and retail meats were determined by using the agar dilution method of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (11). All the antimicrobial agents except for bicozamycin and nalidixic acid were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Bicozamycin and nalidixic acid were kindly supplied by Fujisawa Co. (Osaka, Japan) and Daiich Pharmaceutical Co. (Tokyo, Japan), respectively. The MIC of each antimicrobial agent was interpreted using the CLSI breakpoints. The proportion of S. Schwarzengrund by broiler chicken origin, however, increased from 0% in 2000-2003 (10) to 28.1% in 2005-2007 (unpublished data). The increase in the incidence of S. Schwarzengrund among broiler chickens is considered a threat to chicken meat hygiene. Recently, Aarestrup et al. (1) showed the international spread of multidrug-resistant S. Schwarzengrund in humans caused by contaminated food products imported into Denmark and the United States. In the present study, S. Schwarzengrund isolates from broiler chickens and retail chicken meats were subjected to antimicrobial susceptibility and pulsed-field gel electrophoresis (PFGE) to study the features of Japanese isolates of both origins.

A total of 29 isolates of S. Schwarzengrund were included, 19 from fecal samples of broiler chickens obtained from 1999 to 2007 and 10 from retail chicken meats obtained from 2005 to 2007 (Table 1). Ten broiler chicken isolates were derived from 4 prefectures in Kyushu, 6 from 2 prefectures in Kinki, and 3 from 2 prefectures in Chugoku. All of the chicken meat isolates were from retail meat samples of domestic chicken origin collected at different meat shops in Fukuoka Prefecture (Kyushu). They were stored at −80°C in 10% skim milk.

Minimum inhibitory concentrations (MICs) of the 11 antimicrobial agents (ampicillin, dihydrostreptomycin, kanamycin, gentamicin, oxytetracycline, chloramphenicol, bicozamycin, colistin, nalidixic acid, trimethoprim, and sulfadimethoxine) were determined by using the agar dilution method of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (11). All the antimicrobial agents except for bicozamycin and nalidixic acid were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Bicozamycin and nalidixic acid were kindly supplied by Fujisawa Co. (Osaka, Japan) and Daiich Pharmaceutical Co. (Tokyo, Japan), respectively. The MIC of each antimicrobial agent was interpreted using the recommendations of the CLSI (12). The breakpoints not seen in the CLSI were defined in a previous study (13). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as quality control strains. According to the CDC PulseNet protocol (14), isolates were analyzed for genetic relatedness by PFGE with XbaI and BlnI restriction enzymes. Electrophoresis was performed by the CHEF-DR III System (Bio-Rad Laboratories, Hercules, Calif., USA) with a running condition of 1 phase from 2.2 to 63.8 s at 180 V for 19 h. Molecular Analyst Fingerprinting Plus software, version 1.6 (Bio-Rad Laboratories), was used to compare the PFGE profiles. Similarity and diversity were assessed using the Dice coefficient.
All S. Schwarzengrund isolates used in this study exhibited resistance to both bicozamycin and sulfadimethoxine (≥512 µg/ml). In addition, most of the isolates showed resistance to dihydrostreptomycin and oxytetracycline with or without kanamycin and/or trimethoprim (Table 1). Such resistance patterns, except for bicozamycin resistance, were frequently found in S. Infantis isolates from broiler chickens in Japan (9,10). On the other hand, bicozamycin resistance was rarely found in S. Infantis isolates from broiler chickens between 2000 and 2003 (10). Bicozamycin has been approved as a veterinary medicine in cattle and pigs but not in poultry; also between 1998 and 2007, it was not used to promote the growth of food animals (http://www.famic.go.jp/ffis/feed/sub2_kentei.html [in Japanese]) (15,16). Thus, the prevalence of bicozamycin resistance in S. Schwarzengrund is not likely to be result from bicozamycin use on broiler chicken farms.

Epidemic infection of Salmonella serovars, such as S. Enteritidis PT4 and S. Typhimurium DT104, is a worldwide concern in human and animal health. These serovars have been prevalent in Japan (17,18). Recently, Aarestrup et al. (1) showed that multidrug-resistant S. Schwarzengrund, exhibiting resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, sulfamethoxazole, tetracycline, and trimethoprim/sulfamethoxazole, was found worldwide and was believed to be disseminated internationally by chicken products from Thailand. In the present study, nalidixic acid resistance was found in only one isolate, from broiler chickens in 2007. Even though only a small number of isolates were tested in this study, resistance to ampicillin, gentamicin, chloramphenicol, and colistin was not found in any of them, implying that S. Schwarzengrund isolates in Japan were different from the worldwide strains.

Among the 29 isolates, two unique PFGE patterns were observed by digestion with XbaI restriction enzyme. In addition, PFGE analysis revealed that isolates of one PFGE pattern, classified by XbaI digestion, were divided into three BlnI-digested PFGE patterns; and that those of another pattern, classified by XbaI digestion, were divided into two PFGE BlnI-digested PFGE patterns (Figure 1). PFGE typing revealed a single genotype in 11 of 19 broiler chickens and 7 of 10 retail chicken meats after 2005 (Table 1). The predominant PFGE pattern IIa could be clearly distinguished from that (Ia) of broiler chicken isolates in 1999. In addition, the 11 broiler chicken isolates derived from two different regions of Japan after 2005 exhibited a common resistance to dihydrostreptomycin, kanamycin, oxytetracycline, bicozamycin, and trimethoprim (Table 1). Thus, these results indicate that a genotype of multidrug-resistant S. Schwarzengrund appeared to be disseminated in common among broiler chickens and retail chicken meats in Japan. Until now, S. Schwarzengrund is a less common cause of human salmonellosis in Japan, but the increased incidence of this serovar in broiler chickens may become a threat to human health through the contamination of chicken meats.

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**REFERENCES**


