論文の内容の要旨

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論文題目 **Antimicrobial-resistant bacteria isolated from urban rodents and house shrews in Vietnam and Indonesia**

（ベトナムおよびインドネシアにおけるげっ歯類ならびにスンクスより分離された薬剤耐性菌の解析）

Antimicrobial resistance (AMR) is a global public health concern for both clinical and veterinary medicine. AMR could be used as an indicator for misuse and overuse of antimicrobial agents. The intensive use of antimicrobial agents in humans, livestock and companion animals may affect infection of wildlife animals with AMR bacteria. Small mammals might acquire AMR bacteria in the environment through their habitats. To estimate AMR bacteria present in the environment and risk of public health, I have conducted a study of AMR bacteria in small mammals dwelling in urban areas in Hanoi, Vietnam and Bogor, Indonesia.

1. Antimicrobial-resistant *Escherichia coli* isolated from rodents in Vietnam.

In Vietnam, antimicrobials are extensively used in human medicine and animal farming. Consequently, the high levels of AMR *Escherichia coli* (*E. coli*) have been reported in food, animals and humans. There was a concern about AMR *E. coli* contaminated to the environment in the city. Urban rodents were considered as a suitable indicator for the presence of those bacteria in the environment.

To investigate the prevalence and characteristics of AMR *E. coli* including extended-spectrum beta-lactamases (ESBL)-producing and colistin-resistant *E. coli*. Feces or cloacal swabs were collected from 144 urban rodents caught in Hanoi, Vietnam. Antimicrobial susceptibility tests were conducted according to the Kirby-Bauer disc diffusion method using ten antimicrobial discs. The isolates of ESBL-producing *E. coli* were confirmed by the synergy test of clavulanate diffusion from an amoxicillin-clavulanate disc with cefotaxime and ceftazidime. The resistance genes of β-lactams, sulfonamide and tetracycline were detected by multiplex PCR. The colistin resistance genes were amplified by PCR and identified into *mcr-1*, *mcr-2* and *mcr-3* by sequencing analysis.

A total of 59 AMR *E. coli* was isolated from urban rodents. The prevalence of AMR isolates was observed in hospitals (45.2%: 14/31), markets (41.9%: 36/86) and a cargo station (33.3%: 9/27). There was no statistical difference in the prevalence of AMR *E. coli* between hospitals, markets and a cargo station. The most common resistant phenotype was observed against ampicillin (79.7%: 47/59), followed by tetracycline (78%: 46/59), nalidixic acid (67.8%: 40/59) and sulfamethoxazole-trimethoprim (59.3%: 35/59). Other resistant phenotypes were observed lower than 50%. Multi-drug resistant (MDR) *E. coli* were identified in 42 isolates. The most frequent resistance gene was tetracycline resistance genes, followed by beta-lactams and sulfonamides resistance genes. Furthermore, four MDR *E. coli* were confirmed as ESBL-producing *E. coli*, of which three were carried $\textit{bla}_{\text{CTX-M-1}}$ gene. The colistin resistance gene of *mcr-1* was found in five MDR *E. coli*.

This study has been revealed the prevalence of AMR E. coli, including MDR, ESBL-producing and colistin-resistant E. coli in Hanoi, Vietnam. It indicated an extensive usage of antimicrobial agents in humans in this area.

2. Antimicrobial-resistant *Escherichia coli* isolated from rodents and house shrews in Indonesia.

In Indonesia, misuse and overuse of antibiotics are common in humans, livestock and aquaculture, leading antibiotic resistance in bacteria. The Regional Resistance Surveillance Program administered by 12 Asia-pacific countries showed that Indonesia was the most prevalent country of ESBL-producing *E. coli* in clinical cases. However, few studies have examined AMR *E. coli* isolated from small mammals and environment in Indonesia.

A total of 87 small mammals (79 rodents and eight house shrews) were captured in Bogor, Indonesia. The sensitivity test and the identification of colistin-resistant *E. coli* were performed same as the methods described in the first chapter.

Overall, 20 AMR *E. coli* were identified in small mammals. Of which, 18 and two AMR *E. coli* were isolated from rodents and house shrews, respectively. The most common AMR in *E. coli* was resistant to tetracycline (85%: 17/20) and ampicillin (75%: 15/20). Other resistant phenotypes were ranged from 5% to 35%. Eight out of 20 AMR *E. coli* were identified as MDR *E. coli*. The most frequent resistance gene was tetracycline resistance genes, followed by *β*-lactam genes. ESBL-producing and colistin-resistant *E. coli* were not found from small mammals in Indonesia.

This study could elucidate the increase of MDR *E. coli* in the environment in Bogor, Indonesia. In the city, small mammals dwelling proximity to humans might acquire AMR *E. coli* contaminated in the food or water during their feeding activities, indicating that mammals including humans might be infected with AMR *E. coli* contaminated in the environment.

3. Diarrheagenic *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* from small mammals in Vietnam and Indonesia.

Although most of *E. coli* strains are harmless, there are many case reports that the pathogenic strains caused diarrhea. DEC is transmitted by fecal-oral route and causes diarrhea. In addition, *Salmonella* spp. was also concerned as foodborne diseases in the developing and developed countries. Vaccine for the infection with *Salmonella* spp. is not effective. *S. aureus* could cause a wide range of diseases, including foodborne diseases, skin diseases, blood infection and so on. In general, the treatment of foodborne diseases is currently the use of antimicrobial agents, except for STEC and *S. aureus* infections. Treatment of *S. aureus* infection for other diseases is the use of antimicrobial agents.

DEC was analyzed for the presence of virulence genes associated with the group of enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAEC) by multiplex PCR. *Salmonella* spp. was identified by the bacterial culture method and analyzed against ten antimicrobial agents using the disc diffusion method. *S. aureus* was identified by the bacterial culture method and then confirmed by the detection of 16S rRNA and specific gyrase genes using PCR. Susceptibility test for *S. aureus* was examined by the disc diffusion method using five antimicrobial agents and the minimum inhibitory concentration (MIC) test for cefoxitin and vancomycin. MRSA was confirmed by the MIC of cefoxitin and the detection of methicillin resistance gene.

Two DEC were detected in this study. Of which, an EIEC was a susceptible *E. coli*. Another was an EAEC, which showed an MDR phenotype, resistance to ampicillin, nalidixic acid, sulfamethoxazole-trimethoprim and tetracycline. All of four *Salmonella* spp. isolates were susceptible to all of ten antimicrobial agents examined. Eight and 16 *S. aureus* were isolated from 144 rodents and 87 small mammals captured in Hanoi, Vietnam and Bogor, Indonesia, respectively. The most prevalent in 24 *S. aureus* isolates were resistant to ciprofloxacin, followed by sulfamethoxazole-trimethoprim. All of the *S. aureus* isolates were susceptible to vancomycin. One out of 24 *S. aureus* isolate was identified as MRSA. The remaining isolates were MSSA. The findings show the prevalence of DEC, *Salmonella* spp. and *S. aureus* isolated from small mammals in Vietnam and Indonesia. Control of transmission is crucial for implementing preventive methods of foodborne pathogens.

Small mammals are not purposefully treated with antimicrobials. This study showed the prevalence of AMR *E. coli*, including MDR, ESBL-producing and colistin-resistant *E. coli* in small mammals. It was assumed that they are carrying AMR bacteria as a consequence of exposure to resistant bacteria contaminated in the environment. In addition, foodborne pathogens such as DEC, *Salmonella* spp. and a pathogen for a potential clinical infection (*S. aureus*) were also detected in the feces of small mammals. Since small mammals are dwelling proximity to humans in the urban areas, preventing the infestation of small mammals and improving hygiene are the key drivers to avoid unexpected transmission of AMR bacteria and foodborne pathogens to humans.