Screening of Antimicrobial Resistant Bacteria in Dog Shelters in Thailand

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Abstract

The number of stray dogs in Thailand has been increasing every year. This situation could lead to an increase in microorganisms, including antimicrobial resistant (AMR) bacteria that can be transmitted between humans and dogs. This study evaluated the prevalence of AMR bacteria isolated from rectal swabs taken from dogs living in shelters located throughout Thailand. The most common bacteria isolated from the samples of 159 healthy dogs were Enterobacteriaceae (71.3%). The prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae among the bacterial strains isolated from the dogs was 70.1%. Moreover, *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Staphylococcus aureus* were isolated from the rectal swabs. The percentages of ESBL-producing Enterobacteriaceae in the fecal carriage from dogs living in shelters with and without employed veterinarians were not significantly different (72.2% vs. 69.6%, respectively; P=0.5). The isolated Enterobacteriaceae were usually resistant to ampicillin and ceftriaxone. The percentages for antimicrobial susceptibility of *Edwardsiella tarda, P. aeruginosa, Acinetobacter* spp. and *S. aureus* were also not significantly different between the two types of shelter. Our study revealed that shelter dogs in Thailand may be the reservoir of AMR Enterobacteriaceae. Therefore, people should have awareness of the high prevalence of AMR bacteria among dogs living in shelters, and they should strengthen the efficiency and appropriateness of preventive management at the shelters. These include the rational use of antimicrobials to limit the production of AMR bacteria. The good sanitation and hygiene practices in shelters are required to limit AMR bacterial transmission among the dogs, and from them to humans.

Keywords: antimicrobial-resistant bacteria, dog, dog shelter, fecal carriage, Thailand

ความชุกของแบคทีเรียดื้อยาต้านจุลชีพในสุนัขที่อาศัย ในสถานพักพิงสุนัขในประเทศไทย

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บทคัดย่อ

ประเทศไทยมีจำนวนสุนัขจรจัดเพิ่มขึ้นอย่างต่อเนื่อง สถานการณ์ดังกล่าวอาจนำไปสู่การแพร่กระจายของเชื้อโรคระหว่างมนุษย์และสุนัข รวมทั้งแบคทีเรียดื้อยาต้านจุลชีพ การศึกษานี้สำรวจความชุกของแบคทีเรียดื้อยาต้านจุลชีพจากอุจจาระของสุนัขที่อาศัยอยู่ในสถานพักพิงสุนัขทั่วประเทศไทย แบคทีเรียที่พบมากที่สุดจากตัวอย่างที่เก็บจากสุนัขสุขภาพดี จำนวน 159 ตัว คือ แบคทีเรียกลุ่ม Enterobacteriaceae (ร้อยละ 71.3) และพบ ESBL-producing Enterobacteriaceae จากสุนัขร้อยละ 70.1 นอกจากนี้ ยังพบแบคทีเรีย *Pseudomonas aeruginosa Acinetobacter baumannii* และ *Staphylococcus aureus* จากอุจจาระของสุนัขด้วย ความชุกของ ESBL-producing Enterobacteriaceae ในสุนัขที่อาศัยในสถานพักพิงสุนัขที่มีการจ้างสัตวแพทย์ทำงานประจำ และไม่มีสัตวแพทย์ทำงานประจำ ไม่แตกต่างกันอย่างมีนัยสำคัญ (ร้อยละ 72.2 และร้อยละ 69.6 P=0.5 ตามลำดับ) Enterobacteriaceae ที่พบมักดื้อยา ampicillin และ ceftriaxone ส่วนความไวต่อยาต้านจุลชีพของ *Edwardsiella tarda P. aeruginosa Acinetobacter* spp. และ *S. aureus* ที่พบจากสุนัขของสถานพักพิงสุนัขทั้งสองกลุ่มไม่แตกต่างกัน ผลการวิจัยนี้แสดงว่า สุนัขที่อาศัยในสถานพักพิงสุนัข อาจจะเป็นแหล่งเก็บกักแบคทีเรียกลุ่ม Enterobacteriaceae ที่ดื้อยาต้านจุลชีพ ดังนั้น ผู้เกี่ยวข้องทุกฝ่าย ควรมีความตระหนักถึงการแพร่กระจายของแบคทีเรียดื้อยาต้านจุลชีพในสถานพักพิงสุนัข และควรมีการพัฒนามาตรการที่มีประสิทธิภาพในการป้องกันเชื้อดื้อยาต้านจุลชีพ รวมถึงการใช้ยาต้านจุลชีพอย่างสมเหตุผล เพื่อจำกัดการสร้างแบคทีเรียดื้อยาต้านจุลชีพ การจัดการด้านสุขอนามัยที่ดีในสถานพักพิงจะช่วยจำกัดการแพร่กระจายของแบคทีเรียดื้อยาต้านจุลชีพ ระหว่างสุนัขที่อาศัยรวมกัน และระหว่างสุนัขกับมนุษย์

คำสำคัญ : แบคทีเรียดื้อยาต้านจุลชีพ ประเทศไทยสุนัข สถานพักพิง สุนัข อุจจาระ

INTRODUCTION

The Bureau of Disease Control and Veterinary Services, Department of Livestock Development estimated that the stray dog population in Thailand was about 750,000 in 2016. The stray dog number has been steadily increasing every year (กรมปศุสัตว์, 2559). There is the possible outbreak of many zoonotic diseases, including rabies (Otranto et al., 2009; Liu et al., 2016), toxoplasmosis (Jittapalapong et al., 2007) and leptospirosis (Jittapalapong et al., 2009). Thus, dog shelters were established to solve both the animal welfare and zoonotic disease problems. Antimicrobials are one of the most common drugs given to the dogs living in the shelters. Some enteric bacteria, e.g., *Clostridium difficile*, could be a consequence of antimicrobial usage. The inappropriate use of antimicrobial agents is the important risk factor for inducing antimicrobial resistance (AMR) in bacteria residing in dogs and the environment of shelters (Pesavento et al., 2014). The prevalence of isolated methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) (Gingrich et al., 2011), *Clostridium perfringens* with enterotoxin A gene, and *Salmonella* species (Tupler et al, 2012) isolated from the feces of shelter dogs were previously reported. The extended spectrum β-lactamases (ESBL) and AmpC β-lactamases-producing *Escherichia coli* have also been detected in stray dog feces (Wedley et al., 2017). Transmission of MRSP (van Duijkeren et al., 2011) and ESBL-producing *E. coli* (Wedley et al., 2017) between dogs colonized with such bacteria and people in contact with them could occur. Therefore, people who might be susceptible to AMR bacterial infections are visitors, volunteers and workers in dog shelters (Steneroden et al., 2011).

The prevalence of antimicrobial resistant bacteria in shelter dogs in Thailand are limited data. Thus, these study evaluated the prevalence of AMR bacteria isolated from fecal samples collected from dogs living in shelters located in 5 areas of Thailand in order to determine the magnitude of the AMR problem in companion animals. The data could be used for the development and implementation of a national action plan on AMR as a One Health approach.

MATERIALS AND METHODS

The study protocol (MUVS-2015-61) received approval from the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC).

Sample collection

One hundred and fifty-nine healthy dogs were randomly selected from the dog shelters in the northern area (Lampang Province: n=20), north-eastern area (Nakhon Ratchasima Province: n=29), central area (Bangkok: n=30 and Uthai Thani Province: n=20), eastern area (Chonburi Province: n=20), western area (Kanchanaburi Province: n=20) and southern area (Phuket Province: n=20) of Thailand. There are 200-500 dogs per shelter, with an average of 279 each. All selected dogs were mixed breed, with 115 females (72.3%) and 44 males (27.7%). Their average age was 3.13 years (range: 6 months to 10 years), and average body weight was 15.5 kg (range: 5 to 42 kg). The health status of the shelter dogs was determined by veterinarian before the samples were collected. The samples from dogs that had any clinical sign or abnormal laboratory values were excluded from the experiment.

Isolation of bacteria and antimicrobial susceptibility testing

Each rectal swab was performed by using a sterile swab. All swab samples were placed into Cary-Blair transport medium and sent to laboratory of the Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University for bacterial isolation and antibiotic susceptibility testing.

Each rectal swab was streaked on MacConkey agar supplemented with 4 mg/L of ceftriaxone for selecting third generation cephalosporin resistant Gram-negative bacteria (Heil and Johnson, 2016). *P. aeruginosa* was aerobically culture onto MacConkey agar, blood agar and cetrimide-based media at 37°C for 24-72h. The non-lactose fermented colonies was identified by using the morphology, pigment formation on selective medium, the biochemistry tests and blood agar growth at 42°C (Laine et al., 2009). The *Acinetobacter* spp. was identified by cell and colony morphology, catalase test, oxidase test and motility test. The glucose oxidation, gelatin liquefaction, beta hemolysis, growth at 37°C and 42°C, arginine hydrolysis and susceptibility to chloramphenicol were also performed (Gupta et al., 2015). *P. aeruginosa* and *A. baumannii* could produce acid from glucose, tolerate against citrate and demonstrate alkaline slant/neutral butt on TSI agar. *P. aeruginosa* shown positive oxidase test and motility test. Whereas *A. baumannii* are non-motile bacteria with negative oxidase test. Mannitol salt agar was used to isolate *S. aureus*. Suspected yellow colonies on MSA were continuously characterized by using Gram's stain. *S. aureus* was identified by coagulase, catalase, methylene red, Voges-Proskauer, oxidase and indole tests, and hemolysis in blood agar. The acid productions from glucose, lactose and sucrose were determined. Mannitol salt agar containing methicillin or oxacillin were also used to screening MRSA (Smyth et al., 2005).

Antimicrobial susceptibility tests and the interpretations of the isolated bacteria were performed by the disk diffusion method according to CLSI guideline 2016 (CLSI, 2016) and EUCAST 2016 (EUCAST, 2016) when available. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were used as quality control strain. For ESBL producing bacteria, *K. pneumoniae* ATCC 700603 was used as positive control. The MRSA positive strain was *S. aureus* ATCC 700699. For the cut-off values of the susceptibility of some antimicrobials against certain bacteria that are not available in CLSI and EUCAST, the inhibition zone diameter values were recorded. Drugs susceptibility testing panel for isolated bacteria that belong to Enterobacteriaceae including ampicillin, amoxicillin clavulanate, ceftriaxone, ceftazidime, cefoxitin, ciprofloxacin, trimethoprim-sulfamethoxazole and meropenem. For *P. aeruginosa* and *A. baumannii*, only ceftazidime, ciprofloxacin and meropenem will be performed susceptibility test. Antibiotic susceptibility panel for *S. aureus* comprised of cefoxitin, levofloxacin, clindamycin, erythromycin and tetracycline.

The isolated *E. coli* resistant to cefotaxime (inhibition zone diameter < 27 mm) and ceftriaxone (inhibition zone diameter < 25 mm) were further determined for ESBL production by the Modified Double Disk Synergy Test (MDDST) (Kaur et al, 2013). An amoxicillin-clavulanate disc (20/10 ug) was centrally placed on a Mueller-Hinton agar plate. Cefotaxime, ceftriaxone, cefpodoxime and cefepime disks were placed radially 15-20 mm apart. The isolate that had a synergic inhibition zone of amoxicillin-clavulanate and cephalosporin disk was considered to be ESBL-producing Enterobacteriaceae.

Statistical analysis

The data were presented as descriptive statistics. *Chi-square* statistics were used to compare the rates of antimicrobial susceptibility of the bacteria isolated from the shelters that had a full-time veterinarian and from those without a regular veterinarian. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Three-hundred and fifty-nine bacterial isolates were recovered from the rectal swab samples collected from the 159 dogs. The Enterobacteriaceae (71.3%, n=256) was the most common isolated bacteria, following by *P. aeruginosa* (12.2%, n=44), *A. baumannii* (5.8%, n=21) and *S. aureus* (10.6%, n=38). ESBL-producing Enterobacteriaceae were isolated from 70.1% of the dogs.

The dogs in shelters were divided into two groups. Group 1 comprised dog in the shelters that employed the veterinarian for working day (n=90), and group 2 comprised dogs in the shelters without the employed veterinarian (n=69). The dogs from group 2 were received the medical services from animal clinic or hospitals nearby the shelters. Isolation of Enterobacteriaceae (78.6%, n=184) was most common among the dogs in group 1, following by *P. aeruginosa* (11.5%, n=27), *S. aureus* (7.3%, n=17) and *A. baumannii* (2.6%, n=6). The samples from group 2 showed that the Enterobacteriaceae also commonly isolated (57.6%, n=72), following by *S. aureus* (16.8%, n=21), *P. aeruginosa* (13.6%, n=17) and *A. baumannii* (15%, n=12). The rates of fecal carriage of ESBL-producing Enterobacteriaceae were not significantly different among both groups (72.2% and 69.6%, P=0.5).

The antimicrobial susceptibility profiles of isolated bacteria were shown in Figure 1-3. All isolated *E. coli* and *E. tarda* from both group of shelter dogs were resistant to ampicillin and ceftriaxone. The percentage of meropenem susceptibility of *E. coli* and *E. tarda* were 100. The antimicrobial susceptibility profiles of *E. tarda*, *P. aeruginosa, Acinetobacter* spp. and *S. aureus* were not significantly different between the dogs in the group 1 and 2. *E. coli* isolated from the dogs living in the shelters without the employed veterinarian were more susceptible to cefoxitin and ciprofloxacin (*P*<0.001) (Table 1).

DISCUSSION

The various multidrug-resistant (MDR) bacteria, including ESBL-producing *E. coli*, MRSA and MRSP have worldwide spread among dog populations in many countries. The human nosocomial pathogens such as carbapenemase-producing *E. coli* (Woodford et al. 2014) and *A. baumannii* (Müller, Janssen, and Wieler 2014) are recently found in dogs. The transmission between human and dogs relate to direct contact or indirectly through environmental contamination (Damborg et al. 2016). Therefore, the stray dogs that are close contact animals with human must be concern in part of contaminated environment. The previous study showed that some pathogenic bacteria could be isolated from stray dogs living near the agricultural areas (Jay-Russell et al., 2014).

In Spain, two-thirds of shelter intake dogs were stray dogs before residing in the shelters (Fatjo et al., 2015). The medical history and owner's ability to pay for the necessary care, such as sterilization, are risk factors for dog relinquishments (Weng et al., 2006; Kim et al., 2009; Dolan et al., 2015; Summerton et al., 2015). Many shelter dogs used to experience the poor health care and the history of scavenging or predation to survive before entering the shelters. These contribute the evolution, virulence and emergence of endemic pathogens (Pesavento and Murphy, 2014). The dog shelter establishment is to provide housing in the transient period before their adoption (Barrera et al., 2010) but the dog relinquishments continuously occur. The low adoption rate likely relate to the very long stays of dogs in shelters. Thus, the overcrowding in each cage can cause the bacterial transmission within population (Pesavento et al., 2014).

Our study showed that some pathogenic bacteria in human including, Enterobacteriaceae, *P. aeruginosa, A. baumannii* and *S. aureus* could be isolated from healthy dogs living in shelters with and without employed veterinarian. In addition, the prevalence of fecal carriage of ESBL-producing Enterobacteriaceae (70.1% of 159 healthy dogs) was higher than the corresponding figures revealed in previous reports from Mexico (6% of 53 healthy dogs) (Rocha-Gracia et al., 2015), the Portuguese Republic (15% of 151 healthy dogs) (Belas et al., 2014), France (18.5% of 368 healthy dogs) (Haenni et al., 2014) and the Netherlands (45% of 20 healthy dogs) (Hordijk et al., 2013). In China, the prevalence of ESBL-producing Enterobacteriaceae in companion dogs and cats varied from 39.5% to 55%, depending on the living area (Sun et al., 2010). The observed prevalence of ESBL-producing Enterobacteriaceae fecal carriage among shelter dogs in this study (70.08%) was comparable to the finding of our previous study of stray dogs residing in central area of Thailand (62%) (Boonmasawai et al., 2017) A longitudinal study in the Netherlands observed that the ESBL-producing Enterobacteriaceae could be highly found in dog fecal. These abundance pathogens were continuously shedding without clinical signs for several weeks to months (Baede et al., 2015).

The various patterns of antimicrobial use in each area associate with the prevalence of MDR bacteria in the pet population (Damborg et al., 2016). Due to cephalosporins and fluoroquinolones are commonly used by veterinarian for treatment the canine bacterial infection, Enterobacteriaceae isolated from dogs had trend to resistant to third-generation cephalosporins (Shaikh et al., 2015) and some fluoroquinolones (Albrechtova et al., 2014; Falgenhauer et al., 2016). A prior use of antimicrobials and the status of the dog's living places are risk factors for being carriers of ESBL-producing *E. coli* in dogs. The dogs living in shelters could carry ESBL-producing *E. coli* three times more often than dogs living with owners (Belas et al., 2014). Being female might also be a risk factor for the fecal carriage of ESBL-producing Enterobacteriaceae (Friedmann et al., 2015). The isolates of *E. coli* from the shelter dogs in this study were resistant to ampicillin (100%) with higher rate than the United Kingdom (37.2%) (Wedley et al., 2017). Ampicillin resistance in Enterobacteriaceae in some study could be founded in healthy dogs even if they had not received antimicrobials before because the mechanism of ampicillin resistance might be the harboring of β-lactamase genes in the bacterial population (Costa et al., 2008).

In conclusion, our study revealed that dogs living in shelters in Thailand may be the reservoirs of ESBL-producing Enterobacteriaceae and some AMR pathogenic bacteria. The strategies to prevent the generation and transmission of AMR bacteria in companion animals based on One Health approach are essential requirement (Walther et al., 2017). The rational use of antimicrobials and good sanitary systems could reduce the risk of AMR bacteria emerging, and subsequently spreading among dogs and between dogs and humans. New-intake dogs should be isolated in a disease quarantine area to prevent endemic bacterial transmission by direct contact (Turner et al., 2012). The education and training to strengthen antimicrobial-prescribing competencies in veterinarians is one of important strategies in the global action plan on antimicrobial resistance (Tangcharoensathien et al., 2018). The visitors and workers in the shelters are also the stakeholder of AMR problem. The AMR education and social responsibility are required for more efficient shelter operations. (Vincze et al., 2014).

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Conflicts of interest

The authors have no conflicts of interest to declare.

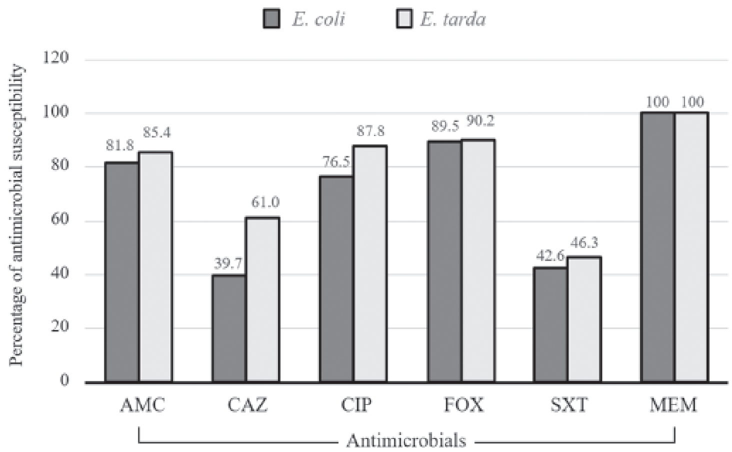

Figure 1 The antimicrobial susceptibility of *E. coli* (n=209) and *E. tarda* (n=41) isolated from shelter dogs.

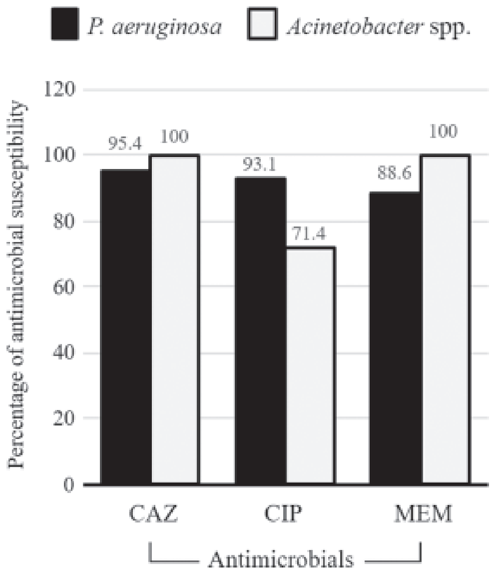

Figure 2 The antimicrobial susceptibility of *P. aeruginosa* (n=44) and *Acinetobacter* spp. (n=21) isolated from shelter dogs.

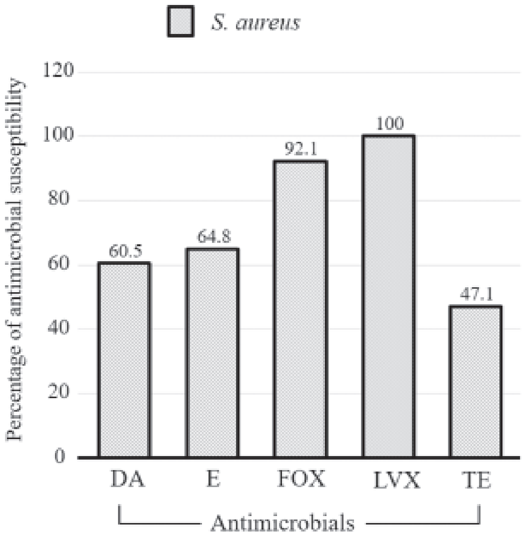

Figure 3 The antimicrobial susceptibility of *S. aureus* (n=38) isolated from shelter dogs.

Table 1 The antimicrobial susceptibility of *E. coli* isolated from group 1 and group 2 shelter dogs.

Antimicrobials	Percentage of antimicrobial susceptibility		*P*-value
	Group 1 (n=139)	Group 2 (n=70)	
AMC	81.3	82.8	0.600
CAZ	46	27.1	0.082
FOX	84.9	98.6	<0.001
CIP	66.9	95.7	<0.001
SXT	38.8	50	0.140

List of abbreviations (Figure 1-3, Table 1): AMC, Amoxicillin-clavulanate; CAZ, Ceftazidime; CIP, Ciprofloxacin; DA, Clindamycin; E, Erythromycin; FOX, Cefoxitin; LVX, Levofloxacin; MEM, Meropenem; SXT, Trimethoprim-sulfamethoxazole; TE, Tetracycline.

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