Short Communication

Clinical characteristics, antimicrobial resistance and treatment outcomes of multidrug-resistant Escherichia coli infection in dogs and cats at a veterinary teaching hospital in Thailand

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Abstract

This retrospective study investigated the clinical characteristics of multidrug-resistant (MDR) *Escherichia coli* and its antimicrobial resistance phenotypes and analyzed the treatment outcomes of MDR *E. coli* infection in dogs and cats. The medical data of dogs and cats diagnosed in 2020 with *E. coli* infection at a veterinary teaching hospital were analyzed. Of 94 cases, the frequency of MDR *E. coli* (66%) infection was higher than that of non-MDR *E. coli* (34%). MDR *E. coli* was significantly more frequently detected in female dogs than non-MDR *E. coli* ($P < 0.026$). The most frequent MDR *E. coli* isolation sites were the urinary tract in dogs and skin wounds in cats. MDR *E. coli* isolates from dogs were highly resistant to ampicillin (96.1%), enrofloxacin (80.4%) and tetracycline (78.4%). Resistance to ampicillin (100%), enrofloxacin (90.9%), marbofloxacin (72.7%) and tetracycline (72.7%) occurred frequently in MDR *E. coli* isolates from cats. Low resistance to amikacin was detected in the MDR *E. coli* isolates from dogs and cats. The rates of clinical cure and non-clinical cure of the MDR *E. coli* and non-MDR *E. coli* cases were not significantly different. The duration of antimicrobial treatment for MDR *E. coli* was significantly longer in cats (12.6 ± 5.85 days) than in non-MDR *E. coli* cases (7 ± 0 days) ($P < 0.048$). Adjunctive therapy was prescribed more frequently in MDR *E. coli* (20%) than non-MDR *E. coli* cases (8.3%). The presence of MDR *E. coli* was high in this study. High resistance to commonly used antimicrobial drugs and treatment complications was observed in this study.

Keywords: *Escherichia coli*, dogs, cats, multidrug-resistant, treatment outcomes

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Introduction

Escherichia coli is the most common pathogenic bacteria isolated from companion animals (Bourely *et al.*, 2020). It is an opportunistic pathogen that comprises intestinal and extra-intestinal groups. Extra-intestinal pathogenic *E. coli* (ExPEC) commonly causes urinary tract infections (UTIs) and skin and soft tissue infections in dogs and cats (Saputra *et al.*, 2017; Bourne *et al.*, 2019). The intensive use of antimicrobials in veterinary practice has increased the antimicrobial resistance problem (Gibson *et al.*, 2008). Moreover, multidrug-resistant (MDR) *E. coli*, including resistance to extended-spectrum β-lactamases, has been increasingly reported in companion animals (Gibson *et al.*, 2008). The sharing of resistant and pathogenic *E. coli* strains between pets and close-contact humans has been observed (Bourne *et al.*, 2019). Therefore, treatment of MDR *E. coli* in the veterinary clinic is challenging and a public health concern. The characteristics and treatment of *E. coli* in ill pets vary depending on the site of the infection, the condition of the animal, the antimicrobial profile, available drugs, prescribing regulations and the experiences of veterinarians in different countries (Weese *et al.*, 2019). Moreover, treatment outcome data of MDR *E. coli* infection in dogs and cats is scarce. Therefore, this study analyzed the clinical characteristics, antimicrobial resistance and treatment outcomes of MDR *E. coli* infections in dogs and cats during 2020 at a veterinary teaching hospital in Thailand. The results will provide information for predicting treatment, the antimicrobial resistance situation and provide potential insight into veterinary hospital management.

Materials and Methods

The medical records of Prasu-Arthron Animal Teaching Hospital, Thailand, between January and December 2020 were included for dogs and cats with an *E. coli* infection. The medical data consisted of general signals, disease or site(s) of infection, sampling sites, antimicrobial therapy, duration of antimicrobial treatment and other treatments. The treatment outcomes were determined during the final visit after the *E. coli* infection was observed. A clinical cure case represented clinical remission related to antimicrobial and adjunctive therapy, improved clinical signs, termination of antimicrobial treatment and no repeated bacterial culture or antimicrobial susceptibility testing (AST). In contrast, a non-clinical cure case meant that there had been no improvement or, perhaps, even worse clinical signs, recurrent infection, a prescription for an antimicrobial agent or bacterial culture and AST was needed.

E. coli was isolated from specimens by routine aerobic culture methods at the Veterinary Diagnostic Center of the Faculty of Veterinary Science, Mahidol University. Briefly, the bacteria were grown on tryptic soy agar (TSA) with 5% sheep blood (Thermo Fisher Scientific, Cambridge, UK), McConkey's agar (Thermo Fisher Scientific) and thioglycollate broth (Thermo Fisher Scientific) and were incubated at 35 ± 2°C for 24–72 hours. Single or multiple colonies were selected based on differences in morphological characteristics and were subcultured on TSA with 5% sheep blood to obtain homogeneous colonies. Species were identified based on colony morphology and conventional biochemical tests. *E. coli* positively reacted to indole, methyl red, motility and the ornithine decarboxylase activity test but reacted negatively to the Voges–Proskauer and citrate utilization tests. In addition, *E. coli* produced an acid reaction with the formation of gas in triple sugar iron test medium. The pure or predominant and mixed growth of two or more species was derived. The presence of bacterial concentrations ≥ 10^3–10^5 colony-forming units/ml was classified as a UTI in the urine specimens (Weese *et al.*, 2019). AST was performed in all *E. coli* isolates using the Kirby–Bauer disk diffusion test method. The interpretations for cefpodoxime (10 µg; resistance (R) ≤ 17 mm), ceftazidime (10 µg; R ≤ 17 mm), enrofloxacin (5 µg; R ≤ 16 mm), marbofloxacin (5 µg; R ≤ 14 mm) and gentamicin (30 µg; R ≤ 12 mm) (Oxoid™, Thermo Fisher Scientific) followed the interpretive categories and zone diameter breakpoints for dogs and cats (CLSI VET01S, 2020). The zone diameter breakpoints of ampicillin (10 µg; R ≤ 13 mm), amoxicillin-clavulanic acid (20/10 µg; R ≤ 13 mm), ceftriaxone (30 µg; R ≤ 19 mm), cefotaxime (30 µg; R ≤ 22 mm), sulfamethoxazole/trimethoprim (1.25/23.7 µg; R ≤ 10 mm), amikacin (30 µg; R ≤ 14 mm), tetracycline (30 µg; R ≤ 11 mm) and doxycycline (30 µg; R ≤ 10 mm) (Oxoid™, Thermo Fisher Scientific) utilized the interpretive categories available for humans in CLSI document M100 (CLSI, 2020). MDR strains were the isolates that resisted at least one agent in three or more antimicrobial classes (Sweeney *et al.*, 2018). An intermediate was defined in a susceptible category following the EUCAST 2019 definition (www.eucast.org).

The chi-square and Fisher's exact tests (when n < 5) were used to compare the differences between MDR and non-MDR *E. coli* cases. The independent *t*-test was used to compare the difference between means of antimicrobial treatment duration of the MDR and non-MDR *E. coli* groups. A *P*-value < 0.05 was considered significant. The statistical analysis was performed using SPSS Statistics 18.0 software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Of the 1,009 dog and cat samples, 629 positive bacterial cultures were derived from various sampling sites (62.3%). A total of 105 cases of *E. coli* infection were observed from ill pets (10.4%, 105/1,009). However, the data of cases that were positive for *E. coli* in the gastrointestinal tract that might be contaminated (n = 6) and incomplete clinical data were excluded (n = 5). Of the 94 cases, *E. coli* was obtained from 74 dogs and 20 cats. *E. coli* was more frequently isolated from female dogs (n = 43) than male dogs (n = 31) and was frequently found in pure breeds and dogs over the age of 10-years (Table 1). In contrast, *E. coli* was significantly detected in male cats (n = 11; female n = 8), mixed breed and 1–10-year-old cats (Table 1). There were 43.6% (41/94) bacterial co-infections with *E. coli*; the most frequently found sites were wounds (61%, 25/41) and the urinary tract (12.2%, 5/41). The predominant co-cultured bacteria were *Enterococcus*

spp. (41.5%, 17/41), *Proteus mirabilis* (19.5%, 8/41) and *Streptococcus* spp. (12.2%, 5/41) (data not shown). The MDR *E. coli* infections (66%, 62/94) consisted of isolates from 51 dogs (68.9%, 51/74) and 11 cats (55%, 11/20). The MDR *E. coli* isolates were significantly more prevalent in female dogs (66.7%) than non-MDR *E. coli* (33.3%) ($P < 0.026$) (Table 1). Samples from the urinary tract (41.2%) and skin (35.3%) were the first and second most frequent sites of MDR *E. coli* isolates in dogs. In cats, MDR *E. coli*-infected samples were more related to skin wound infections (90.9%) than non-MDR *E. coli* (33.3%) ($P < 0.017$). The high prevalence of MDR *E. coli* in this study (66%) was concerning but was in concordance with a previous study in the United States (52% MDR and 42% non-MDR) (Thungrat *et al.,* 2015). Veterinarians should be concerned about public health and provide more information to their staff and pet owners about these infections. MDR *E. coli* was isolated more frequently from female dogs with UTIs than from males, which is consistent with previous studies. MDR *E. coli* are overrepresented in female dogs due to anatomical differences in the urinary tract; specifically, the female urethra is shorter and wider than the male counterpart (Hall *et al.,* 2013). In cats, MDR *E. coli* was often isolated in males and associated with wound infections. The reason for the presence of MDR *E. coli* in wound infections might be the high number of outdoor or stray cats in Thailand that often fight with other cats. This result is different from a previous study reporting that *E. coli* from the urinary tract is more frequent (16.7%), while wounds or abscesses were only positive for *E. coli* in 0.6% of cats (Rzewuska *et al.,* 2015). The routes of ExPEC infection in pets include direct contact with humans and animals, environmental contamination and endogenous transfer from the gastrointestinal tract (Gibson *et al.,* 2008). However, the specific sources of *E. coli* could not be clarified from the medical records in this study. Moreover, it was difficult to distinguish between a true pathogenic infection and contamination by *E. coli* in the suspected bacterial infection cases. Thus, further investigation, examination of bacteria and inflammatory cells in cytology sample, molecular epidemiology, may be needed.

Table 1 Clinical characteristics and treatment outcomes of multidrug-resistant (MDR) *Escherichia coli* and non-MDR *E. coli* infections in dogs and cats

Characteristics	Dogs (n=74)			Cats (n=20)		
	% MDR (n=51)	% Non-MDR (n=23)	*P*-value	% MDR (n=11)	% Non-MDR (n=9)	*P*-value
Sex						
Male	33.3 (17)	60.9 (14)	0.026*	72.7 (8)	33.3 (3)	0.175
Female	66.7 (34)	39.1 (9)	0.026*	27.2 (3)	55.6 (5)	0.326
Unknown	0 (0)	0 (0)	N/A[a]	0 (0)	11.1 (1)	N/A
Breed						
Purebred	56.9 (29)	60.9 (14)	0.746	18.2 (2)	22.2 (2)	0.999
Mixed breed	43.1 (22)	39.1 (9)	0.746	81.8 (9)	77.8 (7)	0.999
Age						
<1 y	3.9 (2)	0 (0)	N/A	27.2 (3)	0 (0)	N/A
1-10 y	31.4 (16)	56.5 (13)	0.040*	72.7 (8)	55.6 (5)	0.642
>10 y	64.7 (33)	43.5 (10)	0.087	0 (0)	22.2 (2)	N/A
Unknown	0 (0)	0 (0)	N/A	0 (0)	22.2 (2)	N/A
Site of infections						
Urinary tract	41.2 (21)	52.2 (12)	0.378	9.1 (1)	44.4 (4)	0.127
Skin (wound and dermatitis)	35.3 (18)	30.4 (7)	0.683	90.9 (10)	33.3 (3)	0.017*
Abdominal cavity	11.8 (6)	13 (3)	0.876	0 (0)	0 (0)	N/A
Other infection [b]	11.8 (6)	4.3 (1)	0.313	0 (0)	22.2 (2)	N/A
Treatment outcomes	% MDR (n=30)	% Non-MDR (n=9)	*P*-value	% MDR (n=5)	% Non-MDR (n=3)	*P*-value
Clinical cure	63.3 (19)	66.7 (6)	0.855	80 (4)	66.7 (2)	0.999
Antimicrobial monotherapy	84.2 (16)	83.3 (5)	0.999	100 (4)	100 (2)	0.999
Adjunctive therapy [c]	15.8 (3)	16.7 (1)	0.999	0 (0)	0 (0)	N/A
Non-clinical cure	36.7 (11)	33.3 (3)	0.999	20 (1)	33.3 (1)	0.999
Antimicrobial monotherapy	72.7 (8)	100 (3)	0.999	0 (0)	100 (1)	N/A
Adjunctive therapy [d]	27.3 (3)	0 (0)	N/A	100 (1)	0 (0)	N/A
Duration of antimicrobial treatment (day) (mean ± standard deviation)	19.33 (±15.56)	21 (±11.61)	0.758	12.6 (±5.85)	7 (±0)	0.048*

* Statistically significant at $P < 0.05$.

[a] Not available.

[b] ear (dogs, n = 4), reproductive tract (dogs, n = 3), plural cavity (cats, n = 2).

[c] marbofloxacin + metronidazole (dog, n = 1), sulfamethoxazole/trimethoprim + metronidazole (dog, n = 1), amoxicillin–clavulanic acid + marbofloxacin+ doxycycline (dog, n = 1), marbofloxacin+ gentamicin sulfate ointment (dog, n = 1).

[d] ceftriaxone + amikacin gel (cat, n = 1), clindamycin+ amoxicillin–clavulanic acid (dog, n = 1), amoxicillin–clavulanic acid+marbofloxacin (dog, n = 1), sulfamethoxazole/trimethoprim+doxycycline (dog, n = 1).

The MDR *E. coli* in dogs was highly resistant to ampicillin (96.1%), enrofloxacin (80.4%), tetracycline (78.4%) and doxycycline (72.5%) (Fig. 1). Resistance < 20% was only observed to amikacin (19.6%). High resistance to ampicillin (100%), enrofloxacin (90.9%), marbofloxacin (72.7%) and tetracycline (72.7%) was observed in the MDR *E. coli* from cats. Cats had the lowest resistance to amikacin (36.4%) (Fig. 1). The resistance rates differed from a previous Australian study of clinical *E. coli*. The authors of that study reported high resistance to amoxicillin-clavulanic acid (dogs = 45.5%, cats = 100%) but low resistance to enrofloxacin (dogs = 9.3%, cats = 3.2%) (Saputra *et al.*, 2017). Low resistance to amikacin and gentamicin in the present study is consistent with previous studies on *E. coli* isolates from companion animals (Saputra *et al.*, 2017; Thungrat *et al.*, 2015). Amikacin is not recommended for routine use but may be useful for treating MDR organisms in bacterial UTIs, respiratory tract infections and superficial bacterial folliculitis in dogs and cats (Hillier *et al.*, 2014; Lappin *et al.*, 2017; Weese *et al.*, 2019). The development of antibiotic stewardship programs and updated antimicrobial use guidelines, particularly for Thailand, are recommended to reduce the spread of MDR *E. coli* (Guzman Ramos *et al.*, 2021).

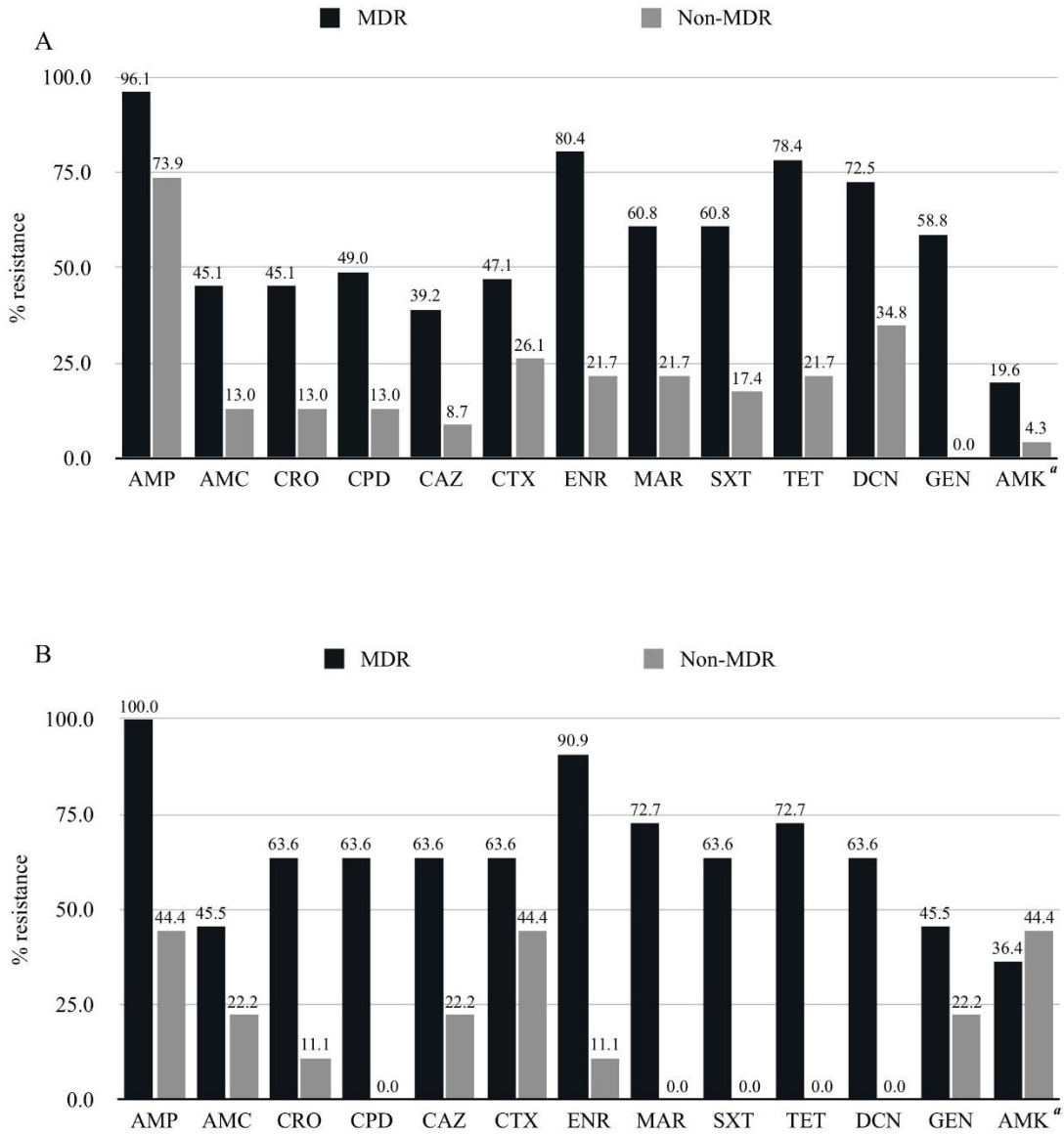

Figure 1 Frequency (%) of antimicrobial resistance of multidrug-resistant (MDR) *Escherichia coli* and non-MDR *E. coli* isolates from dogs (A) and cats (B)

[a] AMP, ampicillin, AMC, amoxicillin-clavulanic acid, CRO, ceftriaxone, CPD, cefpodoxime, CAZ, ceftazidime, CTX, cefotaxime, ENR, enrofloxacin, MAR, marbofloxacin, SXT, sulfamethoxazole/trimethoprim, TET, tetracyclines, DCN, doxycycline, AMK, amikacin, GEN, gentamicin.

A total of 56.5% (35/62) and 37.5% (12/32) of the MDR and non-MDR *E. coli* cases, respectively, were followed up for the final assessment (Table 1). The treatment outcomes in terms of clinical cure and non-clinical cure of MDR and non-MDR *E. coli* were not significantly different in dogs and cats (Table 1). The cases were typically treated with antimicrobial monotherapy, specifically amoxicillin-clavulanic acid, marbofloxacin or enrofloxacin. Adjunctive therapy tended to be more frequently used in dogs and cats with MDR *E. coli* infections (20%, 7/35) than in those with non-MDR *E. coli* infections (8.3%, 1/12) (P = 0.659). Other treatments, such as surgery, ear cleaning and wound dressing, were also performed, leading to the successful treatment of *E. coli* infections. The average antimicrobial treatment duration for MDR and non-MDR *E. coli* in dogs was quite similar at 19 and 21 days, respectively (Table 1). In contrast, the duration of antimicrobial treatment in cats with MDR *E. coli* (12.6 ± 5.85 days) was significantly longer than that of non-MDR *E. coli* (7 ± 0 days) (P = 0.048). In this study, the treatment outcome data was available for 56.5% and 37.5% of the MDR *E. coli* and non-MDR *E. coli* cases, respectively. The cases lost to follow-up could have been due to inconvenience to the owner or economic concerns, or the ill pets may have shown improved clinical signs. Empirical antimicrobial therapy was generally prescribed while waiting for the results from bacterial culture and AST. However, we observed cases that were prescribed with resistant drugs based on the AST but still showed improved clinical signs. This finding was reported by a previous study (Gibson *et al.,* 2008). Apart from antimicrobial use, the clinical cure cases were associated with identifying and removing the underlying cause. Although the rate of clinical cure and non-clinical cure cases and duration of antimicrobial treatment were not significantly different between the MDR and non-MDR *E. coli* cases, pets with a MDR *E. coli* infection were more often treated with adjunctive therapy. In addition, the duration of antimicrobial treatment for an MDR *E. coli* infection was longer than that for a non-MDR *E. coli* infection in cats. This finding highlights that treatment for MDR *E. coli* is more complex than that for non-MDR *E. coli*. More follow-up data would be useful for further study. Moreover, this study used the data available from one veterinary teaching hospital. Future investigations with additional data from many veterinary hospitals will provide more details for managing MDR *E. coli* infections in companion animals.

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