

Efficacy of Fosfomycin on *Escherichia coli* Isolated from Bitches with Pyometra

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ABSTRACT. The aim of this study was to determine the antimicrobial resistance of *Escherichia coli* isolated from the uteri of bitches with pyometra, and 38 *E. coli* isolates were used. The antimicrobials used were ampicillin (ABPC), amoxicillin/clavulanic acid, gentamicin, minocycline, cefazolin, levofloxacin (LVFX), trimethoprim-sulfamethoxazole (ST) and fosfomycin (FOM). Resistance to ABPC occurred most frequently, followed by LVFX and ST. Multi-drug resistance, defined as resistance against 3 or more classes of antimicrobials, was found in 23.7% of all isolates. Nine out of 13 resistant strains were multi-drug resistant, but no strain was found to be resistant to FOM. This suggests that FOM should be administered for *E. coli* from pyometra.

KEY WORDS: antimicrobial resistance, bitch, *Escherichia coli*, fosfomycin, pyometra.

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Pyometra is regarded as one of the most common illnesses in bitches [10, 16, 18]. Its etiology and pathogenesis are complex and only partly understood [11, 14, 16, 17, 19]. The most common bacterium isolated in cases of pyometra is *Escherichia coli* (*E. coli*) [1, 5, 8, 11, 16]. There have been a few studies of resistance to antimicrobials among bacteria isolated from the uteri of bitches with pyometra [4, 7, 9, 15, 20]. The resistant ratio of *E. coli* isolated from pyometra is reported to be various, but is mostly lower than urine samples [9, 18].

Fosfomycin is a cell-wall-active antimicrobial, classified as bactericidal in action, with a broad spectrum including Gram-negative and Gram-positive organisms [3, 13]. Hubka and Boothe suggested that fosfomycin shows effective antimicrobial activity against *E. coli* associated with spontaneous disease in dogs and cats, including isolates expressing multi-drug resistant isolates cultured from the urinary tract [12].

The aim of this study was to determine the antimicrobial resistance of *E. coli* isolated from the uteri of bitches with pyometra.

Samples were taken from 38 bitches that underwent ovariohysterectomy, because of pyometra at Fukuda Animal Hospital (Osaka, Japan) between April 2009 and the end of March 2012. Each sample from a uterus suffering from pyometra was plated onto desoxycholate-hydrogen sulfide-lactose (DHL) agar (Eiken Chemical Co., Ltd., Tokyo, Japan). After incubation at 37°C for 18 hr, two to four red colonies were identified using miniaturized biochemical systems (API System; bioMérieux, Lion, France). One strain was selected from 2 to 4 colonies with the same biological properties. As a result, 38 *E. coli* isolates were obtained from the uterus. *E. coli* isolates were stored in MicroBANK (Pro-

Lab Diagnostic, Richmond Hill, ON, Canada) at –80°C until further use.

Susceptibility to each of 8 antimicrobials was determined using the agar dilution method according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI 2010) [2]. The antimicrobials used were ampicillin (ABPC), amoxicillin/clavulanic acid (CVA-AMPC), gentamicin (GM), minocycline (MINO), cefazolin (CEZ), levofloxacin (LVFX), trimethoprim-sulfamethoxazole (ST) and fosfomycin (FOM).

Resistance to ABPC occurred most frequently (11 isolates, 28.9%), followed by LVFX and ST (7 isolates, 18.4%) (Table 1). Multidrug resistance, defined as resistance against 3 or more classes of antimicrobials, was found in 23.7% of all isolates (Table 2). The most dominant resistant phenotype was ABPC. Resistance to 1 or more of the antimicrobials tested was observed in 13 isolates (34.2%). One strain was resistant to 7 out of 8 drugs, and 2 strains were resistant to 6 drugs. Nine out of 13 resistant strains were multi-drug resistant, but no strain was found to be resistant to FOM.

Resistance to ABPC was the most commonly observed trait among the isolates of *E. coli* from cases of pyometra. We supposed that ABPC is used for long time and many strain got resistant in ABPC, but FOM is not used routinely and many strain did not get resistant in FOM. Hagman and Greko indicated in Sweden that about 10% of strains were

Table 1. Prevalence of antimicrobial resistance among 38 *E. coli* isolates from bitches with pyometra

Antimicrobial	No. of isolates (%)
Ampicillin	11 (29.9)
Levofloxacin	7 (18.4)
Trimethoprim-sulfamethoxazole	7 (18.4)
Amoxicillin/clavulanic acid	5 (13.2)
Minocycline	5 (13.2)
Cefazolin	5 (13.2)
Gentamicin	5 (13.2)
Fosfomycin	0 (0)

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Table 2. Distribution of multidrug resistance among 38 *E. coli* isolates from bitches with pyometra

Antimicrobials								No. of isolates N=38
7	ABPC	CVA-AMPC	GM	MINO	CEZ	LVFX	ST	1
6	ABPC	CVA-AMPC	GM	CEZ	LVFX	ST		1
	ABPC	CVA-AMPC	MINO	CEZ	LVFX	ST		1
5	ABPC	CVA-AMPC	GM	CEZ	LVFX			2
4	ABPC	GM	MINO	ST				1
3	ABPC	MINO	ST					2
	ABPC	LVFX	ST					1
2	ABPC	CVA-AMPC						1
1	ABPC							1
	LVFX							1
	MINO							1
Non-resistant								25

ABPC: ampicillin, CVA-AMPC: amoxicillin/clavulanic acid, GM: gentamicin, MINO: minocycline, CEZ: cefazolin, LVFX: levofloxacin, ST: trimethoprim-sulfamethoxazole.

resistant to ABPC in 2002 [9], and Shimada *et al.* reported in Japan that no multi-drug resistant strain was isolated in 2011 [18]. But, in our results, they were 34%, and multi-drug resistance was higher than the results that they reported. Therefore, it was suggested that multi-drug resistant *E. coli* isolates from pyometra were increased without being varying by country or time. Hubka and Boothe suggested that FOM showed effective antimicrobial activity against *E. coli* associated with spontaneous disease in dogs and cats, including isolates expressing multi-drug resistance cultured from the urinary tract [12]. In our study, FOM showed effective antimicrobial activity against *E. coli* from canine pyometra. This suggests that FOM should be administered for *E. coli* from canine pyometra, but this drug should not be administered to cats [6].

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