

***In vitro* activity of cefovecin, extended-spectrum cephalosporin, against 284 clinical isolates collected from cats and dogs in Croatia**

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ABSTRACT

During the six-month collection period, 284 bacterial strains were recovered from different clinical samples from cats and dogs. Susceptibility to cefovecin was determined by disk-diffusion method. Out of 284 bacterial isolates, 202 were gram-positive bacteria and 82 were gram-negative. The most frequently isolated bacterial species was gram-positive *Staphylococcus pseudintermedius* (152), followed by streptococci (50). The most common gram-negative bacteria were *Pseudomonas aeruginosa* (26), *Proteus* spp. (25) and *E. coli* (22). The highest resistance among gram-positive bacteria was observed in non-hemolytic streptococci (50%) and *Staphylococcus pseudintermedius* (7.2%). Beta-hemolytic streptococci were 100% sensitive to cefovecin. As expected, cefovecin did not show any activity against *P. aeruginosa*. Besides that, the highest resistance was shown by *E. coli* (40.9%) and *Proteus* spp. (8%). All *P. multocida* isolates were sensitive to cefovecin. The results of the conducted survey show no significant differences of cefovecin activity against beta-hemolytic streptococci and *P. multocida* isolates from EU and USA compared to Croatian strains, when tested by the broth microdilution and disk-diffusion method. However, resistance rates in *S. pseudintermedius*, *E. coli* and *Proteus* spp. are much higher among the Croatian strains.

Key words: cefovecin, antimicrobial susceptibility, disk-diffusion, *Staphylococcus pseudintermedius*, *E. coli*, *Proteus* spp.

Introduction

Cephalosporins are widely used in veterinary medicine based on their broad spectrum activity and safety. They are used for the treatment of bacterial infections of the skin, urinary and genital tracts, respiratory tract, infections of bones and joints, soft tissues as well as prophylactic use in abdominal surgery (GREENE and WATSON, 2001). Many antimicrobial agents require administration at least twice a day for at least seven

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days, some even more often and for a longer period (several weeks) depending on the antimicrobial agent and affected organ. Furthermore, some cephalosporin antibiotics are only available for parenteral administration so the owner has to go to a clinic every day, which causes additional stress for the animal and increases the cost of treatment.

Cefovecin (Convenia®, Pfizer Animal Health) is a new third-generation cephalosporin antibiotic approved for use in cats and dogs since 2006 in EU and 2008 in USA. The drug has a very long half-life, so that a single injection lasts 14 days in cats and dogs. Cefovecin differs from other cephalosporins in that it is highly protein bound and has a long duration of activity (STEGEMANN et al., 2006b; STEGEMANN et al., 2006c). As with all cephalosporins, the bactericidal action of cefovecin results from the inhibition of bacterial cell wall synthesis. The major benefit of cefovecin is its long duration of activity. The product is well tolerated and has minor side effects (PASSMORE et al., 2007; PASSMORE et al., 2008; STEGEMANN et al., 2007a; STEGEMANN et al., 2007b).

The objective of this study was to determine the *in vitro* activity of cefovecin against different gram-positive and gram-negative aerobic bacteria isolated from samples from different pathological conditions in cats and dogs. The main purpose of this investigation was to obtain an overview of the susceptibility of most common aerobic bacteria isolated from animals to cefovecin. We considered such a survey important because the use of cefovecin in Croatia began just recently, after its approval in 2008.

Materials and methods

In a six-month period specimens originating from cats and dogs visiting The Clinics of the University of Zagreb Veterinary Faculty were submitted to microbiological examination and a total of 284 different bacterial strains were isolated. Bacterial strains were isolated from: the external ear canal (133), skin (61), urine (21), throat (18), conjunctival sac (13), nostrils (11), infected wounds (9), genital tract (10), interdigital area (6), and rectum (2). Identification of isolated bacteria was carried out according to procedures described by QUINN et al. (1994; 2002). In the present study, 284 bacterial isolates were tested on Mueller-Hinton agar for antimicrobial susceptibility to cefovecin by the Kirby-Bauer's disk-diffusion method recommended by the Clinical and Laboratory Standards Institute (ANONYM., 2008). Disks (Pfizer Animal Health) with 30 µg of cefovecin were used. The following pathogens were tested: *Staphylococcus (S.) pseudintermedius* (formerly identified as *S. intermedius*) (n = 152), non-hemolytic *Streptococcus* spp. (n = 28), beta-hemolytic *Streptococcus* spp. (n = 22), *P. aeruginosa* (n = 26), *E. coli* (n = 22), *Proteus* spp. (n = 25) and *P. multocida* (n = 9). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as control strains (the diameter of inhibition zone around 30 µg cefovecin disk was 26 and 30 mm, respectively).

The culture was incubated for 18-24 hours at 37 °C. The zone of inhibition around disk was measured, and the measurement compared to a data presented in Table 1, that classify the organism into three categories: sensitive, resistant and intermediate sensitive.

Results

The antimicrobial susceptibility testing results of all isolated bacteria are presented in Table 2. Out of 284 bacterial isolates 202 isolates were gram-positive bacteria and 82 were gram-negative. The most frequently isolated bacterial species was gram-positive *S. pseudintermedius* (152) and 141 strains (92.8%) were susceptible to cefovecin. *Streptococci* were, beside *S. pseudintermedius*, most often isolated gram-positive bacteria. Out of 50 isolated strains, 28 were non-hemolytic and 22 strains were β -hemolytic. Interestingly, the highest resistance to cefovecin was present in non-hemolytic strains, where 14 strains (50%) were resistant. β -hemolytic streptococci were 100% sensitive to cefovecin.

Table 1. MIC and zone diameter interpretive criteria for cefovecin (as proposed in Convenia Injectable Lyophile (Canada)[®] package insert)

MIC and zone diameter (30 μ g disk) interpretive chart for cefovecin		
MIC (μ g/mL)	Zone diameter (mm)	Interpretation
≤ 2	≥ 23	susceptible (S)
4	20-22	intermediate (I)
≥ 8	≤ 19	resistant (R)

Table 2. Antimicrobial susceptibility to cefovecin in 284 clinical isolates tested by disk-diffusion method

Bacteria	S		I		R		Total number
	No	%	No	%	No	%	
Gram - positive							
<i>Staphylococcus pseudintermedius</i>	141	92.8	0	0	11	7.2	152
<i>Streptococcus</i> spp. (non-hemolytic, including enterococci)	14	50	0	0	14	50	28
<i>Streptococcus</i> spp. β -hemolytic	22	100	0	0	0	0	22
Total							202
Gram - negative							
<i>Pasteurella multocida</i>	9	100	0	0	0	0	9
<i>Proteus</i> spp.	23	92	0	0	2	8	25
<i>Escherichia coli</i>	13	59.1	0	0	9	40.9	22
<i>Pseudomonas aeruginosa</i>	0	0	0	0	26	100	26
Total							82

All 26 *P. aeruginosa* isolates were resistant to cefovecin. *Proteus* spp. was isolated 25 times and 23 strains (92.0%) were sensitive and two (8%) were resistant. Out of 22 *E. coli* isolates 13 (59.1%) were sensitive and 9 (40.9%) strains were resistant. All *P. multocida* isolates were sensitive to cefovecin.

Discussion

Cefovecin (Convenia[®], Pfizer Animal Health) is a new extended-spectrum cephalosporin that was introduced in small animal practice in Croatia in year 2008. The antimicrobial activity of cefovecin is similar to the other cephalosporin antibiotics, which share a low toxicity and good activity against many gram-positive and gram-negative aerobic bacteria. Cephalexin's in-vitro activity was previously tested in parallel to cefovecin investigating more than 2,600 isolates (STEGEMANN et al., 2006a). Unfortunately, very few papers on this subject are available and it is difficult to discuss results obtained in this study. However, STEGEMANN et al. (2006a) investigated antimicrobial susceptibility to cefovecin in 2641 bacterial strains isolated from dogs and cats. Bacterial isolates from different European countries and USA were predominantly *Staphylococcus intermedius*, *E. coli*, *Pasteurella multocida*, and *Proteus mirabilis*. MIC determination was performed for cefovecin, cephalexin, cefadroxil and amoxicillin-clavulanic acid. Although clinical breakpoints for cefovecin have not yet been submitted to CLSI, the criteria presented in Table 1, proposed in the package insert of Convenia Injectable Lyophile (Canada)[®] (Pfizer Animal Health, Pfizer Canada Inc.), were used.

According to the results of STEGEMANN et al. (2006a) cefovecin exhibited good *in vitro* activity against 501 *S. intermedius* USA and European isolates with MIC₉₀ value of 0.25 µg/mL. Five isolates (<1%) had MIC values ≥8 µg/mL and were considered resistant. In our study, 11 (7.2%) isolates of *S. pseudintermedius* were resistant to cefovecin which is a much higher resistance rate. Five of them were *mecA* positive and considered methicillin-resistant.

Interestingly, the highest resistance to cefovecin was present in non-hemolytic streptococci, where, out of 28 isolates 14 (50%) were resistant. As they were not identified to the species level, the reason for such high resistance might be the possibility that a large proportion of them were actually enterococci. STEGEMANN et al. (2006a) determined that MIC₉₀ for *Enterococcus* spp. was >32 µg/mL, so more than 90% of isolates in that study can be considered resistant to cefovecin. In our study no resistance was noted among β-hemolytic streptococci, which is in complete agreement with previous findings (STEGEMANN et al., 2006a). This is very important, because it is well known that *Streptococcus canis*, a very common inhabitant of dog's skin, and a frequent cause of vaginitis, pyoderma and otitis externa, is β-hemolytic.

Among the gram-negative bacterial isolates, there was no resistance in *P. multocida*, in general sensitive to the majority of antimicrobial agents. This is in agreement with the results obtained by STEGEMANN et al. (2006a), where all 381 strains tested exhibited MICs of ≤ 2 $\mu\text{g/mL}$, which means they were all sensitive to cefovecin. A slightly different result was obtained with *Proteus* spp., where, out of 25 isolates tested, 23 were sensitive (92%) and two strains (8%) were resistant. STEGEMANN et al. (2006a) found only one *Proteus* sp. resistant strain out of 71 tested isolates, which is a much lower rate than determined in this study (8%). Among the 22 *E. coli* strains, isolated mainly from dogs with urinary tract infections, 40.9% were resistant, compared to less than 2% determined by STEGEMANN et al. (2006a). The majority of resistant *E. coli* isolates in our study were also resistant to amoxicillin-clavulanic acid and cephalexin, probably because of the production of extended spectrum beta-lactamase. The reason for such a high resistance rate in our study might be previous treatment with antimicrobials, which was noted in most of dogs with UTI.

Lastly, besides having intrinsic resistance to I and II generation cephalosporins, all *Pseudomonas aeruginosa* isolates were also resistant to cefovecin, which confirms previous findings (STEGEMANN et al., 2006a).

The results of the survey conducted show no significant differences in cefovecin activity against beta-hemolytic streptococci and *P. multocida* isolates from the EU and USA compared to Croatian strains, when tested by broth microdilution and disk-diffusion methods. However, resistance rates in *S. pseudintermedius*, *E. coli* and *Proteus* spp. are much higher among the Croatian strains. The reason might be that cefovecin was already on the market for at least one year before the beginning of this study. Also, isolates tested in the study by STEGEMANN et al. (2006a) were collected between 1999 and 2003, which is five to nine years earlier. Many of the isolates in our study are from problematic cases that were previously empirically treated with antimicrobials until samples were submitted to our laboratory for culture and susceptibility testing.

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SAŽETAK

Tijekom šestomjesečnog razdoblja 284 izolata bakterija izdvojena su iz različitih kliničkih uzoraka podrijetlom od pasa i mačaka. Osjetljivost sojeva na cefovecin određena je disk-difuzijskom metodom. Od 284 izolata 202 bila su gram-pozitivne, a 82 gram-negativne bakterije. Najzastupljenija je bila vrsta *Staphylococcus*

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pseudintermedius (152), a na drugom mjestu streptokoki (50). Najčešće gram-negativne vrste bile su *Pseudomonas aeruginosa* (26), *Proteus* spp. (25) i *E. coli* (22). Najviša stopa rezistencije među gram-pozitivnim bakterijama utvrđena je u nehemolitičkih streptokoka (50%) i u vrste *Staphylococcus pseudintermedius* (7,2%). Beta-hemolitički streptokoki bili su 100% osjetljivi na cefovecin. Kao što je bilo očekivano, cefovecin nije bio djelotvoran na vrstu *P. aeruginosa*. Vrlo visok postotak rezistentnih sojeva utvrđen je i kod vrsta *E. coli* (40,9%) i *Proteus* spp. (8%). Svi izolati *P. multocida* bili su osjetljivi na cefovecin. Ovi rezultati u skladu su s onima dobivenim određivanjem osjetljivosti beta-hemolitičkih streptokoka i sojeva *P. multocida* podrijetlom iz SAD-a i Europske Unije mikrodilucijskom metodom. To se ne može reći za izolate *S. pseudintermedius*, *E. coli* i *Proteus* spp., u kojih je utvrđen znatno viši postotak otpornih sojeva.

Ključne riječi: cefovecin, antimikrobni lijekovi, disk-difuzijska metoda, *Staphylococcus pseudintermedius*, *E. coli*, *Proteus* spp.
