AAC
Journals.ASM.org

Ceftaroline versus Isolates from Animal Bite Wounds: Comparative *In Vitro* Activities against 243 Isolates, Including 156 *Pasteurella* Species Isolates

Ellie J. C. Goldstein,[a,b] Diane M. Citron,[a] C. Vreni Merriam,[a] and Kerin L. Tyrrell[a]

R. M. Alden Research Laboratory, Culver City, California, USA,[a] and David Geffen School of Medicine at UCLA, Los Angeles, California, USA[b]

More than 5 million Americans are bitten by animals, usually dogs, annually. Bite patients comprise ~1% of all patients who visit emergency departments (300,000/year), and approximately 10,000 require hospitalization and intravenous antibiotics. Ceftaroline is the bioactive component of the prodrug ceftaroline fosamil, which is FDA approved for the treatment of acute bacterial skin and skin structure infections (ABSSSIs), including those containing methicillin-resistant *Staphylococcus aureus* (MRSA). There are no *in vitro* data about the activity of ceftaroline against *Pasteurella multocida* subsp. *multocida* and *Pasteurella multocida* subsp. *septica*, other *Pasteurella* spp., or other bite wound isolates. We therefore studied the *in vitro* activity of ceftaroline against 243 animal bite isolates. MICs were determined using the broth microdilution method according to CLSI guidelines. Comparator drugs included cefazolin, ceftriaxone, ertapenem, ampicillin-sulbactam, azithromycin, doxycycline, and sulfamethoxazole-trimethoprim (SMX-TMP). Ceftaroline was the most active agent against all 5 *Pasteurella* species, including *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica*, with a maximum MIC of ≤0.008 μg/ml; more active than ceftriaxone and ertapenem (MIC_{90}s, ≤0.015 μg/ml); and more active than cefazolin (MIC_{90}, 0.5 μg/ml) doxycycline (MIC_{90}, 0.125 μg/ml), azithromycin (MIC_{90}, 0.5 μg/ml), ampicillin-sulbactam (MIC_{90}, 0.125 μg/ml), and SMX-TMP (MIC_{90}, 0.125 μg/ml). Ceftaroline was also very active against all *S. aureus* isolates (MIC_{90}, 0.125 μg/ml) and other *Staphylococcus* and *Streptococcus* species, with a maximum MIC of 0.125 μg/ml against all bite isolates tested. Ceftaroline has potential clinical utility against infections involving *P. multocida*, other *Pasteurella* species, and aerobic Gram-positive isolates, including *S. aureus*.

In 2011, the Humane Society of the United States estimated that 78.2 million dogs and 86.4 million cats were kept as pets in 62% of American households (19). Bites occur in 4.7 million Americans yearly, which extrapolates to one of every two Americans being bitten in their lifetime, usually by a dog (14–16). Animal bite wounds account for 800,000 medical visits annually and comprise the reason for approximately 1% of all emergency department visits (29). It has been estimated that 3 to 18% of dog bites and 28 to 80% of cat bites will become infected (29). Of the 300,000 bite patients who visit an emergency department, approximately 10,000 will require hospitalization and intravenous antibiotics (24, 27). Bite wounds that require attention are often those to the extremities, especially the person's dominant hand, and those caused by larger dogs, which can exert more than 450 pounds/inch^2 of pressure with their jaws, leading to extensive crush injury (14, 15, 18, 27).

While there are a plethora of bacteria isolated from animal bite wounds (2, 27), *Pasteurella* species are present in 75% of infected cat bite wounds (*Pasteurella multocida* subsp. *multocida* in 54%, *P. multocida* subsp. *septica* in 28%) and in 50% of infected dog bite wounds (*P. canis* in 50%, *P. multocida* subsp. *multocida* in 12%, *P. multocida* subsp. *septica* in 10%) (27). However, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA; USA300 clone) in infections shared between pets and human handlers (22) raises the issue of whether treatment of severe animal bite wounds requires MRSA coverage. Currently recommended regimens include amoxicillin-clavulanate orally and ampicillin-sulbactam, carbapenems, and cefoxitin intravenously (26), but all of these lack activity against MRSA.

Ceftaroline fosamil is FDA approved for the treatment of acute bacterial skin and skin structure infections (ABSSSIs), including those containing MRSA. There are scant data about the activity of ceftaroline against *Pasteurella multocida* subsp. *multocida* and *P. multocida* subsp. *septica*, other *Pasteurella* spp., or other bite isolates. Ge et al. (13) reported the *in vitro* activity of ceftaroline against 22 *P. multocida* isolates whose subspecies were not determined and found it to have good activity. As there are no data about the activity of ceftaroline against the specific *Pasteurella* species, including *Pasteurella multocida* subsp. *multocida*, *P. multocida* subsp. *septica*, *P. dagmatis*, *P. canis*, *P. stomatis*, or other *Pasteurella* spp., or its activity against other bite isolates, including *Staphylococcus* spp. and *Streptococcus* spp., we therefore studied the *in vitro* activity of ceftaroline against 243 animal bite isolates, including 156 *Pasteurella* strains.

MATERIALS AND METHODS

Bacterial strains. The organisms were recovered from human clinical samples, identified by standard methods (21), and stored in 20% skim milk at −70°C. They were taken from the freezer and transferred at least twice on blood agar to ensure purity and good growth.

Broth dilution tests. Broth microdilution trays were prepared in-house using a Quick-Spense apparatus (100 μl/well; Sandy Spring Instrument Co. Inc., Germantown, MD) and frozen at −70°C until use. Cell paste from 48-h cultures was suspended in brucella broth, further diluted

Received 29 August 2012 Returned for modification 14 September 2012
Accepted 23 September 2012
Published ahead of print 1 October 2012
Address correspondence to Ellie J. C. Goldstein, ejcgmd@aol.com.

doi:10.1128/AAC.01794-12

TABLE 1 Comparative *in vitro* activity of ceftaroline and 7 other agents against 243 animal bite wound isolates, including 156 *Pasteurella* species[a]

Organism (no. of isolates)	Agent	MIC (μg/ml)		
		Range	50%	90%
P. canis (23)	Ceftaroline	≤0.008	≤0.008	≤0.008
	Cefazolin	0.06–0.5	0.25	0.5
	Ceftriaxone	≤0.015	≤0.015	≤0.015
	Ampicillin-sulbactam	≤0.015–0.125	0.03	0.06
	Ertapenem	≤0.015	≤0.015	≤0.015
	Azithromycin	0.06–0.125	0.125	0.125
	Doxycycline	0.06–0.125	0.125	0.125
	SMX-TMP	0.03–0.125	0.03	0.06
P. dagmatis (13)	Ceftaroline	≤0.008	≤0.008	≤0.008
	Cefazolin	0.125–0.5	0.25	0.5
	Ceftriaxone	≤0.015	≤0.015	≤0.015
	Ampicillin-sulbactam	0.03–0.06	0.06	0.06
	Ertapenem	≤0.015	≤0.015	≤0.015
	Azithromycin	0.03–0.125	0.06	0.06
	Doxycycline	0.125	0.125	0.125
	SMX-TMP	0.03–0.125	0.06	0.06
P. stomatis (20)	Ceftaroline	≤0.008	≤0.008	≤0.008
	Cefazolin	0.125–0.5	0.25	0.5
	Ceftriaxone	≤0.015	≤0.015	≤0.015
	Ampicillin-sulbactam	0.03–0.06	0.06	0.06
	Ertapenem	≤0.015	≤0.015	≤0.015
	Azithromycin	0.03–0.125	0.06	0.06
	Doxycycline	0.125	0.125	0.125
	SMX-TMP	0.03–0.125	0.06	0.06
P. multocida subsp. *multocida* (50)	Ceftaroline	≤0.008	≤0.008	≤0.008
	Cefazolin	0.06–1	0.5	0.5
	Ceftriaxone	≤0.015	≤0.015	≤0.015
	Ampicillin-sulbactam	≤0.015–0.125	0.06	0.125
	Ertapenem	≤0.015–0.03	≤0.015	≤0.015
	Azithromycin	0.03–1	0.25	1
	Doxycycline	0.06–0.5	0.125	0.125
	SMX-TMP	0.015–0.125	0.06	0.125
P. multocida subsp. *septica* (50)	Ceftaroline	≤0.008	≤0.008	≤0.008
	Cefazolin	0.125–1	0.5	0.5
	Ceftriaxone	≤0.015–0.6	≤0.015	≤0.015
	Ampicillin-sulbactam	0.03–0.125	0.125	0.125
	Ertapenem	≤0.015	≤0.015	≤0.015
	Azithromycin	0.03–1	0.125	1
	Doxycycline	0.06–0.25	0.125	0.125
	SMX-TMP	0.015–0.25	0.06	0.125
S. aureus (30)	Ceftaroline	0.06–0.25	0.125	0.125
	Cefazolin	0.06–2	0.5	0.5
	Ceftriaxone	1–16	2	2
	Ampicillin-sulbactam	≤0.015–1	0.5	1
	Ertapenem	0.06–0.5	0.06	0.125
	Azithromycin	1–2	2	2
	Doxycycline	0.03–2	0.06	0.125
	SMX-TMP	0.06–0.125	0.06	0.125
S. epidermidis (12)	Ceftaroline	0.03–0.125	0.03	0.125
	Cefazolin	0.125–4	0.25	4
	Ceftriaxone	1–>16	1	16
	Ampicillin-sulbactam	0.015–2	0.125	1
	Ertapenem	0.06–8	0.125	2
	Azithromycin	2	2	2
	Doxycycline	0.06–8	0.125	1
	SMX-TMP	0.06–8	0.125	0.25

(Continued on following page)

TABLE 1 (Continued)

Organism (no. of isolates)	Agent	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus species[b] (15)	Ceftaroline	0.015–0.125	0.06	0.06
	Cefazolin	0.06–16	0.25	1
	Ceftriaxone	1–16	2	4
	Ampicillin-sulbactam	0.03–1	0.06	0.5
	Ertapenem	0.06–1	0.125	0.125
	Azithromycin	1–4	2	2
	Doxycycline	0.03–2	0.06	0.125
	SMX-TMP	0.06–0.25	0.125	0.25
Streptococcus sanguis (10)	Ceftaroline	≤0.008–0.015	≤0.008	0.015
	Cefazolin	0.06–0.5	0.25	0.5
	Ceftriaxone	0.03–0.5	0.125	0.25
	Ampicillin-sulbactam	0.015–0.06	0.03	0.06
	Ertapenem	0.03–0.125	0.03	0.06
	Azithromycin	0.015–1	0.06	1
	Doxycycline	0.06–8	0.125	4
	SMX-TMP	≤0.008–0.06	0.03	0.06
Streptococcus intermedius (10)	Ceftaroline	≤0.008–0.015	≤0.008	0.015
	Cefazolin	0.125–1	0.25	1
	Ceftriaxone	0.06–0.25	0.125	0.25
	Ampicillin-sulbactam	0.015–0.25	0.03	0.06
	Ertapenem	≤0.015–0.25	0.03	0.06
	Azithromycin	≤0.008–1	0.06	0.5
	Doxycycline	0.03–8	0.06	0.125
	SMX-TMP	0.015–2	0.06	1
Streptococcus mitis (10)	Ceftaroline	≤0.008–0.03	0.015	0.015
	Cefazolin	0.03–2	0.5	2
	Ceftriaxone	0.125–1	0.25	0.5
	Ampicillin-sulbactam	≤0.008–1	0.125	0.25
	Ertapenem	≤0.015–0.25	0.125	0.125
	Azithromycin	0.03–1	0.06	1
	Doxycycline	0.06–2	0.125	1
	SMX-TMP	0.25–>8	1	8

[a] The quality control strains for ceftaroline were *P. multocida* subsp. *multocida* ATCC 12947 and *P. multocida* subsp. *septica* ATCC 51688.
[b] *Staphylococcus* species included *S. cohnii* ($n = 2$), *S. pseudintermedius* ($n = 5$), *S. felis* ($n = 1$), and *S. warneri* ($n = 7$).

in 8.5% saline, and added to the trays with an inoculator device for a final concentration of 1×10^5 to 5×10^5 CFU/ml. Trays for *Pasteurella* and streptococci were supplemented with 3% lysed horse blood. Colony counts were determined on every 10th panel.

All testing was conducted according to procedures in the CLSI M7-A9 and M45-A2 documents (7, 8). Control organisms included *P. multocida* subsp. *multocida* ATCC 12947, *P. multocida* subsp. *septica* ATCC 51688, *Escherichia coli* ATCC 25922, and *S. aureus* ATCC 29213.

Drugs included ceftaroline, cefazolin, ceftriaxone, ertapenem, ampicillin-sulbactam, azithromycin, doxycycline, and sulfamethoxazole-trimethoprim (SMX-TMP), which are commonly used in the treatment of ABSSSIs. Ceftaroline was obtained from Cerexa Pharmaceuticals, and other drugs were obtained from Sigma (St. Louis MO) or USP (Rockville, MD) and reconstituted according to the manufacturers' instructions.

The MIC was defined as the lowest concentration that yielded no visible growth.

RESULTS

Results of the comparative *in vitro* activity of ceftaroline against the study isolates are shown in Table 1. Ceftaroline was the most active agent against all 5 *Pasteurella* species, including *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica*, with a maximum MIC of ≤0.008 μg/ml. It was more active than ceftriaxone and ertapenem (MIC_{90}s, ≤0.015 μg/ml) and more active than cefazolin (MIC_{90}, 0.5 μg/ml), doxycycline (MIC_{90}, 0.125 μg/ml), azithromycin (MIC_{90}, 0.5 μg/ml), ampicillin-sulbactam (MIC_{90}, 0.125 μg/ml), and SMX-TMP (MIC_{90}, 0.125 μg/ml). Ceftaroline was also very active against all *S. aureus* isolates (MIC_{90}, 0.125 μg/ml) and other *Staphylococcus* and *Streptococcus* species, with a maximum MIC of 0.125 μg/ml against all bite isolates tested.

The quality control strains *P. multocida* subsp. *multocida* ATCC 12947 and *P. multocida* subsp. *septica* ATCC 51688 were each run three times, and the ceftaroline MIC was ≤0.008 μg/ml on each occasion.

DISCUSSION

The antimicrobials selected for therapy of infected animal bite wounds must have activity against the components of the biting animals' oral flora, including *P. multocida* and its subspecies. The susceptibility of *P. multocida* can be problematic to the clinician, as the *in vitro* susceptibility of oral cephalosporins cannot be inferred from their susceptibility to intravenous cefazolin (17). In addition, dogs and cats can harbor MRSA in their nasal passages

and orally (1, 20). The transmission of epidemic, potentially invasive MRSA clones between companion animals such as dogs and cats and their owners, household members, and veterinarians has been increasingly reported (4, 10). Consequently, the potential for MRSA to be a component of infected animal bite wounds is cause for concern (22). Currently recommended regimens (26) do not include agents with coverage against MRSA, and clinical regimens for bite wounds with coverage against MRSA have not, to our knowledge, been reported. With up to 20% of the population being colonized with MRSA, the potential for MRSA to be introduced via the victims' own skin flora also needs to be considered.

Ceftaroline fosamil was FDA approved in October 2010 for the treatment of ABSSSIs, including those containing MRSA, but scant data about its activity against *P. multocida*, other *Pasteurella* species, and animal bite isolates in general exist. Ge et al. (13) reported ceftaroline to be active *in vitro* against 22 *Pasteurella multocida* isolates form Luxembourg but gave few clinical data about their source. Our *in vitro* study suggests that ceftaroline has excellent activity against all *Pasteurella* strains (all isolates were susceptible to ≤0.06 μg/ml), including *P. multocida* subsp. *multocida*, *P. multocida* subsp. *septica*, *P. canis*, *P. dagmatis*, and *P. stomatis*. Ceftaroline was also very active against all the *S. aureus* strains (all isolates were susceptible to ≤0.25 μg/ml), *Staphylococcus epidermidis*, *Staphylococcus intermedius*, and *Staphylococcus warneri* strains (all isolates were susceptible to ≤0.125 μg/ml), and streptococci (all isolates were susceptible to ≤0.03 μg/ml). These MIC values compared favorably to those of all the other study agents. Ceftaroline has variable activity against anaerobic bacteria (6), with good activity against anaerobic Gram-positive cocci and beta-lactamase-negative Gram-negative bacilli but poor activity against the *Bacteroides fragilis* group, which are rare animal bite pathogens (27).

S. intermedius is a coagulase-positive *Staphylococcus* species that is part of the canine oral flora and has been isolated from infected dog bite wounds (28). It was isolated from 39% of 135 gingival cultures from indoor canine breeds, which frequently weighed <40 lb, while *S. aureus* was isolated from larger (weight, >40 lb) outdoor working breeds (28). *S. intermedius* can yield a false-positive rapid penicillin binding protein 2a latex agglutination test and can be misidentified as MRSA (23). Adding to the confusion among canine isolates is the description of methicillin-resistant *S. pseudintermedius* (3, 5, 12), which has emerged as a worldwide veterinary canine pathogen. Our *in vitro* data show ceftaroline to have good activity against *S. pseudintermedius*.

Our results with the more typical Gram-positive cocci studied are similar to those previously reported (11, 13, 25). Azithromycin, which has an FDA indication for the treatment of uncomplicated skin and soft tissue infections and has a breakpoint of ≤1 μg/ml for *P. multocida* (8), had an MIC_{90} of 1 μg/ml against *P. multocida* subsp. *multocida* and one of 0.5 μg/ml against *P. multocida* subsp. *septica*. The MIC breakpoint for azithromycin against staphylococci is ≤1 μg/ml by EUCAST standards (9) and ≤2 μg/ml by CLSI standards, and the MIC_{90} for all our staphylococcal strains tested was 2 μg/ml, suggesting some caution in its use for the treatment of infected animal bite wounds.

Ceftaroline has potential clinical utility against infections involving *P. multocida*, other *Pasteurella* species, and aerobic Gram-positive isolates, including *S. aureus*.

ACKNOWLEDGMENTS

This study was supported by a grant from Forest Laboratories.

We thank Eliza Leoncio for excellent technical assistance and Alice E. Goldstein for various forms of assistance.

REFERENCES

1. **Abdel-Moein KA, El-Hairi M, Samir A.** 2012. Methicillin-resistant Staphylococcus aureus: an emerging pathogen of pets in Egypt with a public health burden. Transbound. Emerg. Dis. **59**:331–335.
2. **Abrahamian FA, Goldstein EJC.** 2011. The bacteriology of animal bite wounds. Clin. Microbiol. Rev. **24**:231–246.
3. **Bannoehr J, Guardabassi L.** 2012. *Staphylococcus pseudointermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Vet. Dermatol. **23**:253–266.
4. **Bender JB, Waters KC, Nerby J, Olsen KE, Jawahir S.** 2012. Methicillin-resistant Staphylococcus aureus (MRSA) isolated from pets living in households with MRSA-infected children. Clin. Infect. Dis. **54**: 449–450.
5. **Bond R, Loeffler A.** 2012. What's happened to *Staphylococcus intermedius*? Taxonomic revision and emergence of multi-drug resistance. J. Small Anim. Pract. **53**:147–154.
6. **Citron DM, Tyrrell KL, Merriam CV, Goldstein EJC.** 2010. In vitro activity of ceftaroline against 623 diverse strains of anaerobic bacteria. Antimicrob. Agents Chemother. **54**:1627–1631.
7. **Clinical and Laboratory Standards Institute.** 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 9th ed. CLSI document M7-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Clinical and Laboratory Standards Institute.** 2010. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated fastidious bacteria; approved standard, 2nd ed. CLSI document M45-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **European Committee on Antimicrobial Susceptibility Testing.** 2012. Clinical breakpoints, bacteria v. 2.0. Breakpoint tables for interpretation of MICs and zone diameters. European Committee on Antimicrobial Susceptibility Testing, London, United Kingdom. http://www.eucast.org/clinical_breakpoints/.
10. **Ferreira JP, et al.** 2011. Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. PLoS One **6**:e26978. doi:10.1371/journal.pone.0026978.
11. **Flamm R, Sader HS, Farrell D, Jones RN.** 2012. Summary of ceftaroline activity against pathogens in the United States, 2010: report from Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) surveillance program. Antimicrob. Agents Chemother. **56**:2933–2940.
12. **Frank LA, Loeffler A.** 2012. Methicillin-resistant Staphylococcus pseudointermedius: clinical challenge and treatment options. Vet. Dermatol. **23**:283–291.
13. **Ge Y, Biek D, Talbot GH, Sahm DF.** 2008. In vitro profiling of ceftaroline against a collection of recent bacterial clinical isolates from across the United States. Antimicrob. Agents Chemother. **52**:3398–3407.
14. **Goldstein EJC, Citron DM, Finegold SM.** 1980. Dog bite wounds and infection: a prospective clinical study. Ann. Emerg. Med. **9**:508–512.
15. **Goldstein EJC, et al.** 1978. Bacteriology of human and animal bite wounds. J. Clin. Microbiol. **8**:667–672.
16. **Goldstein EJC.** 1992. Bite wounds and infection. Clin. Infect. Dis. **14**:633–640.
17. **Goldstein EJC, Citron DM, Richwald GA.** 1988. Lack of efficacy of oral forms of certain cephalosporins, erythromycin, and oxacillin against *Pasteurella multocida.* Antimicrob. Agents Chemother. **32**:213–215.
18. **Holmquist L, Elixhauser A.** 2010. Emergency department visits and inpatient stays involving dog bites, 2008. Statistical brief no. 101. Healthcare Cost and Utilization Project Statistical Briefs. Agency for Healthcare Policy and Research, Rockville, MD.
19. **Humane Society of the United States.** 2012. American Pet Products Manufacturers Association (APPMA) 2011-2012 national pet owners survey. The Humane Society of the United States, Washington, DC. http://www.americanpetproducts.org/press_industrytrends.asp.
20. **Loeffler A, et al.** 2005. Prevalence of methicillin-resistant Staphylococcus aureus among staff and pets in a small animal referral hospital in the UK. J. Antimicrob. Chemother. **56**:692–697.
21. **Murray PR, et al (ed).** 2007. Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.

22. **Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S.** 2009. Bite-related and septic syndromes caused by cats and dogs. Lancet Infect. Dis. **9**:439–447.
23. **Pottumarthy S, et al.** 2004. Clinical isolates of Staphylococcus intermedius masquerading as methicillin-resistant *Staphylococcus aureus.* J. Clin. Microbiol. **42**:5881–5884.
24. **Sacks JJ, Kresnow M, Houston B.** 1996. Dog bites: how big a problem? Inj. Prev. **2**:52–54.
25. **Saravolatz LD, Stein G, Johnson LB.** 2011. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus.* Clin. Infect. Dis. **52**:1156–1163.
26. **Stevens DL, et al.** 2005. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin. Infect. Dis. **41**: 1373–1406.
27. **Talan DA, et al.** 1999. The bacteriology and management of dog and cat bite wound infections presenting to emergency departments. N. Engl. J. Med. **340**:85–92.
28. **Talan D, et al.** 1989. *Staphylococcus intermedius* in canine gingiva and canine inflicted human wound infections: laboratory characterization of a newly recognized zoonotic pathogen. J. Clin. Microbiol. **27**:78–81.
29. **Weiss HB, Friedman DJ, Cohen JH.** 1998. Incidence of dog bite injuries treated in emergency departments. JAMA **279**:51–53.