## Title

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# *In Vitro* Activity of Pexiganan and 10 Comparator Antimicrobials against 234 Isolates, Including 93 *Pasteurella* Species and 50 Anaerobic Bacterial Isolates Recovered from Animal Bite Wounds

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**ABSTRACT** Animal bite wounds affect more than 5 million Americans annually, resulting in 300,000 emergency department visits, 10,000 hospitalizations, and an untold number of physician office visits. Various forms of topical therapy are empirically self-employed by many patients prior to seeking medical attention. Pexiganan, a 22-amino-acid synthetic cationic analogue of the peptide magainin II, acts by selectively damaging bacterial cell membranes. We determined the MICs for pexiganan and other antimicrobial agents often used for treatment of bite wounds. Most isolates were from U.S. patients, and ~10% were from European and Canadian patients. The comparator antimicrobials studied were penicillin, amoxicillin-clavulanate, piperacillin-tazobactam, meropenem, clindamycin, doxycycline, moxifloxacin, ceftriaxone, linezolid, and metronidazole. The $MIC_{90}$s of pexiganan were 32 μg/ml (against *Pasteurella multocida* subsp. *multocida*), 16 μg/ml (*P. multocida* subsp. *septica*, *Pasteurella canis*, and *Pasteurella dagmatis*), 8 μg/ml (*Pasteurella stomatis*), 8 μg/ml (*Eikenella corrodens*), 2 μg/ml (*Neisseria weaveri*, *Neisseria zoodegmatis*, and *Moraxella canis-Moraxella lacunata* group), 16 μg/ml (*Bergeyella zoohelcum*), 64 μg/ml (*Bacteroides pyogenes*), 4 μg/ml (*Fusobacterium russii*), 32 μg/ml (*Fusobacterium canifelinum*), and 64 μg/ml (*Prevotella heparinolytica*). The concentration of pexiganan in the cream used was 8,000 μg/ml, more than 60 to 100 times the highest MIC obtained. Pexiganan exhibited a broad range of antimicrobial activity, showing potential for treating animal bite infections. A clinical trial seems warranted.

**KEYWORDS** animal bites, *Eikenella*, *Fusobacterium russii*, *Pasteurella*, pexiganan, *Prevotella heparinolytica*, susceptibility

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More than 5 million Americans each year sustain an animal bite, most often by a dog or cat, leading to approximately 10,000 hospitalizations and 1% (300,000) of all emergency department visits annually (1, 2). Other animal bite patients are commonly seen as outpatients in primary care physician and specialist offices.

The microbiology of infected bite wounds from dogs and cats is similar to that of the organisms that colonize the animals' oral cavity (1, 2). Less frequently, isolates may also come from the environment and a patient's skin. These bite wounds are usually polymicrobial, harboring a broad combination of aerobic and anaerobic microorganisms (3–5), and clinicians select agents to cover this range of organisms.

However, prior to seeking medical attention, 84% of patients will attempt self-therapy, of which, 42% will self-administer topical agents that are ineffective in preventing infection (4, 5). While most patients wash their wounds with water and/or soap very soon after the injury, this broad array of applied agents includes alcohol, hydrogen

peroxide, iodine, and topical antibacterials, such as triple antibiotic ointments. The fact that these patients present with established and often purulent infections suggest that these agents are ineffective in preventing the development of infection.

Magainins are cationic peptides, broad-spectrum antimicrobial agents that naturally occur in animals and act as primary defenses against microbial invaders. Antimicrobial peptides selectively damage bacterial cell membranes through mechanisms that are bactericidal and difficult for bacteria to evade. Pexiganan is a 22-amino-acid synthetic analogue of the peptide magainin II (peptide sequence, GLGKFLKKFGKAFUKLKK-$NH_2$; molecular weight, 2477.21) and is currently in clinical development as a topical agent (pexiganan cream, 0.8%) for mild infections of diabetic foot ulcers. Its spectrum of activity suggests that it may also have potential utility in other skin and soft tissue infections, such as those due to animal bite wounds.

Consequently, we studied the comparative *in vitro* activity of pexiganan against a broad selection of aerobic and anaerobic bacteria recovered from specimens obtained from infected animal bite wounds in patients in the United States, Canada, Sweden, Hungary, Germany, and Holland.

## RESULTS

The MIC ranges, $MIC_{50}$s, and $MIC_{90}$s are shown in Tables 1 and 2. $MIC_{90}$ values were not recorded for any species or organism group where fewer than 10 isolates were tested. There was no difference in MIC values for U.S. and non-U.S. strains (data not shown).

Because many of the non-*Bacteroides* anaerobic organisms grew poorly or not at all in the blood-free broth microdilution, we report only the results from the blood-supplemented pexiganan tests. In the cases where the organism grew in the blood-free broth, the MICs obtained in the blood-supplemented broth tended to be 1 dilution higher in some cases, although in other tests, they were the same or, rarely, lower (data not shown).

Pexiganan had MICs of 4 to 32 $\mu$g/ml for the *Pasteurella* species and 1 to 2 $\mu$g/ml for *Moraxella* and *Neisseria* species. $MIC_{90}$s for anaerobes were generally 32 to 128 $\mu$g/ml. Among the comparator antimicrobial agents, clindamycin had generally poor activity against all *Pasteurella* species tested, with an $MIC_{90}$s of 16 $\mu$g/ml for *Pasteurella multocida* subsp. *multocida* and *P. multocida* subsp. *septica* and 8 $\mu$g/ml for *Pasteurella canis* and *Pasteurella dagmatis*. Doxycycline had relatively good activity against all *Pasteurella* species ($MIC_{90}$s of 0.25 $\mu$g/ml) but $MIC_{90}$s of 2 and 4 $\mu$g/ml against *Bacteroides pyogenes* and *Prevotella heparinolytica*, respectively. Moxifloxacin had excellent activity against all *Pasteurella* species, with $MIC_{90}$s of $\leq$0.03 $\mu$g/ml, but poor activity against *Fusobacterium canifelinum* and *Fusobacterium russii*, with $MIC_{90}$s of $>$16 and 8 $\mu$g/ml, respectively. There was no difference in MICs between the U.S., Swedish, and Hungarian isolates (data not shown). There was no apparent relationship between pexiganan MICs and resistance to any of these agents.

## DISCUSSION

Epidemic rates of animal bite injuries and infections are not surprising, since human-animal contact is a daily occurrence for most people worldwide. Settings for this exposure vary from farms to contact with domestic pets or feral animals; thus, bite injuries can be caused by a wide variety of domestic and wild animals. The Humane Society of the United States estimates that in 2015 in the United States, 65% (79.7 million) of households owned a pet, including 163.6 million dogs and cats (see http://www.humanesociety.org/issues/pet_overpopulation/facts/pet_ownership_statistics.html).

Approximately 4.5 million dog bites occur each year in the United States. Almost 1 out of 5 bites becomes infected (see http://www.cdc.gov/features/dog-bite-prevention/). Among children, the rate of dog-bite-related injuries is highest for those 5 to 9 years old. Children are more likely than adults to receive medical attention for dog bites. Men are more likely than women to be bitten by a dog. Roughly 60% of

**TABLE 1** *In vitro* activities of pexiganan and comparator antimicrobial agents against aerobic organisms

| Organism (no. of isolates) | Antimicrobial agent | MIC data (μg/ml) | | |
|---|---|---|---|---|
| | | Range | $MIC_{50}$ | $MIC_{90}$ |
| *Pasteurella multocida* subsp. *multocida* (30) | Pexiganan | 4–128 | 16 | 32 |
| | Penicillin | ≤0.03–0.125 | 0.125 | 0.125 |
| | Amoxicillin-clavulanate | 0.06–0.25 | 0.25 | 0.25 |
| | Ceftriaxone | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 4–>16 | 16 | 16 |
| | Doxycycline | 0.125–1 | 0.25 | 0.25 |
| | Moxifloxacin | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| *Pasteurella multocida* subsp. *septica* (21) | Pexiganan | 8–32 | 16 | 32 |
| | Penicillin | 0.125–0.125 | 0.125 | 0.125 |
| | Amoxicillin-clavulanate | 0.25–0.25 | 0.25 | 0.25 |
| | Ceftriaxone | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 4–>16 | 16 | 16 |
| | Doxycycline | 0.125–0.25 | 0.25 | 0.25 |
| | Moxifloxacin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| *Pasteurella canis* (16) | Pexiganan | 4–16 | 8 | 16 |
| | Penicillin | ≤0.03–0.125 | 0.06 | 0.125 |
| | Amoxicillin-clavulanate | 0.06–0.25 | 0.125 | 0.25 |
| | Ceftriaxone | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 4–8 | 4 | 8 |
| | Doxycycline | 0.125–0.25 | 0.25 | 0.25 |
| | Moxifloxacin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| *Pasteurella dagmatis* (15) | Pexiganan | 4–32 | 8 | 16 |
| | Penicillin | 0.06–0.25 | 0.125 | 0.2 |
| | Amoxicillin-clavulanate | 0.125–0.25 | 0.25 | 0.25 |
| | Ceftriaxone | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 4–8 | 4 | 8 |
| | Doxycycline | 0.125–0.25 | 0.125 | 0.25 |
| | Moxifloxacin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| *Pasteurella stomatis* (10) | Pexiganan | 4–8 | 8 | 8 |
| | Penicillin | 0.06–0.25 | 0.06 | 0.125 |
| | Amoxicillin-clavulanate | 0.125–0.25 | 0.125 | 0.25 |
| | Ceftriaxone | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 2–8 | 4 | 4 |
| | Doxycycline | 0.125–0.25 | 0.25 | 0.25 |
| | Moxifloxacin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| *Eikenella corrodens* (32) | Pexiganan | 4–16 | 8 | 8 |
| | Penicillin | 0.125–4 | 0.25 | 0.5 |
| | Amoxicillin-clavulanate | 0.125–2 | 0.5 | 2 |
| | Ceftriaxone | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 8–16 | 16 | 16 |
| | Doxycycline | 0.25–4 | 2 | 4 |
| | Moxifloxacin | ≤0.03–0.06 | ≤0.03 | ≤0.03 |

(Continued on next page)

**TABLE 1** (Continued)

| Organism (no. of isolates) | Antimicrobial agent | MIC data (μg/ml) | | |
|---|---|---|---|---|
| | | Range | $MIC_{50}$ | $MIC_{90}$ |
| *Neisseria weaveri* (17) | Pexiganan | 1–2 | 2 | 2 |
| | Penicillin | ≤0.03–0.25 | 0.25 | 0.25 |
| | Amoxicillin-clavulanate | ≤0.03–0.5 | 0.25 | 0.5 |
| | Ceftriaxone | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 2–16 | 4 | 8 |
| | Doxycycline | ≤0.03–0.125 | 0.125 | 0.125 |
| | Moxifloxacin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| *Neisseria zoodegmatis* (15) | Pexiganan | 1–2 | 2 | 2 |
| | Penicillin | 0.06–0.5 | 0.125 | 0.25 |
| | Amoxicillin-clavulanate | 0.06–0.5 | 0.125 | 0.25 |
| | Ceftriaxone | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Clindamycin | 1–4 | 2 | 4 |
| | Doxycycline | ≤0.03–0.25 | 0.06 | 0.25 |
| | Moxifloxacin | ≤0.03–0.06 | ≤0.03 | 0.06 |
| *Moraxella* spp.[a] (16) | Pexiganan | 2–4 | 2 | 4 |
| | Penicillin | ≤0.03–0.5 | 0.06 | 0.25 |
| | Amoxicillin-clavulanate | ≤0.03–0.5 | 0.125 | 0.5 |
| | Ceftriaxone | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Piperacillin-tazobactam | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | 0.25–4 | 2 | 4 |
| | Doxycycline | ≤0.03–0.5 | 0.125 | 0.25 |
| | Moxifloxacin | ≤0.03–4 | ≤0.03 | 0.5 |
| *Bergeyella zoohelcum* (10) | Pexiganan | 4–64 | 8 | 16 |
| | Penicillin | ≤0.03–0.5 | ≤0.03 | 0.125 |
| | Amoxicillin-clavulanate | ≤0.03–0.25 | ≤0.03 | 0.06 |
| | Ceftriaxone | ≤0.03–0.125 | ≤0.03 | 0.06 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–0.06 | ≤0.03 | ≤0.03 |
| | Clindamycin | ≤0.03–0.125 | 0.06 | 0.125 |
| | Doxycycline | 0.06–0.25 | 0.125 | 0.25 |
| | Moxifloxacin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |

[a]*Moraxella* spp.: *Moraxella canis* (11), *Moraxella cuniculi* (1), and *Moraxella lacunata* (4).

animal bites are attributed to dogs, and 10% to 20% are attributed to cats. Cat bites are more common in women and the elderly.

Most of these wounds are minor injuries that go unreported, and the people do not seek medical attention. In industrialized countries, most people with a moderate to severe bite injury will seek some form of medical attention, whether in an emergency department (ED) or in a physician's office (1). Our study showed that clindamycin would be a poor choice for use in bite wounds, as $MIC_{90}$s for all *Pasteurella* species were high, ranging from 4 to >16 μg/ml.

The vast majority of patients initiate some form of self-therapy, especially washing the wound with soap and water, and additionally often employ noneffective topical agents in an effort to prevent infection and avoid medical visitation. Unfortunately, these agents do not have a spectrum of activity that covers the variety of potential pathogens found in the oral flora of a biting animal (4, 5). The susceptibility results in our study are similar to pexiganan MICs reported for similar organisms recovered from other types of soft tissue infections (6, 7). Pexiganan is being evaluated in phase 3 clinical trials as a topical agent at a concentration of 8,000 μg/ml of the active compound for mild infections of diabetic foot ulcers. Experiments have shown that there is no activity for the vehicle, while the pexiganan cream killed 3 logs of bacteria translocated from the human perineum to the forearm over at least a 12-h time frame

**TABLE 2** *In vitro* activities of pexiganan and comparator antimicrobial agents against anaerobic organisms

| Organism (no. of isolates) | Antimicrobial agent | MIC data (μg/ml) | | |
|---|---|---|---|---|
| | | Range | $MIC_{50}$ | $MIC_{90}$ |
| *Bacteroides pyogenes* (15) | Pexiganan | 2–128 | 8 | 64 |
| | Penicillin | ≤0.03–8 | ≤0.03 | 8 |
| | Amoxicillin-clavulanate | ≤0.03–0.5 | ≤0.03 | 0.5 |
| | Ceftriaxone | 0.06–16 | 0.125 | 16 |
| | Piperacillin-tazobactam | ≤0.03–0.25 | ≤0.03 | 0.125 |
| | Meropenem | ≤0.03–0.125 | ≤0.03 | 0.06 |
| | Clindamycin | ≤0.03–2 | ≤0.03 | 0.125 |
| | Doxycycline | ≤0.03–4 | 0.06 | 2 |
| | Moxifloxacin | 0.125–0.25 | 0.125 | 0.25 |
| | Linezolid | 2–4 | 2 | 2 |
| | Metronidazole | 0.125–1 | 0.25 | 0.5 |
| *Prevotella heparinolytica* (16) | Pexiganan | 4–128 | 32 | 128 |
| | Penicillin | ≤0.03–2 | 0.06 | 0.06 |
| | Amoxicillin-clavulanate | 0.125–1 | 0.125 | 0.25 |
| | Ceftriaxone | 0.125–0.25 | 0.25 | 0.25 |
| | Piperacillin-tazobactam | 0.06–0.125 | 0.06 | 0.125 |
| | Meropenem | 0.06–0.125 | 0.06 | 0.125 |
| | Clindamycin | ≤0.03–>16 | ≤0.03 | >16 |
| | Doxycycline | ≤0.03–4 | 0.06 | 4 |
| | Moxifloxacin | 0.25–0.25 | 0.25 | 0.25 |
| | Linezolid | 0.25–2 | 2 | 2 |
| | Metronidazole | 0.06–0.25 | 0.125 | 0.25 |
| *Fusobacterium canifelinum* (10) | Pexiganan | 8–32 | 16 | 32 |
| | Penicillin | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Amoxicillin-clavulanate | 0.06–0.06 | 0.06 | 0.06 |
| | Ceftriaxone | 0.06–0.5 | 0.125 | 0.5 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | ≤0.03–0.125 | 0.06 | 0.125 |
| | Doxycycline | ≤0.03–0.25 | 0.06 | 0.25 |
| | Moxifloxacin | >16–>16 | >16 | >16 |
| | Linezolid | 0.5–1 | 1 | 1 |
| | Metronidazole | 0.06–0.5 | 0.125 | 0.5 |
| *Fusobacterium russii* (10) | Pexiganan | 0.5–4 | 1 | 4 |
| | Penicillin | ≤0.03–0.06 | 0.06 | 0.06 |
| | Amoxicillin-clavulanate | 0.125–0.25 | 0.125 | 0.125 |
| | Ceftriaxone | 0.06–0.5 | 0.125 | 0.5 |
| | Piperacillin-tazobactam | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Meropenem | ≤0.03–≤0.03 | ≤0.03 | ≤0.03 |
| | Clindamycin | ≤0.03–0.06 | 0.06 | 0.06 |
| | Doxycycline | ≤0.03–0.25 | ≤0.03 | 0.06 |
| | Moxifloxacin | 8–16 | 8 | 8 |
| | Linezolid | 1–1 | 1 | 1 |
| | Metronidazole | 0.06–0.5 | 0.125 | 0.5 |

(Dipexium Pharmaceuticals, unpublished data). *Pasteurella* spp. are the most commonly recognized animal bite pathogens and are present in 75% of cat bite wound infections and 50% of dog bite wound infections. Our *in vitro* studies with pexiganan evaluated its activity against 93 strains of *Pasteurella* species and found $MIC_{90}$s of 8 to 16 μg/ml, and the highest MIC was 128 μg/ml. Given the 8,000 μg/ml cream concentration, pexiganan has an excellent multifold therapeutic margin. Additionally, we studied a wide variety of other commonly isolated aerobic and anaerobic animal bite wound pathogens and found them to be equally or even more susceptible to pexiganan. These data suggest a potential therapeutic role for this new topical agent in the initial management of animal bite wounds.

In conclusion, pexiganan was active against a broad spectrum of aerobic and anaerobic bacteria recovered from animal bite wounds in humans. There was no apparent relation to resistance seen with any of the comparator antimicrobials. The

concentration of pexiganan in the cream is 8,000 μg/ml, more than 60 times the highest MIC obtained that was required to inhibit growth of the bite wound isolates. It is likely sufficient to cover organisms present in the infected site. Pexiganan shows potential as an adjunct for treating animal bite wounds and should be validated by a clinical trial.

## MATERIALS AND METHODS

**Isolates.** Most isolates were from U.S. patients, but some were from European and Canadian patients who had clinical bite wound infections (10 of 30 *P. multocida* subsp. *multocida* isolates [Hungary, 5; Sweden, 4; Holland, 1] and 7 of 21 *P. multocida* subsp. *septica* isolates [Hungary, 3; Sweden, 4]). All isolates were identified by standard criteria (8, 9). Many of the isolates were recovered during the past 3 years, although isolates of some of the unusual species were older.

**Antimicrobial agents.** The compounds used were moxifloxacin (Bayer Corp., Pittsburgh, PA), pexiganan (Dipexium Pharmaceuticals, Inc., New York, NY), piperacillin-tazobactam (Wyeth, Co., Madison, NJ), clindamycin (Fluka Chemical Corp., Milwaukee, WI), amoxicillin, clavulanate, metronidazole, ceftriaxone, doxycycline, and penicillin-G (Sigma-Aldrich, St. Louis, MO), and meropenem and linezolid (U.S. Pharmacopeia, Rockville, MD).

**Susceptibility methods.** Organisms were tested by standard methods as detailed by the Clinical and Laboratory Standards Institute (CLSI) unless specified otherwise. Fastidious aerobic organisms were tested by broth microdilution (BMD) (10). Anaerobic and microaerophilic fastidious organisms (*Eikenella* spp.) were tested by agar dilution (AD) (11).

Because pexiganan binds to calcium in agar, thus destroying its activity, we tested *Bacteroides fragilis* by the agar dilution method before starting this study and found very high pexiganan MICs (32 to >512 μg/ml). Repeat testing using BMD with brucella broth resulted in MICs that were in a much lower range, similar to what was reported in previous studies using BMD (6); therefore, the BMD method, which is a standard method for *Bacteroides* but not for other anaerobic species due to issues of inconsistent growth, was used in this study. Because there was a question regarding decreased activity in the presence of blood, duplicate testing was done with and without 5% horse blood.

**Agar dilution method for anaerobes and *Eikenella*.** The comparator antimicrobial agents were reconstituted according to the procedures described in the CLSI M11-A8 document (11). Stock solutions were stored at −70°C until the day of the test. They were thawed, and serial dilutions were prepared and added to molten brucella agar deeps for preparation of the plates. Drug-free plates were included as growth controls. Concentrations for penicillin were from 8 to 0.03 μg/ml, for piperacillin-tazobactam from 32 to 0.03 μg/ml, and for the other drugs from 16 to 0.03 μg/ml.

The organisms were taken from the freezer and subcultured onto blood agar plates at least twice for purity and good growth. On the day of testing, they were suspended in brucella broth to equal the turbidity of the 0.5 McFarland standard and applied to the plates using a Steers replication device that delivers ~2 to 5 μl per spot, for a final concentration of approximately $10^5$ CFU/spot. The plates were incubated in the anaerobic chamber at 36°C for 44 h and examined for growth. The MIC is defined as the lowest concentration of antimicrobial agent that completely inhibits growth or results in a major reduction of growth compared to the drug-free control. Pexiganan was tested by the broth microdilution method (see below).

**Broth microdilution method for aerobes and anaerobes.** The comparator drugs were reconstituted according to the manufacturers' instructions. For all drugs against aerobic organisms, Mueller-Hinton broth supplemented with 5% lysed horse blood was used for testing. For anaerobes only, pexiganan was reconstituted in water and serially diluted in brucella broth with or without 5% lysed horse blood. Concentrations for pexiganan were serial 2-fold dilutions from 512 to 0.25 μg/ml. Concentrations for penicillin were from 8 to 0.03 μg/ml, for piperacillin-tazobactam from 32 to 0.03 μg/ml, and for the remaining drugs from 16 to 0.03 μg/ml. The Quick Spense apparatus was used to dispense 100 μl of the dilutions into 96-well microtiter trays, which were immediately placed in the −70°C freezer for storage. On the day of the test, they were removed from the freezer and thawed at room temperature.

On the day of testing, aerobic strains were suspended in saline to equal the 0.5 McFarland standard, further diluted 1:30 in saline, and added to the trays using a 96-pronged inoculation device that delivered 10 μl to each well, for a final concentration of approximately $5 \times 10^4$ CFU/well. The plates were incubated in an ambient atmosphere at 35°C for 20 h. Anaerobic isolates were suspended in brucella broth to equal the turbidity of the 0.5 McFarland standard and further diluted 1:15 in saline before adding 10 μl to each well, for a final concentration of approximately $1 \times 10^5$ CFU per well. The anaerobic isolates were incubated in the anaerobic chamber at 35°C for 44 h, and the *Eikenella corrodens* plates were incubated in 5% $CO_2$ for 48 h. After incubation, the trays were examined for growth using an inverted mirror. The MIC was considered the lowest concentration that completely inhibited growth or caused a marked reduction of growth compared to the drug-free control well. The susceptibility of test isolates to the comparator agents was determined per the interpretive breakpoints (6). Interpretive breakpoints for pexiganan have not been defined.

The quality control strains *Bacteroides fragilis* ATCC 25825 and *Clostridium difficile* ATCC 700057 were included each day of testing anaerobes, and *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were included with the aerobic testing.

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