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Susceptibility of Bite Wound Bacteria to Seven Oral Antimicrobial Agents, Including RU-985, a New Erythromycin: Considerations in Choosing Empiric Therapy

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The susceptibility of 93 aerobic and 59 anaerobic bacteria isolated from human and animal bite wounds was determined by agar dilution. No agent tested (penicillin, oxacillin, cephalexin, sulfamethoxazole-trimethoprim, minocycline, erythromycin, and RU-965) was consistently active against all isolates. A total of 21% of the *Bacteroides* species, all isolated from human bites, were resistant to penicillin; 14 and 18% of the *Pasteurella multocida* isolates were resistant to erythromycin and oxacillin, respectively.

Bite wounds are common injuries and account for approximately 1% of all emergency room visits (5). Infectious complications are frequent and include cellulitis, septic arthritis, osteomyelitis, and even fatal sepsis. Isolates are most often those from the oral flora of the biter and include both aerobic and, in 33 to 55% of cases, anaerobic bacteria (10, 11). To date, the information available regarding the susceptibility of bacteria isolated from bite wounds comes from studies that have focused on a single organism, such as *Pasteurella multocida*, DF-2, or *Eikenella corrodens* (2, 7, 8, 16, 17). In addition, these studies have often used veterinary isolates and strains obtained from general clinical specimens other than bite wounds.

We determined the susceptibility of 93 aerobic and 59 anaerobic bacteria to seven oral antimicrobial agents, including a new macrolide antibiotic, RU-985. This new agent has a structure and an antibacterial spectrum similar to that of erythromycin, but gives higher and more sustained plasma and tissue levels, according to the manufacturer (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.).

The purpose of this study was (i) to use only strains isolated from bite wounds, (ii) to study recently isolated strains to look for the emergence of resistance, (iii) to study the susceptibility of these isolates to oral antibacterial agents since many of these wounds are treated with oral antibiotics and outpatient follow-up, (iv) to supply these data in a single paper since no previous study combines this information, and (v) to extrapolate the potential utility for empiric therapy of the various agents studied.

MATERIALS AND METHODS

All 152 strains tested were recent isolates and were identified by standard criteria (1, 13, 14; V. L. Sutter, D. M. Citron, M. A. C. Edelstein, and S. M. Finegold, *Wadsworth Anaerobic Bacteriology Manual*, 4th ed., in press). The sources of the isolates were: dog bites, 83; cat bites, 23; human bites, 42; squirrel bite, 1; and bites of unknown origin, 3. The numbers and species of isolates tested are given in Table 1.

Standard laboratory powders were kindly supplied by the following companies: penicillin G, cephalexin, and erythromycin, Eli Lilly & Co., Indianapolis, Ind.; oxacillin, Bristol Laboratories, Syracuse, N.Y.; minocycline, Lederle Laboratories, Pearl River, N.Y.; sulfamethoxazole-trimethoprim (SXT), Hoffman-La Roche Inc., Nutley, N.J.; and RU-965, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.

Strains were taken from frozen stock culture and transferred twice to ensure purity and good growth. Because of the diversity of the isolates tested, strains of various types were handled differently, according to accepted appropriate methods for the particular organism. Anaerobic bacteria were cultured and inocula were prepared by the methods outlined in the *Wadsworth Anaerobic Bacteriology Manual* (in press). Isolates of *E. corrodens* were cultured and inocula were prepared by the methodology previously established by our laboratory (8). Other aerobic isolates were tested by standard procedures (14).

Mueller-Hinton agar supplemented with hemin (10 μg/ml) was the basal medium for all aerobic isolates. The plates for *E. corrodens*, the alpha-hemolytic streptococci, *Haemophilus* species, and *Actinobacillus* species were also supplemented with 5% sheep blood. Plates for testing the activity of SXT were supplemented with 5% laked horse blood instead of sheep blood. Plates used for anaerobic bacteria contained brucella agar supplemented with vitamin K_1, hemin, and 5% laked sheep blood, except for SXT testing in which case 5% laked horse blood was used instead of sheep blood. Freshly prepared solutions of twofold dilutions of antimicrobial agents were incorporated into the media described above to yield final concentrations from 64 to 0.06 μg/ml, except RU-965, in which the highest concentration was 32 μg/ml because of the solubility limitation. SXT was prepared in a sulfamethoxazole-to-trimethoprim ratio of 19:1.

The plates were inoculated with a Steers replicator (Craft Machine Inc., Chester, Pa.). Control plates without antimicrobials were inoculated before and after each series of drug-containing plates. Plates with aerobic bacteria were incubated at 35°C in an aerobic environment for 24 h and

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TABLE 1. Activity of seven agents against aerobic and anaerobic bite wound isolates

Organism	No. of isolates	Antimicrobial agent	MIC (μg/ml)[a]		
			Range	50%	90%
Staphylococcus aureus	17	Penicillin G	≤0.06–64	4	32
		Oxacillin	0.12–0.5	0.25	0.5
		Cephalexin	0.5–4	1	2
		Minocycline	0.25–4	0.25	4
		SXT	1–4	1	4
		Erythromycin	0.25–>64	0.5	>64
		RU-965	0.5–>32	2	>32
Pastuerella multocida	22	Penicillin G	<0.06–32	≤0.06	0.25
		Oxacillin	<0.06–>64	1	8
		Cephalexin	≤0.06–2	0.25	1
		Minocycline	≤0.06–4	0.25	0.25
		SXT	≤0.06–8	0.5	2
		Erythromycin	≤0.06–>32	1	4
		RU-965	2–>32	8	32
Alpha-hemolytic streptococci	12	Penicillin	≤0.06–0.5	0.125	0.25
		Oxacillin	≤0.06–2	0.5	1
		Cephalexin	0.12–4	1	2
		Minocycline	0.12–16	0.25	8
		SXT	≤0.06–4	0.5	1
		Erythromycin	≤0.06–0.12	0.06	0.12
		RU-965	0.12–1	0.25	0.5
Eikenella corrodens	12	Penicillin	0.12–2	0.5	2
		Oxacillin	0.25–>64	32	≥64
		Cephalexin	1–>64	16	64
		Minocycline	0.12–4	1	2
		SXT	0.5–64	0.5	8
		Erythromycin	0.12–8	4	8
		RU-965	4–>32	32	32
Gram-negative aerobic fermenters[b]	15	Penicillin	≤0.06–4	0.12	4
		Oxacillin	≤0.06–32	4	32
		Cephalexin	≤0.06–4	1	4
		Minocycline	≤0.06–0.5	0.25	0.5
		SXT	≤0.06–1	0.5	1
		Erythromycin	≤0.06–4	0.5	4
		RU-965	1–32	4	32
Gram-negative aerobic nonfermenters[c]	15	Penicillin	≤0.06–8	0.12	4
		Oxacillin	≤0.06–32	4	32
		Cephalexin	≤0.06–4	1	4
		Minocycline	≤0.06–1	0.25	1
		SXT	≤0.06–2	1	2
		Erythromycin	≤0.06–8	1	8
		RU-965	1–32	8	32
Nonpigmented *Bacteroides* species	18	Penicillin	<0.06–16	≤0.06	8
		Oxacillin	≤0.06–32	1	32
		Cephalexin	0.5–16	1	8
		Minocycline	≤0.06–8	≤0.06	4
		SXT	1–>64	8	64
		Erythromycin	≤0.06–1	0.25	1
		RU-965	0.12–16	0.5	4
Pigmented *Bacteroides* species	16	Penicillin	≤0.06–8	≤0.06	1
		Oxacillin	≤0.06–16	0.5	16
		Cephalexin	≤0.06–4	0.5	2
		Minocycline	≤0.06–32	≤0.06	1
		SXT	≤0.06–32	2	32
		Erythromycin	≤0.06–1	0.06	1
		RU-965	≤0.06–16	0.25	4
Fusobacterium species	15	Penicillin	≤0.06–1	≤0.06	0.5
		Oxacillin	≤0.06–4	0.12	2.0
		Cephalexin	≤0.06–1	0.25	1
		Minocycline	≤0.06–0.25	≤0.06	0.12

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TABLE 1—*Continued*

Organism	No. of isolates	Antimicrobial agent	MIC (μg/ml)[a]		
			Range	50%	90%
		SXT	1–≥64	32	64
		Erythromycin	2–64	16	32
		RU-965	2–>32	32	>32
Anaerobic gram-positive cocci	10	Penicillin	≤0.06–0.5	0.12	0.25
		Oxacillin	≤0.06–2	0.5	2
		Cephalexin	0.25–>64	2	32
		Minocycline	≤0.06–8	0.12	4
		SXT	1–≥64	4	>64
		Erythromycin	≤0.06–≥64	0.25	>64
		RU-965	0.25–>32	2	>32

[a] MICs required to inhibit 50 and 90% of isolates as indicated.
[b] Includes EF-4, 7; *Actinobacillus actinomycetemcomitans*, 5; and *Haemophilis* species, 3.
[c] Includes *Moraxella* species, 6; M-5, 4; II-j, 2; *Neisseria* species and *Branhamella* catarrhalis, 2; and *Flavobacterium* species, 1.

then examined. Anaerobic bacteria were incubated for 48 h in GasPak (BBL Microbiology Systems, Cockeysville, Md.) or Anapak (Scott Laboratories, Inc., Richmond, Calif.) jars and then examined. *E. corrodens*, alpha-hemolytic streptococci, *Haemophilus* species, and *Actinobacillus actinomycetemcomitans* strains were incubated in 5 to 10% CO_2 for 48 h and then examined. Control strains (*Staphylococcus aureus* ATCC 25923, *Bacteroides thetaiotaomicron* ATCC 29741, *B. fragilis* ATCC 25285, and *E. corrodens* ATCC 23834) were tested simultaneously with the appropriate plates and environments.

RESULTS AND DISCUSSION

The results of the study are summarized in Table 1. These data suggest that the selection of an empiric oral antimicrobial regimen for human and animal bite wounds remains problematic. No agent tested was consistently active against the wide variety of aerobic and anaerobic bacteria that are potential pathogens in bite wounds.

Almost all animal bite wound isolates were susceptible to penicillin G, with the exception of *S. aureus*, which is present in 10 to 25% of such wounds (4, 7). *Bacteroides* species are the most frequently isolated anaerobic bacteria from both animal and human bite wounds. While all the *Bacteroides* species isolated from animal bite wounds were susceptible to penicillin, 47% (7/15) of those isolated from human bite wounds were resistant to penicillin. These isolates were found to produce beta-lactamase.

Some centers have advocated the use of two agents as empiric therapy until specific bacteriologic information becomes available (5, 7, 9). The fixed combination of amoxicillin-potassium clavulanate has been shown to be as effective as penicillin with or without dicloxacillin in the therapy of bite wounds (12), but it was not tested in our current study. Minocycline was relatively active against most of the strains tested. A total of two strains of alpha-hemolytic streptococci, one strain of *Peptostreptococcus* species, and approximately 5% of the *Bacteroides* species were resistant to minocycline. Minocycline and other tetracyclines, such as doxycycline, might offer an alternative to combination therapy or be used as primary therapy in the penicillin-allergic patient. However, tetracyclines are contraindicated in children and pregnant and breast-feeding women.

Oxacillin, erythromycin, and, to a lesser extent, cephalexin showed relatively poor activity against various bite pathogens. Despite their recommended use by some authors (3, 4, 15), data from our microbiological study suggest that if these agents are used, careful monitoring of wound bacteriology and therapeutic response would be prudent.

Elenbaas et al. (6) reported a prospective, double-blind study that evaluated the activity of oral oxacillin compared with that of a placebo in the empiric therapy of dog bite wounds. They noted that "oxacillin was not associated with improved outcome" and that the only two infections that occurred in the 46 evaluated patients were in the oxacillin group. While oxacillin was chosen as the test antibiotic because of its "effectiveness against gram-positive organisms and *P. multocida*" (6), our study showed oxacillin to have relatively poor in vitro activity against bite isolates. It was consistently less active than penicillin G against all isolates, both aerobic and anaerobic, with the exception of *S. aureus*. Resistance to oxacillin was demonstrated in 18% of *P. multocida*, 24% of *Bacteroides* species, and >50% of other fastidious aerobic gram-negative strains. *E. corrodens*, which may be found in up to 25% of human bite wounds, is consistently resistant to oxacillin and relatively resistant to cephalexin (8); therapeutic failure has been associated with the use of both oxacillin and cephalexin in human bites (7).

SXT exhibited moderate activity against most aerobic isolates, but was inactive against most (71%) anaerobic isolates. If SXT were to be used to treat bite wounds, an additional agent, such as metronidazole, would be required to cover anaerobic bacteria.

The spectrum of activity of RU-965 was similar to that of erythromycin, but it was consistently less active against both aerobic and anaerobic isolates. Erythromycin was inactive against most strains of *E. corrodens* and *Fusobacterium* species, and 14% of *P. multocida* strains. Almost all pigmented and nonpigmented *Bacteroides* species and *S. aureus* isolates were susceptible to ≤4 μg of RU-965 per ml, and aerobic streptococci were susceptible to ≤1 μg of RU-965 per ml. *P. multocida*, *Moraxella* spp., *Haemophilus* spp., *E. corrodens*, and *Fusobacterium* spp. required >4 μg of RU-965 per ml for inhibition. The clinical usefulness of RU-965 in bite wounds would depend on achievable tissue levels.

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LITERATURE CITED

1. **Blachman, U., and M. J. Pickett.** 1978. Unusual aerobic bacilli in clinical bacteriology. Scientific Development Press, Los Angeles.
2. **Butler, T., R. E. Weaver, T. K. V. Ramani, C. T. Uyeda, R. A. Bobo, J. S. Ryu, and R. B. Kohler.** 1977. Unidentified gram-negative rod infection: a new disease of man. Ann. Intern. Med. **86**:1–5.
3. **Callaham, M.** 1980. Prophylactic antibiotics in common dog bite wounds: a controlled study. Ann. Emerg. Med. **9**:410–414.
4. **Callaham, M. L.** 1978. Treatment of common dog bites: infection risk factors. J. Am. Col. Emerg. Phys. **7**:83–87.
5. **Douglas, L. G.** 1975. Bite wounds. Am. Fam. Physician **11**:93–99.
6. **Elenbaas, R. M., W. K. McNabney, and W. A. Robinson.** 1982. Prophylactic oxacillin in dog bite wounds. Ann. Emerg. Med. **11**:248–251.
7. **Goldstein, E. J. C., M. Barone, and T. A. Miller.** 1983. *Eikenella corrodens* in hand infections. J. Hand Surg. **8**:563–566.
8. **Goldstein, E. J. C., and D. M. Citron.** 1984. Susceptibility of *Eikenella corrodens* to penicillin, apalcillin, and twelve new cephalosporins. Antimicrob. Agents Chemother. **26**:947–948.
9. **Goldstein, E. J. C., D. M. Citron, and S. M. Finegold.** 1980. Dog bite wounds and infection: a prospective clinical study. Ann. Emerg. Med. **9**:508–512.
10. **Goldstein, E. J. C., D. M. Citron, and S. M. Finegold.** 1984. Role of anaerobic bacteria in bite wound infections. Rev. Infect. Dis. **6**(Suppl.):S177–S183.
11. **Goldstein, E. J. C., D. M. Citron, B. Wield, U. Blachman, V. L. Sutter, and S. M. Finegold.** 1978. Bacteriology of human and animal bite wounds. J. Clin. Microbiol. **8**:667–672.
12. **Goldstein, E. J. C., J. F. Reinhardt, P. M. Murray, and S. M. Finegold.** 1984. Animal and human bite wounds: a comparative study. Augmentin vs penicillin +/− dicloxacillin. Postgrad. Med. J. Special Suppl.:105–110.
13. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobic laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
14. **Lennette, E. H., A. Balows, W. J. Hausler Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
15. **Peeples, E., J. A. Boswick, and F. A. Scott.** 1980. Wounds of the hand contaminated by human or animal saliva. J. Trauma **20**:383–389.
16. **Stevens, D. L., J. W. Higbee, T. R. Oberhofer, and E. D. Everett.** 1979. Antibiotic susceptibilities of human isolates of *Pasteurella multocida*. Antimicrob. Agents Chemother. **16**:322–324.
17. **Weber, D. J., J. S. Wolfson, M. N. Swartz, and D. C. Hooper.** 1984. *Pasteurella multocida* infections: report of 34 cases and review of the literature. Medicine (Baltimore) **63**:133–154.