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Colonization of Human Wounds by *Escherichia vulneris* and *Escherichia hermannii*

FRANCIS D. PIEN,[1]* STEVEN SHRUM,[2] J. M. SWENSON,[3] B. C. HILL,[3] CLYDE THORNSBERRY,[3] AND J. J. FARMER III[2]

Straub Clinic and University of Hawaii-John A. Burns School of Medicine, Honolulu, Hawaii 96813,[1] and Enteric Bacteriology Section[2] and Antimicrobics and Infection Mechanisms Branch, Hospital Infection Programs,[3] Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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In this report we present clinical descriptions of 12 Hawaiian patients from whom *Escherichia vulneris* or *E. hermannii* strains were isolated. All but two patients had soft-tissue infections with multiple bacteria, particularly *Staphylococcus aureus*. The other two had purulent conjunctivitis associated with *S. aureus* and infected malignant peritonitis with multiple organisms, respectively. In none of the cases were the *Escherichia* spp. found in abundant quantities or considered pathogenic. In preliminary animal pathogenicity studies, 12 strains each of *E. vulneris* and *E. hermannii* failed to cause serious symptoms in 4-week-old mice when 10^7 cells were injected intraperitoneally. When 10^6 cells were used, none of these bacterial strains injected into mouse soft tissue was capable of producing persistent wound infections. Susceptibility studies of 40 strains of these bacteria to 20 different antimicrobial agents showed that they were susceptible to third-generation cephalosporins as well as to most other cephalosporins, aminoglycosides, trimethoprim, and sulfamethoxazole-trimethoprim; these strains were only marginally susceptible or resistant to penicillin, tetracycline, chloramphenicol, and nitrofurantoin.

Two new *Escherichia* spp., *E. vulneris* and *E. hermannii*, have recently been described. These species were mainly recovered from human wounds (1, 2). Approximately 10% of these isolates were from Hawaii. This report of 12 Hawaiian cases delineates the clinical significance of these new *Escherichia* spp. We determined the susceptibility of these bacteria to 20 antimicrobial agents and performed preliminary pathogenicity studies in mice.

MATERIALS AND METHODS

Patients. Twelve patients from whom *E. vulneris* and *E. hermannii* strains were isolated were identified at the Straub Clinic, Honolulu, Hawaii, during the period 1979 through 1982. Six of the patients were seen by one of us (F.D.P.); clinical data for the other cases were obtained by retrospective case review. The identity of all isolates was confirmed at the Hawaii State Department of Health Laboratory in Honolulu; eight of these isolates were also studied at the Enteric Bacteriology Laboratories, Centers for Disease Control (CDC), Atlanta, Ga.

Bacterial isolates. *E. vulneris* and *E. hermannii* strains from the Hawaiian patients were identified as previously described (1, 2). Additional strains of *E. hermannii* and *E. vulneris* were isolated for laboratory studies at the Enteric Bacteriology Section, CDC, from a wide variety of sources of geographic areas. Twelve strains of each species were used in animal experiments, and 20 each were used to evaluate susceptibility to antimicrobial agents. Other bacterial species from the CDC collection used in the animal experiments were *E. coli* ATCC 29522, *Klebsiella pneumoniae* 9449-76, *Citrobacter freundii* JJF fecal strains, *Proteus mirabilis*, *Vibrio parahaemolyticus* 0681-83, *V. alginolyticus* 1820-83, *V. damsela* 0023-81 and 2227-81, *V. metschnikovii* 2167-78, *V. anguillarum* 9011-83, and *V. fluvialis* 0679-83. Eight other members of the family *Vibrionaceae* that do not cause human infections were also used: *Photobacterium leiognathi*, *P. angustum*, *V. campbellii* 909-79, *V. natriegens* 9101-79, *V. nereis* 9103-79, *V. nigripulchritudo* 9104-79, *V. fischeri* 9064-79, and *V. harveyi* 9539-78.

Animals. Four-week-old ICR mice (Animal Production Facility, CDC), each weighing between 27 and 36 g, were used. In trial 1 they were anesthetized with diethyl ether. In all other trials they were anesthetized by intramuscular injection of 0.1 ml of a one- to fourfold dilution of Innovar-Vet (Pitman-Moore, Washington Crossing, N.J.).

Animal pathogenicity. Both *Escherichia* spp. were evaluated for their ability to cause wound infection and for lethality after intraperitoneal injection. Strains were grown in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) for 18 to 24 h at 36°C. Plate counts indicated about 10^8 organisms per ml (range, 7.1×10^7 to 3.4×10^8 CFU per ml). In trial 1, each strain was tested for its ability to cause soft-tissue infections after 0.025 ml of the brain heart infusion culture was injected in the following sites: subcutaneously in the forehead, subcutaneously in the right rear footpad, intramuscularly in the right rear leg, and intradermally in the base of the tail. In trial 2, the same injections were performed; 0.05 ml of culture was also placed on a fresh 5- to 10-mm elliptical wound made with scissors. In trial 3, 0.1 ml of culture was injected intraperitoneally. In trials 1, 2, and 3, each of the 24 strains of *E. hermannii* and *E. vulneris* was tested; one strain was tested per animal. In trial 4, any culture of *E. hermannii* or *E. vulneris* that had any apparent effect compared with that of the control (uninoculated brain heart infusion broth) was retested in three mice. In trial 5 (which was done simultaneously with trial 3), 0.1 ml of a brain heart infusion culture of all the other species listed above was injected intraperitoneally; one strain was tested per mouse.

Antimicrobial susceptibility tests. Antimicrobial susceptibilities were determined by broth microdilution; standard methods as described by the National Committee for Clinical

* Corresponding author.

TABLE 1. Clinical characteristics of *E. vulneris* patients in Hawaii

Patient no.	Age (yr)/ sex	Type of infection	Culture source	Associated bacteria
1	8/M	Recurrent cellulitis	Leg	Many *S. aureus*
2	30/M	Soccer soft-tissue injury	Foot	Many *S. aureus* and group A streptococci, few enterococci and *Enterobacter cloacae*
3	57/F	Spontaneous abscess	Foot	Many *S. aureus*
4	27/F	Cellulitis secondary to psoriasis	Leg	Rare *S. epidermidis*, *Acinetobacter lwoffi*
5	20/M	Soft-tissue injury by auto fender	Wrist	Few *S. epidermidis*
6	19/M	Crush injury from 30-lb rock in Tonga	Foot	Rare *S. epidermidis*
7	65/M	Spontaneous boil	Toe	Many *S. aureus*, few *Enterobacter agglomerans*

Laboratory Standards (4) were used. Antimicrobial powders specified for in vitro testing were obtained from the manufacturers.

RESULTS

Clinical significance. Tables 1 and 2 summarize the clinical features of our Hawaiian cases. Most cases were abscesses in active young persons (average age, 23 years). Many of these skin infections resulted from outdoor accidents. In all cases, except for the peritoneal fluid isolate, *Escherichia* colonies were found only rarely. In many cases, the *Escherichia* strains were isolated in association with *Staphylococcus aureus*; in all patients, other bacterial pathogens were present, usually in much greater numbers than for the *Escherichia* spp. Wound infections resolved with dicloxacillin or erythromycin, although the *Escherichia* isolates were resistant to these antibiotics. The case of infant conjunctivitis was most probably caused by the heavy growth of *S. aureus*. The patient with malignant peritoneal effusion had had several bacteria and yeasts isolated on many occasions. In none of our Hawaiian patients were the *Escherichia* isolates considered pathogenic.

Lethality for mice. None of the strains of *E. hermannii* or *E. vulneris* caused death when a dose of 10^7 cells was injected intraperitoneally. Three strains of each species caused the mice to appear slightly ill, but this lasted only 1 day. In contrast, the following strains were lethal: *E. coli*, *K. pneumoniae*, *V. fluvialis*, *V. parahaemolyticus*, and both strains of *V. damsela*. The following organisms made the animals appear slightly ill but were not fatal: four *Vibrio* spp. associated with human infections, two *Photobacterium* spp., *C. freundii*, and *Proteus mirabilis*. Six members of the family *Vibrionaceae* not associated with human infection caused no symptoms.

Wound infections in mice. None of the 24 strains of *E. hermannii* and *E. vulneris* caused persistent wound infections in any mice. There were a few instances of mild swelling at an injection site, but these lasted only 1 day and did not occur when the strain was injected again in three additional mice.

Antimicrobial susceptibility testing. Results for the drugs tested by microdilution are given in Table 3 as MIC ranges and MICs at which 50 and 90% of strains are inhibited. Using the guidelines suggested by the National Committee for Clinical Laboratory Standards for interpreting MICs (4), we found that all strains were susceptible to amikacin, cefoperazone, cefotaxime, ceftazidime, gentamicin, moxalactam, nitrofurantoin, sulfamethoxazole-trimethoprim, tobramycin, and trimethoprim. One isolate of *E. vulneris* was more resistant to the first- and second-generation cephalosporins than were other isolates of this species. The third-generation cephalosporins, the aminoglycosides, trimethoprim, and trimethoprim-sulfamethoxazole were the most active agents against both species. The least active drugs were carbenicillin, chloramphenicol, penicillin, and tetracycline.

DISCUSSION

Both *E. hermannii* and *E. vulneris* were described as new species in 1982 (1, 2). *E. vulneris* was formerly known at CDC as Enteric Group I; it also codes in the profile system of Analytab Products, Inc., as API Group 2. Unlike other *Escherichia* spp., *E. vulneris* is negative for indole production and the presence of orthinine decarboxylase. Unlike *Enterobacter* spp., *E. vulneris* is methyl red positive, does not utilize citrate on Simmons citrate medium, and gives a negative Voges-Proskauer reaction. Of 50 clinical isolates of *E. vulneris* received by CDC over a 23-year period, 74% were from wounds, 10% were from the respiratory tract, 8% were from blood, and the rest were from miscellaneous

TABLE 2. Clinical characteristics of *E. hermannii* patients in Hawaii

Patient no.	Age/sex	Type of infection	Culture source	Associated bacteria
1	2 mo/M	Chronic conjunctivitis	Conjunctiva	Many *S. aureus* and *Corynebacterium* sp.; few enterococci
2	22 yr/M	Laceration due to motorcycle accident	Knee	Many *Enterobacter cloacae* and non-group A beta-hemolytic streptococci
3	1 yr/M	Recurrent impetigo	Cheek	Many *S. aureus*; few enterococci, *A. lwoffi*, and *Enterobacter agglomerans*
4	24 yr/M	Spontaneous abscess	Heel	Many group A streptococci, few *S. epidermidis*
5	29 yr/M	Malignant peritonitis, gastric carcinoma with jejunocolonic cutaneous fistula	Peritoneal fluid	Many *C. freundii*, *Candida* sp., and *K. pneumoniae*

TABLE 3. Range of MICs, MIC_{50}s, and MIC_{90}s for 20 strains each of *E. hermannii* and *E. vulneris*[a]

Drug	MIC (μg/ml) for:					
	E. hermannii			*E. vulneris*		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Ampicillin	4->16	16	16	1.0–8	4	8
Penicillin	8->16	16	32	8->16	16	>16
Carbenicillin	>128			16->128	128	>128
Cephalothin	≤0.5–1.0	1.0	1.0	1.0–16	2	8
Cefoxitin	0.5–2	1.0	1.0	1.0->32	4	8
Cefamandole	≤0.25–0.5	≤0.25	≤0.25	≤0.25–2	0.5	1.0
Cefoperazone	≤0.5			≤0.5–4	≤0.5	1.0
Cefotaxime	≤0.25			≤0.25		
Cefatazidine	≤1.0			≤1.0		
Moxalactam	≤0.25			≤0.25–2	≤0.25	0.5
Gentamicin	≤0.5			≤0.5		
Tobramycin	≤0.25–2	≤0.25	0.5	≤0.25–0.5	≤0.25	≤0.25
Amikacin	0.5–1.0	0.5	0.5	≤0.5–1.0	≤0.5	≤0.5
Chloramphenicol	8–16	8	16	4–16	8	16
Nitrofurantoin	32–64	64	64	16–64	32	64
Tetracycline	2->16	2	4	2–8	4	8
Trimethoprim	≤1.0–2	≤1.0	≤1.0	≤1.0–2	≤1.0	≤1.0
Trimethoprim-sulfamethoxazole	≤2.4/0.12			≤2.4/0.12		

[a] MIC_{50} and MIC_{90} are MICs at which 50 and 90% of strains are inhibited, respectively.

sources (2). Because of the high propensity of the species for human wounds, it was given the species name *vulneris* (Latin for wound). Recently, Dye et al. described two young male patients who grew both *E. vulneris* and *Enterobacter agglomerans* from wounds sustained in outdoor accidents (3). However, because of the known association of *Enterobacter agglomerans* with traumatic wound infections (5), the exact role of *E. vulneris* in producing tissue necrosis is unclear in reference 3. In our current series, all seven of our *E. vulneris* isolates were from soft-tissue infections. In none of these cases was *E. vulneris* found in large numbers; in most wounds, *E. vulneris* was associated with heavy growths of *S. aureus*.

E. hermannii was formerly known at CDC as Enteric Group 11 and was usually reported in the clinical laboratory as a yellow-pigmented *E. coli* strain (1). Of 25 human *E. hermannii* isolates received by CDC, 12 were from wounds, 6 were respiratory isolates, 5 were from stool specimens, and 1 each was from blood and spinal fluid specimens. (1). Three of our five isolates were from abscesses and were mixed with large numbers of pathogenic bacteria. The fourth isolate was from a case of *S. aureus* conjunctivitis, and the fifth was from a case of mixed bacterial peritonitis. In none of these infections was *E. hermannii* considered to be the primary pathogen.

The two *Escherichia* spp. were not pathogenic for mice, as determined in our preliminary studies. Although several other bacteria were lethal for this animal model, no significant pathogenicity was observed with *E. vulneris* or *E. hermannii* strains. As indicated by our data for isolates from humans, we were unable to induce wound infections with these bacteria.

Both species vary in their susceptibility to beta-lactam antibiotics. The isolates were very susceptible to the third-generation cephalosporins and, for the most part, were susceptible to the first- and second-generation cephalosporins. These bacterial strains were, however, resistant or only marginally susceptible to ampicillin and penicillin. These data are consistent with the report of Dye et al., who found that MICs of ampicillin for their two isolates of *E. vulneris* were 8 and 4 mg/ml, respectively (3).

In our study, we found the two *Escherichia* spp. to be only marginally susceptible or resistant to both chloramphenicol and tetracycline. In general, *E. hermannii* and *E. vulneris* strains appear to be resistant to more antibiotics than are community-acquired *E. coli* strains. The question of whether it is necessary to treat these bacteria at all, especially in immunocompromised hosts, awaits further clinical reports.

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