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Isolation of *Yersinia enterocolitica* (0:5,27 biotype 2) from a Common Garter Snake

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ABSTRACT: *Yersinia enterocolitica* serotype 0:5,27 biotype 2 was isolated from the intestinal contents of a common garter snake (*Thamnophis sirtalis*). The isolate possessed virulence associated phenotypes in all tests conducted. It was susceptible to amikacin, ampicillin/sulbactam, aztreonam, cefoperazone, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, gentamicin, imipenem, mezlocillin, norfloxacin, piperacillin, ticarcillin/clavulanic acid, trimethoprim-sulfamethoxazole, tobramycin, chloramphenicol and tetracycline. The isolate harbored the virulence plasmid.

Key words: *Yersinia enterocolitica*, common garter snake, virulence phenotype, antimicrobial susceptibility, virulence plasmid, *Thamnophis sirtalis*.

Yersinia enterocolitica and related species are ubiquitous and widely distributed. The organisms frequently have been isolated from domestic, wild and zoo animals, birds and invertebrates (Hurvell, 1981; Mingrone and Fantasia, 1988; Brittingham et al., 1988). Although the organisms are relatively common in the environment, only certain biotypes and serotypes of *Y. enterocolitica* are consistently pathogenic for humans (Cornelis et al., 1987). Swine have been implicated as the reservoir of human pathogenic strains (Tauxe et al., 1987). The strains isolated from wild and zoo animals usually do not possess the characteristics of human pathogens (Hurvell, 1981; Mingrone and Fantasia, 1988). To our knowledge, there are no published reports on the isolation of *Y. enterocolitica* from snakes.

This study was conducted in the course of an investigation on the occurrence of western equine encephalitis virus in snakes in Saskatchewan, Canada. The 201 garter snakes (*Thamnophis sirtalis*) examined were collected from hibernaculae in southwestern Saskatchewan (51°27′N, 109°08′W) in the spring of 1988. The entire large intestine and part of the ileum of each exsanguinated snake was removed aseptically and cut into very small pieces. About 1 g of intestinal tissue samples from each snake was inoculated into 10 ml of 0.067 M phosphate buffered saline (PBS), pH 7.6. The tubes were kept at 4 C; samples were streaked onto Cefsulodin, Irgasan, Novobiocin (CIN) agar plates (Difco Laboratories, Detroit, Michigan, USA) at 7 and 14 days. The specimens also were checked for *Salmonella* spp. following enrichment in selenite-F broth and plating on Salmonella-Shigella agar (Ewing, 1986). Suspect colonies were identified by API 20E system (Analytab Products, Plain View, New York, USA) and by the methods of Ewing (1986). Any isolates were serotyped by slide agglutination at the National *Yersinia* Reference Laboratory, Toronto, Canada.

The antimicrobial susceptibility of yersiniae was determined by the API Uniscept® type 3 (Analytab Products) and by the disk susceptibility test (National Committee for Clinical Laboratory Standards, 1984). Beta-lactamase production was determined using Nitrocefin impregnated Cefinase® discs (Baltimore Biological Laboratories, Microbiology Systems, Cockeysville, Maryland, USA). The following virulence associated characteristics of the isolate were determined by the methods of Kwaga and Iversen (1992): calcium dependency, serum resistance, autoagglutination, latex particle agglutination, salt aggregation, pyrazinamidase activity, crystal violet binding, congo red binding, heat-stable enterotoxin production, HEp-2 cell line invasion, HEp-2 toxicity, and mouse lethality. Plasmid DNA was isolated using the rapid, small-scale alkaline lysis method as described by Maniatis et al. (1982). Restriction enzymes (*Eco*RI, *Bam*HI, and

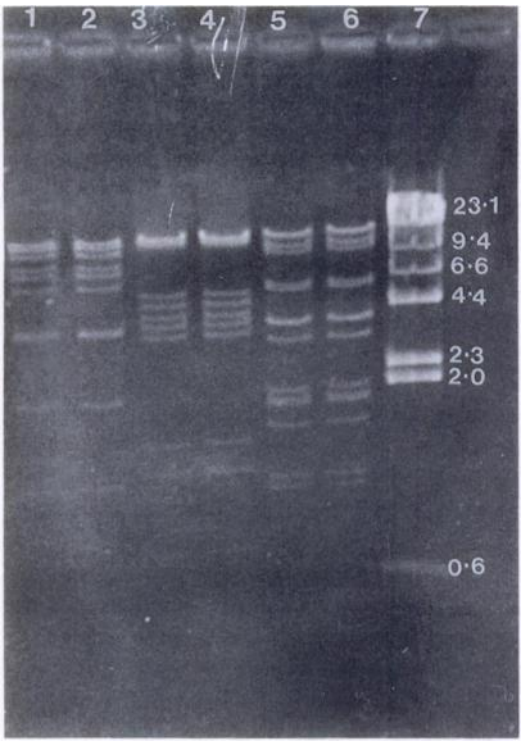

FIGURE 1. Plasmid DNA from snake (Lanes 1, 3, 5) and from a reference strain (C9044) (Lanes 2, 4, 6) were digested with *Eco*RI, *Bam*HI, and *Hind*III, respectively. Lane 7 is Lambda DNA molecular size marker digested with *Hind*III (Bethesda Research Laboratories, Gaithersburg, Maryland). The numbers to the right are the molecular sizes of the fragments of the size marker in kilobases.

*Hin*dIII) were used according to the 1990 instruction manuals of the manufacturers (Boehringer Mannheim, Germany; Sigma, St. Louis, Missouri, USA). Restricted DNA was separated through 1% agarose gel in Tris-Borate buffer and stained with ethidium bromide for visualization and photographing (Maniatis et al., 1982).

No *Salmonella* was isolated. One *Y. enterocolitica* isolate was recovered. It belonged to serogroup 0:5,27 biotype 2. It was positive for beta-lactamase production. The *Y. enterocolitica* was susceptible to amikacin, ampicillin/sulbactam, aztreonam, cefoperazone, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, gentamicin, imipenem, mezlocillin, norfloxacin, piperacillin, ticarcillin/clavulanic acid, trimethoprim-sulfamethoxazole, tobramycin, chloramphenicol and tetracycline; and resistant to ampicillin, ampicillin/clavulanic acid, cefazolin, cefoxitin, cephalothin, clindamycin, erythromycin, oxacillin, penicillin, vancomycin, and streptomycin. This antibiotic susceptibility pattern observed for the isolate was similar to those that have been found in in vitro tests against yersiniae (Hornstein et al., 1985; Kanazawa et al., 1987). The isolate had characteristics of virulence in all the tests of virulence phenotypes described above. It also had a plasmid of about 70 kilobases (Kb) in size (Fig. 1), typical of virulent strains (Cornelis et al., 1987). The restriction fragment patterns of the plasmid were similar to those of a reference strain of human origin (Fig. 1) and to swine isolates (Kwaga and Iversen, unpubl.) belonging to the same serogroup affiliation. This was contrary to the findings of Mingrone and Fantasia (1988) in which isolates from wild and zoo animals were negative for virulence associated characteristics. The yersiniae from that study also generally did not harbor the typical virulence plasmids. It was fortuitous that we isolated a virulent strain of *Y. enterocolitica* from a garter snake. It is difficult to determine the source of this bacterium in the snake, but it may have originated from a contaminated run-off water or from sewage. In Saskatchewan, swine have harbored various bioserotypes of *Y. enterocolitica* including serotype 0:5,27 biotype 2 (Kwaga et al., 1990).

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