

# *Edwardsiella tarda*—identification and clinical significance

## REPORT OF TWO CASES

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THE family *Enterobacteriaceae* is composed of a group of gram-negative bacilli, members of which are among the most frequently encountered organisms in specimens submitted to the clinical microbiology laboratory for culture. Edwards and Ewing<sup>1</sup> described this family of microorganisms in detail and presented a classification based on the study of numerous biochemical and serologic characteristics of more than 11,000 strains. Recently this classification was revised,<sup>2</sup> and now includes a new tribe, *Edwardsiellae*, with a single genus *Edwardsiella*. The type and only species is *Edwardsiella tarda*.

Our paper presents a report of two cases of the isolation of *Edwardsiella tarda* from clinical material, a short review of pertinent scientific reports, and a description of clinically useful biochemical reactions that may be used to distinguish this new genus from other biochemically related members of *Enterobacteriaceae*.

## REPORT OF CASES

**Case 1.** A 15-year-old Caucasian boy was admitted to the Cleveland Clinic Hospital in July 1967 because of an injury sustained four days earlier while swimming in a lake in Ontario, Canada. The patient had struck a submerged log, and a large wood splinter entered his right thigh. He consulted a local physician, who made a clinical diagnosis of gas gangrene, and gave him injections of penicillin, streptomycin, and tetanus toxoid. The boy was referred to the Cleveland Clinic for further treatment, with possibly the use of hyperbaric oxygen.

On admission to the Cleveland Clinic Hospital, his temperature was 100.8 F and he was in acute distress. Results of the physical examination were normal with the exception of the wound, which was draining a foul, fecal-smelling, brown material. Culture of the wound revealed, in addition to *Clostridium perfringens*, a gram-negative bacillus subsequently identified as *Edwardsiella tarda*. The patient was treated with polyvalent *Clostridium*, antitoxin, penicillin, and chloramphenicol, as well as surgical debridement and drainage of the wound. He responded to this treatment and was discharged from the hospital 15 days after admission, with his wound healed.

**Case 2.** A 53-year-old Caucasian man, was first seen at the Cleveland Clinic in January 1968, because of long-standing hepatomegaly and intermittent diarrhea since July 1967. He had spent the last 32 years almost continuously in Central and South America. In December 1967, the diarrhea became severe and was accompanied by fever. Treatment with chloramphenicol elicited no clinical response. Cultures of feces were not made at that

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time. Mild diarrhea and low-grade fever persisted. He was hospitalized primarily because of hepatomegaly. Biopsy of the liver revealed nutritional cirrhosis. The hepatic function studies disclosed the following values: serum bilirubin, 1.6 mg per 100 ml; serum glutamic oxaloacetic transaminase (SGOT), 84 units; serum albumin, 3.1 g per 100 ml;  $\gamma$ -globulin, 1.7 g per 100 ml; sulfobromophthalein test, 34 percent retention; and prothrombin time, 13 sec (control 12 sec).

Examination of feces revealed rare ova of *Trichuris trichiuria*. Stool culture on *Salmonella-Shigella* medium yielded *Edwardsiella tarda*. Diarrhea was controlled while the patient was hospitalized, and he was discharged from the hospital and advised to continue therapy aimed at alleviating this hepatic problem.

#### REVIEW OF PUBLICATIONS

Cultures exhibiting biochemical features of the currently recognized genus *Edwardsiella* were collected and studied by Ewing and associates<sup>3</sup> in 1959. These organisms were simply referred to as "bacterium 1483-59." According to Ewing and associates,<sup>3</sup> Sakazaki in 1962 reported the isolation of a new group of *Enterobacteriaceae* which he termed the Asakusa group. Those cultures were isolated chiefly from snakes, and were similar to bacterium 1483-59, with a few exceptions in the biochemical reactions. King and Adler<sup>4</sup> in 1964 described the isolation of a bacterial strain with characteristics of bacterium 1483-59 from a man hospitalized with enteric fever and acute gastroenteritis. The group name of Bartholemew suggested by them represented the county in Indiana wherein the strain was isolated.

Up to 1965, Ewing and associates<sup>3</sup> studied in detail 37 strains submitted to the National Communicable Disease Center. The strains originated in widely separated geographic areas—the United States (16 states), Brazil, Ecuador, Israel, and Japan. Of the 37 isolates, 34 were obtained from man, the others from animals including cattle and reptiles. Of the strains isolated from man, the majority (25) were recovered from the feces from patients with a history of diarrhea. Eighteen patients had no history of gastrointestinal symptoms. Of the extraintestinal isolations, wounds were found to harbor *Edwardsiella tarda* in five instances. Single isolations from blood and urine, and two from unknown sources were also noted.

Recently, Sonnenwirth and Kallus<sup>5</sup> described a fatal case of primary meningitis due to *Edwardsiella tarda* in a 31-year-old Caucasian woman.

#### DISCUSSION

Previous reports<sup>3</sup> have already pointed out the similarity between biochemical reactions of *Edwardsiella tarda* and other members of *Enterobacteriaceae*, notably *Salmonella*, *Arizona*, *Citrobacter*, and *Proteus*. All these organisms are characterized by the production of hydrogen sulfide ( $H_2S$ ) and, in most cases, a failure to ferment lactose (as much as 60 percent of *Arizona* and *Citrobacter* strains promptly ferment lactose). Since in most diagnostic laboratories these two characteristics are the major basis for the prompt recognition of potential *Salmonellae*, a rapid

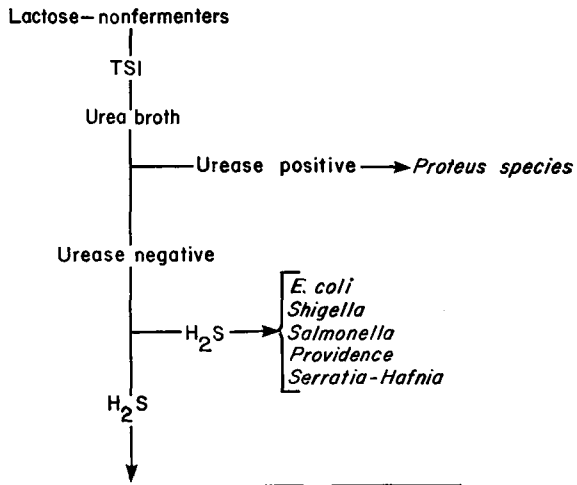
means for differentiating these groups is required. Urease activity of the *Proteus* group quickly segregates these organisms from the rest. In addition, polyvalent *Salmonella* antiserum can provide information leading to a presumptive serologic identification of members of the *Salmonella* group. The urease test and serologic typing can be accomplished in less than six hours and should not significantly delay institution of therapy. It is to be remembered that serologic tests performed before biochemical verification can only be of presumptive value. In the event that urease activity cannot be demonstrated, and agglutination of the organism in question does not take place, further tests are required for definitive identification. Based upon the extensive studies of the biochemical reactions of *Edwardsiella tarda*, *Salmonella*, *Arizona*, and *Citrobacter* by Ewing and associates,<sup>3</sup> a scheme for the differentiation of these groups of organisms was designed (Fig. 1) by us.

All lactose nonfermenting colonies are inoculated to a triple sugar iron agar (TSI) slant, and urea broth or agar. Those organisms producing in TSI an alkaline reaction of the slant, an acid butt, and H<sub>2</sub>S, but remain urease negative after 18 hr of incubation, are tested with polyvalent *Salmonella* antiserum. If agglutination occurs, group-specific antisera are used and a presumptive preliminary report of *Salmonella* with the appropriately designated serologic group is issued. Whether the agglutination test is or is not reactive, the following media are inoculated so that definitive identification can be made: lysine decarboxylase broth, malonate broth, semisolid agar medium or other suitable medium for demonstration of indol (e.g., SIM), Simmon's citrate agar, mannitol CTA agar, and arginine dehydrolase broth.

The reactions produced in these media have been chosen as being clearly positive or negative in more than 90 percent of the strains studied by Ewing and associates.<sup>3</sup> The expected results for the four groups under consideration are listed in Figure 1. With the exception of the arginine dehydrolase reaction, which may require three days' incubation, all reactions can be read, and are valid after 18 hr of incubation.

In vitro antibiotic sensitivity tests were performed on the isolates of *Edwardsiella tarda* strains in the two cases reported here. Both isolates were tested by the single-disk method and were susceptible to ampicillin, cephalothin, tetracycline, kanamycin, novobiocin, streptomycin, and chloramphenicol. In addition, the isolate from patient 2 was tested in vitro by the tube dilution method with a microtiter technic. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the drugs tested are shown in Table 1.

In the in vitro dilution studies of Sonnenwirth and Kallus,<sup>5</sup> they tested the susceptibility of their strain of *Edwardsiella tarda* (isolated from the cerebrospinal fluid) to chloramphenicol, tetracycline, ampicillin (MIC 0.78 µg per milliliter; MBC 1.56 µg per milliliter for each drug); and kanamycin (MIC 12.5 µg per



Medium or test	<i>Salmonella</i>	<i>Edwardsiella</i>	<i>Arizona</i>	<i>Citrobacter</i>
Polyvalent <i>Salmonella</i> Antiserum	+	-	-	-
Lysine decarboxylase	+	+	+	-
Malonate	-	-	+	-
Indol	-	+	-	-
Citrate	+	-	+	+
Mannitol	+	-	+	+
Arginine dehydrolase	+ (delayed)	-	+ (delayed)	+ (delayed)
	↓ <i>Salmonella</i> species *	↓ <i>E. tarda</i>	↓ <i>A. arizonae</i>	↓ <i>C. freundii</i>

\* Further serologic tests required for definitive identification.

Fig. 1. Scheme for the differentiation of lactose nonfermenting hydrogen sulfide producing *Enterobacteriaceae*.

milliliter; MBC 25 µg per milliliter). These authors believed that, while still in the effective range, the values obtained for kanamycin were remarkably high. In our study, kanamycin appears to have been much more effective (MIC 0.95 µg per milliliter). Both chloromycetin and tetracycline, while showing a good inhibitory effect, were not bactericidal in amounts less than 50 µg per milliliter. The in vitro susceptibility to cephalothin and polymyxin B has not been previously reported. Cephalothin is highly effective, while the organism revealed a remarkable insensitivity to polymyxin B, the MIC and MBC values being in excess of 100 µg per milliliter.

**Table 1.**—Results of *in vitro* tube dilution antibiotic susceptibility tests of *Edwardsiella tarda*

Drug	Minimum concentration, $\mu$ /gml	
	Inhibitory	Bactericidal
Ampicillin	0.095	0.095
Tetracycline	0.095	50.0
Kanamycin	0.095	0.095
Cephalothin	0.19	0.39
Chloramphenicol	12.5	50.0
Polymyxin B	100.0	100.0

The significance of *Edwardsiella tarda*, as an etiologic agent of disease in man is as yet not certain. The recently reported case of fatal meningitis due to this organism,<sup>6</sup> proves that it can indeed be severely pathogenic. The reported association of *Edwardsiella tarda* with acute diarrhea and gastroenteritis, as well as its isolation from wounds, is of particular interest in view of the cases reported here. Certainly the few documented cases thus far reported do not confirm, they only suggest, that this organism is an etiologic agent of gastroenteritis.

Serum from patient 2 was made available for serologic study five months after his bout of severe diarrhea. H and O antigens of the *Edwardsiella tarda* strain isolated from this patient were prepared in formalinized saline (H antigen) and alcohol-saline (O antigen) using the method of Kauffmann.<sup>6</sup> The Widal reaction, in the standard tube agglutination test, was positive with a serum dilution of 1:160 for both H and O agglutinins. Sera from normal individuals tested at the same time were negative.

While the interpretation of a single determination must be made with caution, an agglutinin titer of 1:160 five months after an episode of severe diarrhea is highly suggestive, if not definitely indicative, of infection with *Edwardsiella tarda*.

The significance of *Edwardsiella tarda* isolated from wound cultures is not clear. In case 1, *Edwardsiella tarda* was isolated from an obviously contaminated wound affected by gas gangrene due to *Clostridium perfringens*. There appears to be no significance in the association of these two organisms.

It is apparent that further study of *Edwardsiella tarda* is indicated. Increased awareness of this new species and a convenient means to differentiate it from biochemically similar organisms should lead to further investigation.

#### SUMMARY

Two cases in which *Edwardsiella tarda* was isolated from clinical specimens are presented, with a brief review of the pertinent medical writings. A scheme is presented for identification of this organism and its differentiation from other

organisms with similar basic biochemical characteristics. Elevated serum antibody titers to H and O antigens of *Edwardsiella tarda* in a patient with diarrhea, from whom *Edwardsiella tarda* was isolated, strongly suggest that this organism can be an etiologic agent of acute gastroenteritis.

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