

***Edwardsiella tarda* in a study of juvenile diarrhoea**

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Edwardsiella tarda is the name proposed by Ewing, McWhorter, Escobar & Lubin (1965) for a new group of organisms within the Enterobacteriaceae, studied since 1959 and referred to by them as 'bacterium 1483-59'. These bacteria were similar to those named the 'Asakusa' group by Sakazaki & Murata (1962) and Sakazaki (1965), and the 'Bartholomew' group by King & Adler (1964). The major characters which distinguish these organisms from existing genera are that they give a negative phenylpyruvic acid test and are mannitol-negative and produce abundant H₂S.

Of the human strains of Ewing *et al.* (1965) 25 out of 34 had been isolated from faeces, the others from extra-intestinal sites, but a history of diarrhoea was available in only five instances. One of the two animal strains in their study came from a bovine case of diarrhoea. The one strain described by King & Adler (1964) was isolated from the faeces of a patient with both enteric fever and acute gastro-enteritis. In contrast, of the 256 strains of Sakazaki (1965) 250 came from animals, almost wholly snakes, and only five from human gastro-enteritis cases. The incidence of *Ed. tarda* and its role in human diarrhoea is not precisely known, and it seems worth recording the isolation of such organisms during a special study of juvenile diarrhoea at present in progress at Vellore.

MATERIALS

Children below the age of 5 years in a rural area and an urban area were selected for a detailed bacteriological study during the period June 1963 to September 1965 to evaluate specifically the etiological role in juvenile diarrhoea of the Arizona, Citrobacter (including the Bethesda-Ballerup subgroup) and Providence genera of the Enterobacteriaceae. Also included were children with diarrhoea attending the paediatric outpatient service and those admitted to the children's ward of the Christian Medical College (C.M.C.) Hospital. Rectal swabs were taken from the diarrhoeal cases before antimicrobial therapy was begun.

A control group of children without diarrhoea in both the rural and urban areas were also investigated subsequently. Specimens collected monthly from non-

diarrhoeal children in the rural area were examined from September 1965 to January 1966 and in the urban area from September 1965 to July 1966.

METHODS

The isolation and identification methods used at C.M.C. were those of Bhat & Myers (1962) with a few modifications. The direct plating media were sheep blood agar (BA), MacConkey agar (MA), deoxycholate citrate agar (DA) and bismuth sulphite agar (BS). Enrichment in Selenite F broth was also used. After incubation overnight at 37° C., suspect colonies were subcultured to the set of preliminary screening tests shown in Table 1. These tests included the use of lysine iron agar (LIA) specifically recommended by Edwards & Fife (1961) for the identification of Arizona strains.

Table 1. *Reactions of Edwardsiella tarda in screening tests*

Test	<i>Ed. tarda</i> 4 strains
TSI: Butt (glucose)	AG
Slant (lactose/sucrose)	—
H ₂ S	+
LIA: Lysine decarboxylase	+
H ₂ S	+
Urease	—
Indole	+
Motility	+

AG = acid and gas.

The selected additional biochemical tests used at C.M.C. were similar to those included in the set of detailed tests (Carpenter, Lapage & Steel, 1966) in use at the Dysentery Reference Laboratory (D.R.L.), with minor exceptions. At C.M.C. nutrient broth with bromthymol blue as indicator was used for the fermentation tests, whereas at D.R.L. 1% (w/v) peptone water with Andrade indicator was used. Each medium contained 0.5% (w/v) of the various carbohydrates and in both laboratories the cultures were incubated for 21 days. The methyl red (MR) and Voges-Proskauer (VP) tests were incubated at 37° C. for 3 days at C.M.C., and for 3 days at 30° C. at D.R.L. For the phenylpyruvic acid (PPA) and malonate tests at C.M.C. the phenylalanine malonate medium of Difco Laboratories Inc., U.S.A., was used. In both laboratories the method of Møller (1955) was used for the decarboxylase tests.

RESULTS

During the period June 1963 to September 1965, from 1491 episodes of diarrhoea in 513 children (748 episodes in 265 rural children and 743 in 248 urban children) two proved strains of *Ed. tarda* and two presumptive strains (subsequently not available for detailed study) were isolated. No such strains were isolated from the control children without diarrhoea in the subsequent survey. The latter consisted

of 371 specimens from 141 rural children in the period September 1965 to January 1966, and 950 specimens from 178 urban children from September 1965 to July 1966. (Details of other intestinal pathogens will be published elsewhere.)

Clinical history of patients

Case 1. This was an 8-month-old male from the urban area with a history of having watery greenish yellow stools without blood and mucus for 3 weeks before the organism, subsequently identified as *Ed. tarda*, was isolated from a faecal specimen in December 1963. Other recognized intestinal pathogens were not isolated on this occasion. Apart from this episode of diarrhoea, the child had had eight others during the period of study, one of which had been associated with the known pathogen, *Salmonella enteritidis*.

Case 2. This was a 16-month-old female child from the rural area situated about 14 miles from the urban area in the study. This child, when first examined, had a history of diarrhoea of 3 days' duration, and a rectal swab yielded *Shigella flexneri 2a*. From the follow-up specimen, taken 2 weeks later in December 1963 when the child had recovered from her diarrhoea, the shigella strain was not isolated again, but the strain of *Ed. tarda* was cultured, and so this strain was isolated during convalescence from bacillary dysentery. During the period of the study, this child had had six episodes of diarrhoea, two of which had been associated with shigella infections, but specific pathogens had not been isolated during the other four attacks

Cases 3 and 4. These were a 4-year-old male child and a 3-year-old female child in two nearby hamlets in the rural area under study, both children having a history of diarrhoea for 3 days. The presumptive strains of *Ed. tarda* were unlikely to be directly linked epidemiologically as they were isolated from the children in October 1964 and January 1965 respectively. *Sh. flexneri 2a* was isolated from the female child at the same time.

Reactions of the strains

The initial biochemical and serological examination of the first two strains, isolated in 1963, suggested that the strains did not belong to any of the recognized genera of the Enterobacteriaceae, and the report of a reference laboratory to which they were submitted confirmed this, but the organisms were not specifically identified. However, in 1966, a review of the reactions of the strains by one of us (K.P.C.*), in consultations at Vellore, strongly indicated that the strains might be *Ed. tarda*, and subsequently the strains were examined in detail to confirm their identification. At this time, unfortunately, only the strains from the first two cases were viable.

Colonial appearance

On BA the colonies of the four strains were small, greyish, smooth, low dome-shaped and non-haemolytic. On MA and DA they were small and pale non-lactose-fermenting and after incubation for 48 hr. the colonies on DC developed a dark

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centre. On BS they were greyish with a tiny brownish halo and metallic sheen. The colonies were rather smaller than salmonellas or shigellas on comparable media. All strains survived enrichment in selenite broth.

Biochemical reactions

The reactions of the four strains in the screening tests are given in Table 1. Table 2 combines the detailed reactions of the first two strains and some of the reactions of the second two strains in the tests done at C.M.C. and D.R.L.

Table 2. *Detailed biochemical reactions of Edwardsiella tarda*

Test	<i>Ed. tarda</i>	Test	<i>Ed. tarda</i>	
Glucose	AG	Simmons's citrate	-	
Lactose	-	Christensen's citrate	+	
Mannitol	-	Malonate	-	
Maltose	AG	Gluconate	-	
Glycerol	(a)	Gelatin liquefaction	-	
Sucrose, dulcitol, salicin, xylose, adonitol, arabinose, cellobiose, dextrin, inositol, raffinose, rhamnose, sorbitol, trehalose	}	Growth in KCN	-	
		Nitrate reduction	+	
		β -galactosidase	-	
		Catalase	+	
		Oxidase	-	
PPA	-	Decarboxylases:	Arginine	-
MR	+		Lysine	+
VP	-		Ornithine	+
		Hugh & Leifson	Fermentation	

(a) = weak acid production after incubation for more than 48 hr.

Serological reactions

As the reactions in the screening medium, LIA, were similar to those of the *Salmonella* and Arizona groups, the strains were specifically tested in polyvalent salmonella O sera covering groups A to E (Lederle Laboratories, U.S.A.) and in seven pools of Arizona O sera (kindly provided by Dr Joan Taylor, Colindale) but the four strains did not agglutinate in these sera nor in the sera available for any other recognized pathogenic group of the Enterobacteriaceae. Subsequently, Dr R. Sakazaki, Tokyo, typed the first strain (F197/63) as *Ed. tarda* 016: H1, and the second strain (F236/63) as H4 but the O group could not be determined as the strain was by then serologically rough.

Drug sensitivity pattern

The strains were sensitive to chloramphenicol, streptomycin, tetracycline, neomycin, ampicillin and furazolidone but resistant to sulphonamides and colomycin.

DISCUSSION

Strains of *Ed. tarda* were isolated from only four out of 832 children under five years during an intensive survey extending over approximately 3 years. These strains were isolated from children with diarrhoea or an immediately antecedent history of it, and none was isolated from the control children without diarrhoea.

From one of the children whose specimen yielded this organism the accepted pathogen, *Sh. flexneri* 2a, was isolated in addition, and another child was convalescent from a shigella infection. Dorigan & Deinhardt (personal communication) likewise reported the simultaneous isolation of *Sh. sonnei* and *Ed. tarda* from a marmoset. It is not possible from the C.M.C. study to decide whether *Ed. tarda* is a specific pathogen in its own right or a secondary invader in an otherwise physiologically abnormal intestine. However, it would appear from this study that this organism is rare in human intestinal infections and indeed no other wild strains from patients in either Britain or elsewhere have been received by D.R.L. from 1945 to 1966. It is possible from the published records of the isolation of this organism (King & Adler, 1964; Ewing *et al.* 1965; Sakazaki, 1965; Wallace, White & Gore, 1966) that it may have a specific geographic distribution possibly dependent on the reservoir being mainly in such animals as snakes, seals and alligators, and that man is only an accidental host. This is analogous to the Arizona group as no human infections caused by these organisms have been recorded in Britain up to the end of 1965 (J. Taylor, personal communication).

The reactions given in Tables 1 and 2 conform to those of stock strains of *Ed. tarda* (kindly supplied by Dr W. H. Ewing, Atlanta, U.S.A.) and to those of other published reports.

From Table 1 it is clear that the reactions of *Ed. tarda* in the screening tests are similar to those of the *Salmonella* and Arizona groups except for the indole reaction, though indole-positive salmonella strains do occur. These groups typically show rapid decarboxylation of lysine and abundant H₂S production in LIA, and in this study the LIA medium proved a very useful screening medium for the various H₂S-producing organisms of the Enterobacteriaceae which give identical reactions in triple sugar iron (TSI) agar.

Ed. tarda ferments very few carbohydrates but in its other biochemical reactions it gives, apart from H₂S production, the basic pattern of *Escherichia coli*, or the Alkaescens-dispar group which it closely resembles by its reactions in the two citrate media. As it ferments so few carbohydrates, and particularly fails to ferment mannitol, it may be superficially confused with strains of *Proteus* and Providence from which it is easily distinguished by its negative urease and PPA reactions, and with *Plesiomonas shigelloides* (Eddy & Carpenter, 1964) from which it is distinguished by the negative oxidase reaction.

Though *Ed. tarda* appears to be rare in human intestinal infections it is possible that it is not being recognized as it has been only recently described and has unusual characters.

The accepted major pathogens of the Enterobacteriaceae are isolated from only a small proportion of patients with diarrhoea, and it is urged, therefore, that more specific attention should be paid to the isolation of other enterobacteria, such as *Ed. tarda* for example, to determine further their etiological role in intestinal infections.

SUMMARY

Four strains of *Edwardsiella tarda*, a recently described member of the Enterobacteriaceae, were studied. They were isolated from four out of 832 children during a 3-year survey of juvenile diarrhoea at Vellore, S. India. Three of the four children had diarrhoea when the organisms were isolated and one was convalescent from bacillary dysentery. From this study and a review of the literature it appears that *Ed. tarda* is found only infrequently in man and that its main reservoir may be in animals such as snakes and seals. It is suggested that this organism should be specifically looked for in cases of diarrhoea to determine its etiological role, particularly when the major intestinal pathogens are not isolated.

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