

## EDWARDSIELLA TARDA INFECTIONS IN MAN

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### Summary

During a period of 7 years, 13 Strains of *Edwardsiella tarda* were isolated from intestinal as well as extra intestinal infections in man — 8 strains from faeces, 2 from blood, 2 from wound swabs and one from urine. The clinical features, biochemical characteristics and antibiotic susceptibility to 18 chemotherapeutic drugs were studied. *E. tarda* exists as a normal commensal in the intestines of man and animals but it has been associated with a number of infections in man. Because of its potential pathogenicity, greater awareness of the organism is necessary in order to elucidate its role in human infections.

Since 1959, an unusual biotype of *Enterobacteriaceae*, mannitol negative, hydrogen sulphide positive, was frequently isolated from reptiles. Sakazaki<sup>1</sup> in 1962 studied 153 cultures of this organism and suggested that they should be placed in a single biochemical group of the family *Enterobacteriaceae* and proposed the name "Asakusa" for the group — a place name where the original strain was isolated. This organism was also labelled "bacterium 1483-59".<sup>2</sup>

King and Adler in 1964<sup>3</sup> described the isolation of a culture of bacterium 1483-59, which they labelled the "Bartholomew group". This was from a man hospitalized with enteric fever and acute gastroenteritis.

Ewing *et al*<sup>2</sup> studied 37 strains of this organism and came to the conclusion that they belonged to the family of *Enterobacteriaceae*. They suggested that a new genus should be created for this organism and proposed the name *Edwardsiella*. Further it was suggested that the specific epithet *tarda* (from Latin, slow, implying inactivity) be adopted.

*Edwardsiella tarda* (*E. tarda*) has been isolated from the intestinal flora of man as well as snakes,<sup>1</sup> sea lion and alligators<sup>4</sup> and from pig bile.<sup>5</sup> It has been described as the causative agent of red fin disease in eels and has been isolated in abundance from diseased eels cultured in various areas of Japan<sup>6</sup> and diseased channel catfish in the USA.<sup>7</sup>

In recent years, *E. tarda* has been associated with a number of clinical infections — juvenile

diarrhoea,<sup>8</sup> neonatal meningitis,<sup>9</sup> liver abscess and septicaemia, mild diarrhoea as well as wound infections.<sup>10</sup>

The following is a report on 13 strains of *E. tarda* isolated from clinical material at the University Hospital, Kuala Lumpur between April 1970 and October 1977. Their biochemical characteristics, antibiotic susceptibility patterns and relevant clinical data are included.

### MATERIALS AND METHODS

#### Source and identification of cultures

Clinical specimens were cultured routinely using the following methods:—

Stools were plated on blood agar, desoxycholate citrate agar and MacConkey agar. Selenite broth was also inoculated.

Blood cultures were obtained by venepuncture and placed in 'Liquoid' broth and cooked meat media.

Pus swabs were Gram stained, and cultured on blood, chocolate and MacConkey agar.

Urine viable counts were performed on blood and MacConkey agar plates using the urostrip method.<sup>11</sup>

Cultures were incubated for 1 to 2 days at 37°C.

Suspect colonies from each plate were picked, Gram stained, inoculated into Kligler's iron agar slopes (Difco), and MacConkey agar plates. Detailed biochemical tests were carried out according to the methods of Cowan & Steel.<sup>12</sup>

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*Antibiotic Susceptibility Tests*

The strains were tested against 18 chemotherapeutic agents by the disc diffusion method as described by Bauer and associates,<sup>1,3</sup> but using Oxoid Diagnostic Sensitivity Agar. Disc potencies of drug used (in micrograms, except penicillin which is in units) were as follows: penicillin G (1), ampicillin (10), cephaloridine (25), tetracycline (25), chloramphenicol (25), streptomycin (10), kanamycin (30), gentamicin (10), carbenicillin (100), polymixin B (100), erythromycin (5), sulphadimidine (200), cotrimoxazole (25), nalidixic acid (30), nitrofurantoin (200), rifampicin (30), tobramycin (10) and neomycin (30). Inhibition zone diameters were measured in millimeters and recorded as sensitive (susceptible and intermediate zones of Bauer *et al*<sup>1,3</sup>) or resistant.

*Clinical observation*

Patients' case records were reviewed for information such as age, sex, source of specimen, presence of other pathogens in mixed cultures, antibiotic treatment as well as other relevant clinical data.

RESULTS

*Edwardsiella tarda* is a motile gram negative rod. The colonies at 24 hours on blood agar appear as smooth, slightly opaque and about 2mm in diameter with an entire edge. On MacConkey agar they appear as colourless, smooth colonies, also about 2mm in diameter with an entire edge.

The biochemical reactions of the 13 isolates are presented in Table I. All isolates were highly consistent in most of their reactions except for fermentation of sucrose. One strain only fermented sucrose at 24 hours with production of acid and gas. Most reactions were complete in 1 to 2 days.

The results of antibiotic susceptibility tests showed that all 13 strains were uniformly resistant to penicillin, erythromycin and polymixin B, but sensitive to all the other 15 antibacterial agents tested — ampicillin, cephaloridine, tetracycline, chloramphenicol, streptomycin, kanamycin, gentamicin, carbenicillin, sulphadimidine, trimethoprim-sulphamethoxazole, nalidixic acid, nitrofurantoin, rifampicin, tobramycin and neomycin.

TABLE I  
BIOCHEMICAL REACTIONS OF 13 STRAINS OF *EDWARDSIELLA TARDA*.

Test or substrate.			
Motility (37°C)	+	L-rhamnose	—
Catalase	+	Salicin	—
Oxidase	—	D-sorbitol	—
Growth in KCN medium	—	sucrose	—*
Citrate (Simmon's)	—	trehalose	—
Malonate	—	D-xylose	—
Gas from glucose	+	ONPG	—
Acid from		Indole	+
adonitol	—	Urease	—
L-arabinose	—	Methyl red	+
D-arabitol	—	Voges Proskaur	—
cellobiose	—	Kliger's iron agar	
dulcitol	—	alkaline slant	+
glycerol	+	acid butt + gas	+
inositol	—	H <sub>2</sub> S	+
lactose	—	Arginine dihydrolase	—
maltose	+	Lysine decarboxylase	+
D-mannitol	—	Ornithine decarboxylase	+
D-mannose	+	phenylalanine deaminase	—
mucate	—	gelatin	--
raffinose	—	nitrate to nitrite	+

\*1 strain produced acid from sucrose

The clinical sources of the 13 isolates are shown in Table II. The organisms were isolated mainly from stool — 8 strains. Two were from blood cultures and two from wound swabs. The 2 isolates from wound swabs were recovered as pure cultures exhibiting heavy growth. Only one of the 13 isolates was from urine (colony count exceeding  $10^5$  per ml).

TABLE II  
SUMMARY OF FINDINGS IN 13 PATIENTS FROM WHOM *E. TARDA*  
WAS ISOLATED

No.	Age (yr) Sex	Clinical Data	Source of culture material	Findings
1.	34 M	One month's history of lower abdominal colic with bloody diarrhoea. Final diagnosis: Ulcerative colitis with gastroenteritis.	Stool	<i>E. tarda</i> (twice)
2.	28 M	One week's history of diarrhoea and abdominal colic. Watery stools with blood and mucus. No fever. Patient became asymptomatic without any antibiotic treatment. Diagnosis: gastroenteritis.	Stool	<i>E. tarda</i>
3.	41 F	Pain in the right iliac fossa for 2½ months. Diarrhoea with mucus and blood in stools for one week. Surgical drainage of appendicular abscess performed. Developed diarrhoea on the sixth post-operative day. Patient died. Final diagnosis: purulent peritonitis.	Stool	<i>E. tarda</i>
4.	2½ M	2 days' history of passing watery stools with no blood or mucus. Slight dehydration present. Patient's symptoms improved with intravenous fluid therapy. No antibiotics were administered. Diagnosis: gastroenteritis	Stool	<i>E. tarda</i>
5.	21 M	Chronic diarrhoea for 2 months.	Stool	<i>E. tarda</i> <i>Salmonella</i> species <i>Plesiomonas</i> <i>shigelloides</i>
6.	68 M	Five days history of severe epigastric pain associated with fever, chills and rigors. A perforated gall-bladder was found at surgery and cholecystectomy performed. Blood for culture was taken before surgery. Patient was treated with ampicillin	Blood Pus gall- bladder	<i>E. tarda</i>   <i>Escherichia</i> <i>coli</i>

TABLE II  
SUMMARY OF FINDINGS IN 13 PATIENTS FROM WHOM *E. TARDA* WAS ISOLATED (CONT'D)

No.	Age(yr) Sex	Clinical Data	Source of culture material	Findings
		and kanamycin. Final diagnosis: Empyema of the gall-bladder.		
7.	22 F	History of discharge from (R) ear since childhood. Recurrence of ear discharge with headache, fever, chills, rigors and vomiting for 1 week. Blood for culture taken. (R) radical mastoidectomy performed. Treated with chloramphenicol. Final diagnosis: (R) chronic suppurative otitis media	Blood	<i>E. tarda</i>
8.	28 M	Abcess formation on dorsum of hand following a nail prick. Ampicillin was given and abcess healed.	Wound swab	<i>E. tarda</i>
9.	18 M	Inflamed appendix removed at surgery. Developed wound infection at site of suture-pus taken for culture. Treated with cotrimoxazole. Secondary suture performed.	Wound swab	<i>E. tarda</i>
10.	19 M	Mentally retarded patient with 2 days history of pain and pruritus of penis and scrotum together with difficulty in micturition and bleeding per urethra. Treated with sulphadimidine and symptoms improved.	Urine for viable count.	$>10^5$ <i>E. tarda</i> /ml.
11.	20 F	Follow-up stool for culture after an episode of Salmonella food-poisoning.	Stool	<i>E. tarda</i>
12.	21 F	Follow-up stool for culture after an episode of Salmonella food-poisoning.	Stool	<i>E. tarda</i>
13.	27	Cock in a Nurses' Hostel. No history of diarrhoea. Routine stool for culture following an outbreak of salmonella food-poisoning.	Stool	<i>E. tarda</i> (twice)

## DISCUSSION

Taxonomically *E. tarda* has been placed in the family *Enterobacteriaceae*.<sup>2</sup> It resembles the

*Salmonellae* in some respects but has distinctive biochemical and serological characteristics. Cowan & Steel<sup>1,2</sup> feel that the creation of a new genus *Edwardsiella* seems to be unnecessary. It is their opinion that this organism should be regarded as a biotype of *Escherichia coli*, because the features that distinguish it from *Escherichia* are said to be mainly quantitative; *E. tarda* produces hydrogen sulphide (H<sub>2</sub>S) on triple sugar iron agar (TSI). *E. coli* is negative on TSI but produces H<sub>2</sub>S when tested by a more sensitive method.

*E. tarda* has been isolated from the stools of patients without symptoms of diarrhoea,<sup>2</sup> as well as from normal reptiles<sup>4</sup> and animals.<sup>5</sup> This is reminiscent of *Salmonella* and *Arizona* which can be isolated from the intestines of normal man and animals. The last three patients in this series definitely exhibited intestinal carriage as there were no symptoms of diarrhoea when the organisms were isolated.

Gilman and co-workers in 1971<sup>1,4</sup> studied 208 cases of bloody diarrhoea among Malaysian jungle dwelling Orang Asli. *Entamoeba histolytica* was isolated from 76 patients and from 25 of these *E. tarda* was also isolated. They put forward the theory that "*E. tarda* isolation in amoebic dysentery is a finding incidental to the creation by the amoeba of a micro-environment favourable to *E. tarda* and quite unrelated to diarrhoea". This only adds weight to the fact that intestinal carriage of *E. tarda* does exist, but the frequency of this in the general population is not known.

The above does not exclude the fact that *E. tarda* can give rise to infections in man. There are two criteria that can be used to ascertain whether the organisms isolated from a specimen is the cause of the illness in that patient. One is the rise in antibody titre to that particular organism during or soon after the infection. In the present series no antibody studies were carried out. A search of the literature revealed only one paper<sup>1,4</sup> where antibodies to *E. tarda* were measured, but the specificity of the antibodies, however, was not conclusively demonstrated.

The second criteria is that the patient should recover from the infection after suitable anti-microbial therapy. In this series, this criteria has been fulfilled by case numbers eight and nine where the wounds healed after treatment with cotrimoxazole, and case number nine whose

urinary tract symptoms cleared after treatment with sulphadimidine.

Case number 6 was diagnosed as empyema of the gall-bladder. *E. tarda* was isolated from blood culture on one occasion only but pus from the gall bladder taken at surgery grew only *Escherichia coli*. It is highly unlikely that the *E. tarda* in the blood culture was a contaminant. One can only postulate that there had been a transient bacteraemia. This leads one to the pertinent question — can intestinal carriage of *E. tarda* give rise to a transient bacteraemia under certain conditions? The answer is probably yes since this does occur with other organisms such as *Salmonellae* and *Bacteroides*.

The antibiotic sensitivity of the 13 strains were remarkably uniform being sensitive to 15 and resistant to only 3 anti-microbial agents — penicillin, erythromycin and polymixin B. The 12 strains studied by Koshi<sup>1,5</sup> had a varied antibiotic sensitivity pattern with 2 strains being resistant to all the common antibiotics.

There is no doubt that *E. tarda* is potentially pathogenic to man but it must also be emphasised that asymptomatic carriage can and does occur.

Case number 5 is interesting in that there is evidence of a mixed infection. The pathogenic role of the organisms isolated, *E. tarda*, *Salmonella* and *Plesiomonas shigelloides*, either singly or together, is difficult to determine. After treatment with chloramphenicol, the diarrhoea stopped, and follow up stools were negative for any entero-pathogens.

To the author's knowledge this is the first report on the role of *E. tarda* in a variety of intestinal and extra-intestinal infections in humans in Malaysia. *E. tarda* infections are not recognised often, possibly because the organism is overlooked and discarded as a non-pathogen without the necessary biochemical tests being performed.

Animal studies should be initiated to elucidate the disease process, if any, and the mechanisms by which *E. tarda* causes pathogenic effects.

#### ACKNOWLEDGEMENT

The author is grateful to the late Dr. K. Patricia Carpenter, Director, Dysentery Reference Laboratory, Colindale and Dr. Don J. Brenner,

Chief, Enteric Section, Centre for Disease Control, Atlanta, Georgia, for confirming the identity of the organisms.

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