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Isolation and characterization of *Yersinia enterocolitica* from diarrhoeic human subjects and other sources

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***Yersinia enterocolitica*, an important gastrointestinal pathogen, was isolated from 3% of the 1189 stool samples collected from pediatric diarrhoeic patients and 32.9% of the 492 throat swabs collected from swine in Delhi. *Y. enterocolitica* was also isolated from groundwater, waste water and river Yamuna. In addition, *Y. intermedia* and *Y. frederiksenii* were also isolated from human stool and swine throat samples. All the *Y. enterocolitica* strains belonged to biotype 1A. This study represents first isolation of *Y. enterocolitica* from swine throat swabs, groundwater and surface water in India.**

YERSINIA enterocolitica, an emerging enteric pathogen, is associated with various clinical manifestations, ranging from self-limited gastroenteritis to more invasive syndromes like terminal ileitis and mesenteric lymphadenitis[1]. *Y. enterocolitica* is commonly transmitted to humans by contaminated food and water. Swine, being the major reservoir of *Y. enterocolitica*, represent the principal source of contamination[2]. In swine, *Y. enterocolitica* is isolated most frequently from throat. Although prevalence of *Y. enterocolitica* in temperate areas of world is well documented, there is very little information from tropical and subtropical countries, including India[2]. Isolation of *Y. enterocolitica* from India has been reported sporadically from stools of diarrhoeic patients[3–7], milk[8], swine intestinal contents[4] and rectal swabs[9], pork[10] and sewage effluents[11]. However, there is paucity of comprehensive studies on the isolation of *Y. enterocolitica* from India. The present study conducted over a period of three years reports isolation of *Y. enterocolitica* from pediatric diarrhoeic patients, swine throat samples, groundwater, waste water and surface water in Delhi.

A total of 1189 stool samples were collected from diarrhoeic children attending the All India Institute of Medical Sciences and Kasturba Gandhi Hospital, Delhi. In addition, 71 stool samples were also taken from non-diarrhoeic patients. Two millilitres of the stool sample was added to 18 ml of sterilized phosphate buffered saline and refrigerated at 4°C for 2 weeks. For swine, 492 samples of throat swabs were collected from four slaughterhouses located in different parts of Delhi. Each swab was transferred to 90 ml of cold enrichment broth (phosphate buffered saline with 1% sorbitol and 0.15% bile salts) and kept at 4°C for 3 weeks. A total of 179 groundwater samples were collected from handpumps, located primarily in slum areas, from all over the Delhi. Seventy-three waste water samples were taken from small and medium size waste water drains located in various parts of the city. A total of 44 surface water samples were collected from river Yamuna from the entire stretch of river running through Delhi (19 samples) and also from upstream Delhi (15 samples) and downstream Delhi (10 samples). Fifty millilitres of water sample was put in 450 ml of cold enrichment broth and kept at 4°C for 3 weeks.

After cold enrichment, samples were streaked onto CIN (Cefsulodin–Irgasan–Novobiocin) agar (Hi Media, Mumbai) plates and incubated at 25°C for 24 h (ref. 12). The presumptive *Yersinia* isolates, which showed bull's eye colony morphology on CIN agar, were subjected to four biochemical tests, viz. urease, Kligler's iron agar, differential motility and Voges-Proskauer. The isolates conforming to the above tests were subjected to detailed biochemical characterization using 46 biochemical tests[13]. Only one isolate from each positive sample was put to detailed tests. Since serotyping of *Yersinia* is a very complex process and its antisera are not available commercially, all the clinical isolates were sent to WHO *Yersinia* Reference Center, Pasteur Institute, Paris

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(France) for serotyping. In addition, all the *Yersinia* isolates from various sources have been deposited at WHO *Yersinia* Reference Center.

Results of the isolation of *Y. enterocolitica* from diarrhoeic human subjects and other sources are summarized in Table 1. From a total of 1189 diarrhoeal stool samples studied, 36 (3%) samples were found to be positive for *Y. enterocolitica*. However, out of 71 non-diarrhoeal stool samples no *Y. enterocolitica* strain could be isolated. Of the 492 throat swab samples collected from swine, *Y. enterocolitica* was isolated from 32.9% samples. *Y. enterocolitica* was also isolated from groundwater, waste water and surface water. Other species namely *Y. intermedia* and *Y. frederiksenii* were isolated from 4 (0.3%) and 7 (0.5%) diarrhoeal stool samples respectively. In addition, *Y. intermedia* was isolated from 23 (4.6%) and *Y. frederiksenii* from 9 (1.8%) swine throat samples. Most *Yersinia* isolates showed characteristic biochemical reactions that were typical of the respective species. However, some *Y. intermedia* isolates of swine origin showed atypical reactions: six isolates utilized lactose, one failed to show lipase activity, while another one was positive for lactose and negative for lipase. Two of the swine *Y. frederiksenii* isolates failed to reduce nitrate. All *Y. enterocolitica* isolates from groundwater and waste water fermented rhamnose and utilized citrate. Also, four *Y. enterocolitica* isolates from swine oral swabs and one surface water isolate were positive for rhamnose and citrate. All the *Y. enterocolitica* isolates from human, swine and water were of biotype 1A. Of the 36 *Y. enterocolitica* strains isolated from diarrhoeic stools, 8 were of serotype O : 6, 30-6, 31, 19 of serotype O : 6, 30 and 9 were untypable (Table 2). *Y. intermedia* isolates from clinical samples were of biotype 1 (3 strains) and biotype 2 (1 strain). Among the swine *Y. intermedia* isolates, 18 belonged to biotype 1, 2 isolates to biotype 4, while 3 isolates were of rare biotype 8. Of the isolates from clinical samples, six *Y. frederiksenii* belonged to serotype O : 35, one *Y. intermedia* to serotype O : 7, 8-8, whereas the rest were non-agglutinable.

The isolation rate of *Y. enterocolitica* from human diarrhoeal stools (3%) and swine oral swabs (32.9%) is quite comparable to that reported from other parts of the world where *Y. enterocolitica* is considered a major gastrointestinal pathogen[1]. Isolation of *Y. enterocolitica* from groundwater is significant in view of the fact that in several slum areas of Delhi, handpumps are the major source of drinking water. Slightly higher frequency of isolation of *Y. enterocolitica* from part of Yamuna transversing Delhi and downstream (3 isolates from 29 samples) than from upstream region (1 isolate from 15 samples) may be attributed to discharge of sewage and sewage effluents from Delhi into Yamuna. In an earlier study from our laboratory, we reported isolation of *Y. enterocolitica* from sewage effluents collected from several sewage treatment plant in the city[11]. All the isolates of *Y. enterocolitica* belonged to biotype 1A, the pathogenicity of which is currently a matter of controversy. These strains generally lack the classical phenotypic and genotypic

Table 1. Summary of *Y. enterocolitica* isolated from human, swine and water samples

Sample	No. of samples processed	No. of samples positive for *Y. enterocolitica* (%)
Diarrhoeal stools	1189	36 (3)
Non-diarrhoeal stools	71	0
Swine throat swabs	492	162 (32.9)
Groundwater	179	5 (2.8)
Waste water	73	9 (12.3)
River Yamuna	44	4 (9)

Table 2. Details of *Y. enterocolitica* isolates from diarrhoeic patients

Isolate	Serotype	WHO Reference Center No.
C16	6, 30-6, 31	IP27359
C17	6, 30-6, 31	IP27360
C20	6, 30-6, 31	IP27361
C27	6, 30-6, 31	IP27362
C51	6, 30-6, 31	IP27363
C64	6, 30-6, 31	IP27364
C92	6, 30-6, 31	IP27365
C93	6, 30-6, 31	IP27366
C94	NAG*	IP27381
C112	NAG	IP27382
C114	NAG	IP27383
C130	NAG	IP27385
C161	NAG	IP27386
C167	NAG	IP27387
C192	NAG	IP27388
C760	6, 30	IP27403
C764	6, 30	IP27404
C770	6, 30	IP27405
C772	6, 30	IP27406
C777	6, 30	IP27407
C782	6, 30	IP27408
C791	6, 30	IP27425
C792	6, 30	IP27426
C801	6, 30	IP27427
C842	6, 30	IP27428
C845	6, 30	IP27429
C855	6, 30	IP27430
C871	6, 30	IP27431
C876	6, 30	IP27432
C927	6, 30	IP27433
C931	6, 30	IP27434
C945	6, 30	IP27481
C963	NAG	IP27482
C975	6, 30	IP27483
C998	6, 30	IP27484
C1021	NAG	IP27485

*NAG, Non-agglutinable.

markers of *Y. enterocolitica* pathogenicity[14]. However, recent studies have shown that biotype 1A strains produce heat stable enterotoxin[15], invade cultured epithelial cells[16] and resist killing by macrophages[17]. *Y. enterocolitica* serotype O : 6,30 belonging to biotype 1A, observed in this study, has been implicated in nosocomial and milk-borne outbreaks in certain parts of the world[18]. This serotype has also been isolated from extraintestinal infections in human beings[19]. All these observations point to its pathogenic potential. This serotype has not been reported earlier from India.

The present study provides a report of isolation of *Y. enterocolitica* from swine oral swab, groundwater and surface water in India. The isolation of *Y. intermedia* and *Y. frederiksenii* from human diarrhoeic stools, and *Y. intermedia* from swine also represents their first isolation from these sources in India. The clinical significance of *Y. intermedia* and *Y. frederiksenii* is controversial[20] and further studies are awaited in this aspect. Some of the atypical biochemical characteristics especially lactose positivity of *Y. intermedia* and nitrate negativity of *Y. frederiksenii* isolates were noteworthy. It would be worthwhile to work on the prevalence of *Y. enterocolitica* in human, animals and environment in this part of the continent.

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Microstructural and fluid inclusion constraints on the evolution of Jakhri Thrust Zone in the Satluj valley of NW Himalaya

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The regional structures, microstructure and fluid inclusion trail pattern have been employed to work out the evolution of the Jakhri Thrust Zone (JTZ) exposed in the Satluj valley of NW Himalaya. It is a NE-dipping, SW-propagating out-of-sequence thrust cutting across the folded Lesser Himalayan crystalline nappe and lies in the seismically active inner Lesser Himalayan zone. The recrystallization microstructure in the footwall quartzite suggests deformation in the lower green schist facies condition with a progressively decreasing finite strain away from the thrust. The microstructures and fluid inclusion trails (secondary) show analogous pattern and have formed during the same deformation event in the footwall. The CO_2–H_2O and H_2O–NaCl fluid inclusions have been identified. The former has been re-equilibrated during the peak deformation whereas the latter has evolved and been re-equilibrated during exhumation. The isochores of CO_2–H_2O and H_2O–NaCl inclusions suggest an isothermal exhumation path from a depth of ~15–17 km, considering a lithostatic condition. These results suggest that the JTZ is a deep-seated thrust, probably a steep imbrication on the main decollement fault.

THE Jakhri Thrust Zone (JTZ) lies in the Satluj valley of NW-Himalaya (Figure 1). The tectonic status of the JTZ is debated and recent fission track data on zircon–apatite cogenetic pairs from the hanging wall of the JTZ suggest it to be active during past < 4.5 Ma (ref. 1), which is younger than the age MBT, i.e. ca. 10 Ma (ref. 2). The

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