Implications of *Yersinia enterocolitica* biotyping

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Abstract
The utility of a simple biotyping scheme to differentiate pathogenic and non-pathogenic strains of *Yersinia enterocolitica* was determined for 79 patients who were admitted to or attended a reference children's hospital in western Canada. Biotyping defined predominantly two subsets of *Y enterocolitica*. 'Pathogenic' strains were more likely to have been obtained from younger patients (mean age 61.9 months) who experienced an acute gastrointestinal illness that was occasionally associated with bloody diarrhoea or a surgical procedure. Growth of *Y enterocolitica* from selective solid bacteriological growth media were often in the moderate to heavy range (82.0%). In contrast, 'non-pathogenic' strains were more often obtained from older patients (mean 116.0 months) who were already recognised to have suffered from a chronic illness and who were likely to have been admitted to hospital. Moderate to heavy growth of bacterium in stool specimens were infrequently (17.4%) obtained from the latter patients. The use of a simple biotyping scheme for the differentiation of *Y enterocolitica* strains has the potential to improve patient care.

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Yersinia enterocolitica is an important aetiological agent of paediatric gastroenteritis. Considerable progress has been made in the understanding of the molecular biology of the bacterium and the clinical spectrum of associated illnesses. Despite these gains, controversy has continued to surround the practical laboratory definition of pathogenic strains.

Early studies of gastrointestinal yersiniosis were, in retrospect, hindered by the lack of appreciation for the species diversity of what was then known singularly as *Y enterocolitica*.^{1 2} This species designation was subsequently modified to the extent that several other species have been defined (for example, *Y frederiksenii*, *Y intermedia*, and *Y kristensenii* among others), and there is general agreement that these latter species are unlikely to be enteric pathogens. Further work determined however that, of the strains that were appropriately designated *Y enterocolitica*, pathogenic and non-pathogenic variants could again be differentiated.³ This differentiation was believed to be possible through the use of serotyping, biotyping, direct determination of the presence of a virulence plasmid, or indirect determination of phenotypic traits that are virulence plasmid dependent.⁴⁻⁶ The practical utility and clinical relevance of these methods for delineating pathogenic or non-pathogenic

isolates has been questioned.^{7 8} Variability in the bacterial expression of virulence markers has been recognised and may account for some of the confusion in this area.⁹

A practical biotyping method for discriminating putative pathogenic and non-pathogenic *Y enterocolitica* has been described and its value more recently affirmed.^{3 9} This typing method could conceivably be completed within a 24-48 hour period after a stool isolate has been defined as *Y enterocolitica*. Such rapid and accurate designation would potentially affect decision making for both medical management and hospital infection control. We detail herein our experience with the biotyping scheme and explain its implications for patient management.

Patients and methods

Seventy nine isolates of *Y enterocolitica* were isolated from individual patients over seven years (1986-92). The identifications of these bacteria were confirmed by two independent biochemical systems. Many isolates were also confirmed as *Y enterocolitica* by a reference laboratory, and serotyping was available for some strains as well.

Additional biotyping was performed in retrospect on isolates that had been maintained frozen at -70°C. Bacterial isolates had been passaged ≤3 times before preservation and were subcultured once before biotyping. The extended biochemical tests included aesculin hydrolysis, salicin fermentation, and pyrazinamidase activity.^{5 9} Each assay was incubated at 25°C for 48 hours. The designation of 'pathogenic' biotype was given to those strains that were negative in all three tests whereas 'non-pathogenic' biotypes were positive in all three assays.

Demographic and clinical features of patients from whom pathogenic and non-pathogenic biotypes were isolated

	Pathogenic* biotypes (n=56)	Non-pathogenic* biotypes (n=23)	p Value
Gender			
Boys	32	12	0.88
Girls	24	11	
Mean (range) age in months	61.9 (3-179)	116.0 (14-220)	<0.001
Acute illness*	52/52	12/23	<0.001
Bloody diarrhoea	13/52	1/23	0.05
Co-pathogens	4/56	3/23	0.41
Chronic underlying illness	8/54	21/23	<0.001
Surgical procedure	7/56	1/23	0.43
Semiquantitation in culture†			
3+	34	1	<0.001
2+	7	3	
1+	8	8	
VL	1	11	

*See definitions in text.

†3+, 2+, and 1+ refer to growths in tertiary, secondary, or primary areas of solid selective bacteriological media. VL refers to the presence of very few colonies, usually fewer than 5.

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Patient records were reviewed in retrospect in order to examine the relationship of biotype to disease process, but the reviewer was blinded with respect to the specific biotype. An 'acute illness' was defined as recent onset of a diarrhoeal illness or acute abdominal pain with or without diarrhoea. Patient data were also examined for gender, age, history of bloody diarrhoea, chronic underlying illness, resultant surgical procedure, and site of medical care, that is, inpatient or outpatient. These details were obtained in full for most patients despite the retrospective review. The inability to acquire all of the data for each patient is reflected by the denominator for frequency calculations in the table. Co-pathogens were recorded, although the extent of co-pathogen search was not consistent among these patients because of the retrospective information gathering. Laboratory records of semiquantitation of the bacterial isolates on primary solid selective and differential media were available for 73 patients. Semiquantitation was recorded as VL, 1+, 2+, and 3+. The VL designation referred to a few colonies (<5), whereas 1+, 2+, and 3+ referred to isolation in primary, secondary, and tertiary areas of bacteriological agar plates. Throughout the seven years when isolates were acquired, cefsulodin-irgasan-novobiocin media were utilised specifically for the isolation of *Y enterocolitica*. Cold enrichment techniques were not employed at any time.

The χ^2 and *t* tests (two tailed) were used to assess the probability of a difference between patient groups.

Results

Details of demographic and clinical variables associated with pathogenic and non-pathogenic biotypes are illustrated in the table.

Patients with the pathogenic biotype were significantly younger and less likely to have been admitted to hospital with a chronic illness. The pathogenic biotype was more frequently associated with an acute, presumably gastrointestinal, illness and also more commonly associated with overt bloody diarrhoea. These patients were often febrile (64.2%) and a history of emesis was not uncommon (38.5%). Among children of ages ≥ 3 years, whose clinical history was likely to be more reliable, abdominal pain was frequent (81.3%). Altogether 42.3% of patients with abdominal pain had the discomfort well localised to the right lower quadrant of the abdomen; most of the others gave descriptions of pain that was crampy and diffuse or periumbilical; an intussusception was documented radiologically in one patient. For those isolations where results were recorded, there was clearly a predominance of greater semiquantitations among specimens that yielded the pathogenic biotype.

Other putatively pathogenic microorganisms were uncommonly identified in the clinical specimens of patients regardless of whether the concomitantly isolated *Y enterocolitica* biotype was classified as pathogenic or non-pathogenic. The other potential aetio-

logical agents of gastroenteritis included: (a) along with pathogenic – giardia, *Campylobacter jejuni*, enteropathogenic *Escherichia coli*, and *Clostridium difficile*, and (b) along with non-pathogenic – *Aeromonas* spp, *C difficile*, and a pure heavy growth of *Candida albicans*. All of these other agents were isolated in singular instances.

Approximately 40% of patients with pathogenic biotypes were treated on an outpatient basis only, whereas all of the patients with non-pathogenic biotypes had been admitted to hospital, often with a severe chronic underlying illness. Among the latter, most (63.6%) had *Y enterocolitica* cultured from stool specimens that had been acquired well into the hospital admission. The chronic illnesses most often included malignancies (n=8), nephropathies (n=4), failure to thrive (n=3), and chronic abdominal pain (n=3). The identification of *Y enterocolitica* as a presumable enteric pathogen in a patient, who was already admitted to hospital for another illness, often prompted a change to alternate infection control precautions.

Eight surgical procedures among all patients were recorded. Among the patients with pathogenic biotypes, all surgical interventions occurred among patients with acute abdominal pain. At the time of the operative procedure, the clinical diagnoses included terminal ileitis (n=4), mesenteric adenitis (n=2), or peritonitis (n=1). Three appendices were removed and two of these had evidence of mild inflammation. The laboratory isolations of *Y enterocolitica* were known only after surgery in all of these patients. The single patient with a non-pathogenic strain who underwent surgery had a longstanding history of recurrent severe abdominal pain, and intraoperatively, a large mesenteric cyst was discovered to be responsible for both the pain and an associated intermittent bowel obstruction.

Of the total 79 *Y enterocolitica* isolates, only two strains did not clearly fall within the pathogenic and non-pathogenic biotype patterns. Both of these were pyrazinamidase positive and aesculin/salicin negative. One such patient was admitted to hospital with a short history of abdominal pain and emesis, and physical examination found evidence of tenderness and guarding in the right lower abdominal quadrant. This patient was subjected to an operative procedure, and cultures of a lymph node yielded a pure growth of *Y enterocolitica*. The other strain with an odd biotype was isolated from the stool of a 3 year old new diabetic child who did not have active diarrhoea but had had only one watery stool. This patient did not subsequently manifest diarrhoea and an enterovirus was isolated from stool as well.

Information on the serotyping of 18 isolates was available. Pathogenic biotypes were of serotypes 3 (n=8), 8 (n=2), 28 (n=1), 5,27 (n=1), and non-typable (n=1). Non-pathogenic biotypes were of serotypes 5 (n=1), 6,30 (n=1), 7,8 (n=1), and non-typable (n=2). These results are consistent with the designation, by others, of pathogenic and non-pathogenic serotypes.

Discussion

The designation of pathogenic and non-pathogenic variants of *Y. enterocolitica* has been hindered to some extent by the changes that may occur for some phenotypic traits after in vitro passage.⁹ Although serotyping has generally proved to be a valuable and consistent tool for differentiating *Y. enterocolitica* isolates, the technique has been restricted to reference centres; therefore, the results have been mainly of retrospective value. The use of genetic probes for defining virulence is of potential benefit,¹⁰ but a practical method with this technology for routine laboratory use is not yet available. The definition of a simple, relatively rapid, and economical scheme for determining pathogenic and non-pathogenic strains is best met at this time by the biotyping protocol as defined herein and as justified by others.^{3,5,9} Although we support the clinical value of this current biotyping scheme, it is acknowledged that considerable efforts in the form of previous biotyping schemata,^{1,3} have been directed towards achieving this goal.

As for most biotyping schemata where few biochemical traits are being used, there are always exceptions. From our data, it appears that these exceptions are few (2.5%). The high degree of consensus between pyrazinamidase and salicin/aesculin biochemicals has been previously demonstrated.^{5-7,9} Although it has been proposed that salicin/aesculin negative and pyrazinamidase positive strains are non-pathogenic,⁶ others have published some evidence to the contrary.⁹ The clinical details of one of our patients with the latter biotype confirms the pathogenic potential in view of having had isolated the organism in pure growth from a mesenteric node. The infrequency of such intermediate biotypes does not detract from the overall implementation of this simple scheme.

Two previous reports have raised the majority of potential opposition to the utility of biotyping.^{7,8} In a study of predominantly adult patients, Noble *et al.* suggested that proposed virulence markers (including biotype) did not correlate well with clinical symptoms.⁷ It is probable, however, that such inability to associate any subset with disease could be attributed to the fact that only a minority (12.5%) of their *Y. enterocolitica* isolates were of the putatively pathogenic (salicin, aesculin, and pyrazinamidase negative) biotype. Their serotyping data also confirms that the majority of their *Y. enterocolitica* isolates were likely to be non-pathogenic. It is probable that the use of cold enrichment enhanced the ability to find non-pathogenic serotypes/biotypes in their patient population. More compelling evidence, which detracts from the utility of biotyping, has been published by Morris *et al.*⁸ In a Chilean paediatric population, a study of birth cohorts found a greater association of the non-pathogenic biotype with diarrhoea. It is evident, however, that the demographic characteristics of their study patients were considerably different from those of our own. The marked difference in mean age between their birth cohort and our patients is but one

important example; standards of public health are certain to be important as well. We hypothesise that our findings are much more likely to be representative for Canada and the US, especially given that our data are supportive of the general experience in western Europe.^{11,12}

Pathogenic biotypes are in general isolated from stools of younger children who manifest an acute gastrointestinal illness that is occasionally accompanied by bloody diarrhoea and that occasionally leads to a surgical procedure. Isolates from these patients will be commonly present in moderate to heavy quantities. In contrast, the patient who yields a non-pathogenic biotype is more likely to be older, and the growth of *Y. enterocolitica* from solid selective media is often light to scant. This patient will often have a chronic underlying illness that has prompted hospital admission. During the complicated course of the latter patient's hospital stay, non-pathogenic *Y. enterocolitica* is found essentially as a coincidence in the context of greater overall investigations to which such a patient will be subjected. Given that this patient commonly has an underlying illness and is already admitted to hospital, physicians will be biased to prescribe an antibiotic and/or towards considering that such a patient should be handled with enteric precautions. Both of the latter approaches will complicate the case of an already complex illness and may inappropriately influence resource utilisation. We propose that the implementation of the simple biotyping scheme will lead to more sound patient care.

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