J. King Saud Univ., Vol. 3, Science(2), pp. 91-100, Riyadh (1411 A.H./1991)

Incidence of *Yersinia enterocolitica* in Some Food and Environmental Samples in Saudi Arabia

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(Received 3 February 1990; accepted for publication 7 October 1990)

Abstract. Alkali treatment, after enrichment in an appropriate selective medium, was used to isolate *Yersinia enterocolitica* from food and environmental samples in Riyadh, Saudi Arabia. A total of 33 isolates were obtained from 106 specimens of sewage water, irrigation water and chicken cecal contents, whereas no isolates were obtained from 110 samples of milk, chocolate milk, drinking water and rain water. All of the known *Y. enterocolitica* biotypes were represented at rates of approximately 51,21,12,12 and 3% for biotypes 2,4,3,1 and 5 respectively. Nineteen of the isolates (58%) were virulent as estimated by the known virulence criteria of this species.

Introduction

Yersinia enterocolitica is a major cause of acute gastroenteritis, terminal ileitis and mesenteric lymphadenitis (for reviews see Botone [1] and Bercovier *et al.* [2]). Strains of *Y. enterocolitica* have been isolated from a wide variety of foods as well as from animal and environmental sources such as: Water [3-4], milk and milk products [5-7], vegetables [8], meats [9-10], rodents [11], swine [12], animals and human beings [13-15]. *Y. enterocolitica* has the ability to grow at low temperatures, whereas, other enteric bacteria fail to grow or even decline in numbers at low temperature [16]. Therefore, cold enrichment techniques were originally used for its isolation [16,17]. Unfortunately, cold enrichment is of limited clinical value because of the length of the time required for isolation. Recently, several other, more rapid techniques have been used to isolate *Y. enterocolitica* from a variety of sources [18-19]. These techniques start with an enrichment in a selective medium, then treatment of one loopful of the enriched cells with an alkaline solution (0.5% KOH in 0.5% NaCl). The treated cells are then plated directly on selective or non-selective medium. Alkali treatment has been found to be very useful in destroying other gram-negative bacteria which are more sensitive to alkaline conditions than *Y. enterocolitica* [20-21].

A survey, extending over 3 years, of four major hospitals in Riyadh, Saudi Arabia has shown that this bacterium has never been isolated although the isolation has been attempted. *Y. enterocolitica* isolation rates do vary throughout the world [1]. Studies indicate that the food-and waterborne *Y. enterocolitica* infections in humans are essentially transmitted by the consumption of raw meats, raw milk, raw oysters, vegetables and water. Therefore, this study was undetaken to determine the incidence of the organism in food and environmental samples in the Riyadh area.

Materials and Methods

Isolation Procedures

A) Isolation of *Y. enterocolitica* from chicken

The intestines of 58 chickens were transferred to this laboratory under cooled conditions immediately after slaughter. About 1 gm of the cecal contents from each chicken was taken and immersed into test-tubes containing 10 ml of peptone-sorbitol-bile salts broth [22] and incubated at 26°C for 72 h. One loopful (0.04 ml) of the enrichment was mixed with 0.1 ml of 0.5% KOH in 0.5% NaCl [20-21] and immediately streaked on a blood agar base plate. The plates were incubated for 48 h at 26°C.

B) Isolation of *Y. enterocolitica* from milk and chocolate milk

Thirty-four pasteurized cow's milk (shelf life 4 days) and 32 ultra-high temperature chocolate-milk (shelf life 4 months) cartons were obtained from different companies. 10 ml of the milk or chocolate-milk samples were mixed with 90 ml of peptone-sorbitol-bile salts broth and processed as described above.

C) Isolation of *Y. enterocolitica* from drinking, sewage, irrigation and rain waters.

This method was performed essentially as described by Marinelli *et al.* [3] with the following modification. 24,20,30 and 18 samples of drinking water (mixture of tube-well and desalinated sea water), naturally accumulated rain water, untreated sewage water and irrigation water (tube-well) of farms with cows and sheep, respectively, were taken from different geographical locations using sterile cotton balls covered with a sterile cloth (radius of 6 cm). Each cotton ball was allowed to float in the above water environments for 48 h and then was immersed in 50 ml sterile peptone-sorbitol-bile salts broth [22] and incubated at 26°C for 72 h, after which the cotton ball was pressed with a sterile glass rod and 2 ml samples of this enrichment were transferred to 9 ml of 0.5% KOH in 0.5% NaCl [20-21]. Three loopfuls of this alkaline mixture were immediately plated on blood agar base. The plates were incubated at 26°C for 48 h.

Identification and Biotyping of the *Y. enterocolitica* Isolates

Colonies resembling *Y. enterocolitica* from the above sources were restreaked on blood agar base for the confirmation of their purity. The pure isolates were transferred to a blood agar base slants and subjected to the biochemical studies. Altogether, each isolate was tested for the following 15 parameters: Motility at 37°C, motility at25°C,urease, indole, glucose, xylose, lactose, trehalose, production of an acid slant and acid butt (without H_2S) in a triple-sugar-iron agar slant, production of an alkaline slant and acid butt in a lysine-arginine-iron agar slant, growth in selective and differential deoxycholate-sodium chloride (DYS) medium [23] with bright red colonies, binding of Congo red, nitrate reduction, lecithinase and ornithine decarboxylase. Details concerning the media and test procedures have been described elsewhere [24]. The parameters listed above formed the basis for identification [2] and biotyping [25] of *Y. enterocolitica* according to established criteria.

Plasmid Screening

Bacteria were grown overnight in 2 ml of brain heart infusion broth at 25°C and used for the isolation of the virulence plasmid as described by Portnoy *et al.* [26]. Agarose gel electrophoresis was carried out in 0.7% gels at 130 V for 3 h. After staining with ethidium bromide (1 μg/ml), plasmid DNA was visualized under UV illumination.

Intraperitoneal Test

Cells were grown at 37°C in Higuchi and Carlin medium [27], then washed and diluted with 0.85% saline. An 0.1 ml amount of each bacterial dilution was injected intraperitonelly into each of three Swiss albino suckling mice (College of Pharmacy, King Saud University). Death of mice between 2-7 days at an LD_{50} of less than 150 bacterial cells was considered a positive intraperitoneal test [28].

Table 1. Occurrence of *Y. enterocolitica* in some food and environmental samples

Source	Number of tested samples	Number of Positive samples	% positive
Milk	34	0	0
Chocolate Milk	32	0	0
Drinking water	24	0	0
Rain Water	20	0	0
Sewage water	30	7	23.3
Irrigation water	18	3	16.6
Chicken	58	23	39.6

Sereny Test

Three Swiss albino mice (6-8 weeks old) were infected for each *Y. enterocolitica* strain using one loopful of 10^9 bacterial cells per ml saline (0.5% NaCl). The control eye received on loopful of sterile saline. Induction of conjunctivitis in the test mice was considered a positive Sereny test [28].

Peroral Test

An aqueous suspension of 10^8 bacterial cells /ml was prepared for each strain. Three Swiss albino mice (6-8 weeks old) were deprived of drinking water for 12 h and then were allowed to drink *ad libitum* from the above suspension. Death of two or more mice was considered a positive peroral test [28].

Results

Isolation of *Y. enterocolitica*

The frequency of isolation of *Y. enterocolitica* for each source is shown in Table 1. A total of 33 isolates were obtained from sewage water, irrigation water and chicken cecal contents. *Y. enterocolitica* was not recovered from milk, chocolate milk, drinking water and rain water.

Biochemical Reactions

Biochemical reactions for all isolates are shown in Table 2. The isolates were all positive for urease and glucose and they were all motile at 25°C and non-motile at 37°C. The isolates grew in: DYS medium with bright red colonies, triple-sugar-iron agar with an acid slant and acid butt, and lysine-arginine-iron agar with an alkaline slant and acid butt. They varied with respect to the biochemical reactions for indole, xylose, lactose, trehalose, nitrate reduction, lecithinase and ornithine decarboxylase and these variations formed the basis of biotyping [25].

Biotyping

The biotypes are also shown in Table 2. Biotypes 1,2,3 and 4 were isolated from chicken. Biotypes 1,2 and 5 were isolated from sewage, whereas, only biotype 2 was isolated from irrigation water. Approximately 51,21,12,12 and 3% of the isolates were from biotypes 2,4,3,1 and 5 respectively.

Virulence Studies

Results of the virulence studies are shown in Table 2. Nineteen isolates (58%)

Table 2. Biochemical tests, biotypes and virulence tests of the *Y. enterocolitica* isolates[a].

Biochemical and virulence tests	Isolate source and number											
	Chicken							Sewage			Irrigation	
	1,8,21,22	2,7,18	3-5	6,13,17	9-12,14-16	19-20	23	1,3-4	2	5	6-7	1-3
Motility at 37°C	−	−	−	−	−	−	−	−	−	−	−	−
Motility at 25°C	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+	+	+	+
Indole	−	+	−	−	+	+	−	+	−	+	+	−
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	−	+	+	−	+	+	+	+	−	+	+	+
Lactose	−	+	+	−	+	+	+	+	−	+	+	+
Trehalose	+	+	+	+	+	+	+	+	−	+	+	+
TSI[b]	+	+	+	+	+	+	+	+	+	+	+	+
LAI[c]	+	+	+	+	+	+	+	+	+	+	+	+
DYS medium[d]	+	+	+	+	+	+	+	+	+	+	+	+
Congo red[e]	−	+	+	+	+	−	−	−	+	−	+	−
Nitrate reduction	+	+	+	+	+	+	+	+	−	+	+	+
Lecithinase	−	+	−	−	−	−	−	−	−	+	−	−
Ornithin decarboxylase	+	+	+	+	+	+	+	+	−	+	+	+
Biotype	4	1	3	4	2	2	3	2	5	1	2	2
Virulence plasmid	−	+	+	+	+	−	−	−	+	−	+	−
Sereny test	−	+	+	+	+	−	−	−	+	−	+	−
Peroral test	−	+	+	+	+	−	−	−	+	−	+	−
Intraperitoneal test	−	+	+	+	+	−	−	−	+	−	+	−

[a] – Positive results are indicated by +, negative by −.

[b] – Triple-sugar-iron agar test (TSI) with and acid slant and acid butt was considered positive.

[c] – Lysine-arginine-iron agar test (LAI) with an alkaline slant and acid butt was considered positive.

[d] – DYS medium with a bright red colonies was considered positive.

[e] – Positive Congo red test means that the cells were able to bind the dye.

possessed the virulence plasmid, bound Congo red, and produced positive Sereny, peroral and intraperitoneal tests in mice. Most of the virulent isolates were obtained from chicken, whereas, no virulent isolates were obtained from farm water.

Discussion

Worldwide studies performed during the last two decades indicate that *Y. enterocolitica* infections are commonly transmitted by raw milk, raw meats, raw oysters, water and vegetables [5,7,8,13,29]. Recognition of transmission of *Y. enterocolitica* to humans by the above vehicles has necessitated the implementation of good selective techniques for the isolation of this bacterium and for its separation from other bacterial contaminants.

The first technique to be used for the isolation of *Y. enterocolitica* was cold enrichment [16]; this technique, however, is no longer used because the length of incubation can lead to the growth of the contaminants and it is not of immediate clinical value. Aulisio *et al.* [23] proposed an alkali method for the rapid recovery of *Yersinia* spp. from foods and environmental samples. Alkali treatment has been adopted by many authors [20,21] following the enrichment in an appropriate selective medium [9,22,23].

Preliminary studies in our laboratory [30] have shown that *Y. enterocolitica* cells grew or remained viable in: chicken and beef ground meat, choclate milk, milk, phosphate buffer and water. The preliminary studies also have shown that *Y. enterocolitica* in foods were less susceptible to heat injury than when in water or buffer.

In the present investigation, 33 *Y. enterocolitica* isolates were obtained from chicken, sewage and irrigation water. Most of the isolates were from chickens and this shows that swine are not the only animal reservoirs of *Y. enterocolitica* [12]. We were unable to isolate *Y. enterocolitica* from pasteurized milk and chocolate milk. Francis *et al.* [31] found that *Y. enterocolitica* does not survive pasteurization, other authors, however, reported that viable *Y. enterocolitica* may persist after pasteurization if the organism is present in large numbers [5,7,29,31]. These findings may explain the absence of *Y. enterocolitica* in the milk and chocolate milk samples analyzed in this study. The absence of *Y. enterocolitica* in the drinking water and rain water could be due to chlorination of the first and lack of exposure of both to any subsequent animal or human contamination.

This study has shown a good correlation between the presence of the virulence-associated plasmid, the Congo red binding, and the positive Sereny, peroral and intraperitoneal tests; these findings are in agreement with the results obtained by several authors [28,32-34]. However, there was no correlation between biotype and

virulence characteristics. Further, the results provided were not sufficient for strain discrimination.

The findings of this study suggest that *Y. enterocolitica* should be present in a proportion of the stool and blood specimens investigated in the clinical laboratories of our hospitals. However, they may go undetected because of the lack of effective isolation procedures or because of their similarity to other enterobacteria with respect to most of the biochemical reactions. Our study should be extended to more food, animal and environmental samples throughout the year in order to determine the reservoirs and vehicles of transmission and the extent of the seasonal variation of this bacterium.

Acknowledgement. This research (Botany/1404/22) was supported by the Research Center, College of Science, King Saud University, Riyadh, Saudi Arabia. We would like to thank Mr. Shamsher Khan for reading the manuscript.

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مدى وجود البكتيريا يرسينيا انتيروكوليتيكا في بعض عينات الأطعمة والبيئة في المملكة العربية السعودية

علي عبدالله السلامة و شريف عزت مكي

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(استلم في ٨ رجب ١٤١٠هـ، قُبل للنشر في ١٨ ربيع الأول ١٤١١هـ)

ملخص البحث. لقد استخدمت المعاملة القاعدية بعد عملية الإكثار في بيئة انتخابية مناسبة لعزل البكتيريا يرسينيا انتيروكوليتيكا من بعض عينات الأطعمة والبيئة المأخوذة من الرياض، بالمملكة العربية السعودية. لقد حصل على ٣٣ عزلة لهذه البكتيريا من عينات مياه المجاري، مياه الزراعة، محتويات المصران الأعور للدجاج. بينما لم يحصل على أية عزلة من ١١٠ عينة من الحليب، حليب الشكولاتة، ماء الصنبور، ماء الأمطار. هذه العزلات شملت كل الأنواع الحيوية لهذه البكتيريا وبمعدل ٥١، ٢١، ١٢، ١٣، ٣٪ للأنواع الحيوية ٢، ٤، ٣، ١، ٥ على التوالي. وبناءً على معايير الضراوة المعروفة لهذا النوع البكتيري فإن ١٩ عزلة من هذه العزلات (٥٨٪) ضارية.