ORIGINAL ARTICLE

Isolation of *Yersinia enterocolitica* biotype 1 A from raw meat products

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Key words

Yersinia enterocolitica • Meat products • Food

Summary

A survey has been carried out for the presence of Yersinia enterocolitioca in raw meat products.
One hundred and twenty raw beef, chicken and ham samples were assayed for the presence of Yersinia enterocolitica by the 4 degrees °C enrichment method after 2 weeks of incubation using phosphate buffered saline. Yersinia enterocolitica biotype 1 A non agglutinable (NAG) was isolated from a sample of beef and identified by Api 20 E System and additional biochemical tests, Lipase, β-D-Glucosidase and Pyrazinamidase. The Author related about pathogenicity of Yersinia enterocolitica biotype 1 A that may cause symptoms similar to that caused by virulent biotypes. The presence of Yersinia enterocolitica in raw meat products represents a health risk for consumers therefore was suggested to yersiniosis control and further surveillance studies on epidemiology of such emerging pathogens.

Introduction

Yersinia enterocolitica belonges to a family of rod-shaped bacteria and is an important food and water-borne gastrointestinal pathogen that most commonly causes diarrhea, fever, vomity, abdominal paint, terminal ileitis and mesenteric lymphadenitis. Infection occurs most often in young children and cause a variety of symptoms that typically develop in 4 to 7 days and may last 1 to 3 weeks or longer. *Yersinia enterocolitica* is rapidly emerging worldwide as an enteric pathogen and has become a major cause of diarrhea in most of the industrialized world. Any raw animal food (raw milk, poultry, fish, meat), may carry *Yersinia enterocolitica* and cause illness when the food is eaten. It can grow in properly refrigerated foods and survive in frozen foods for long periods: infection is most often acquired by eating contamined food, especially raw or undercooked products. The characteristics of *Yersinia enterocolitica* are: Family Enterobacteriaceae, Gram-, Oxidase-, ferments Glucose, growth temperature 0-45 °C, optimum 30-35 °C, pH 4-10, optimum 7.6. We reported isolations of *Yersinia enterocolitica* in the past from chicken, pork, dogs and human [1-5]. Based on data from the Foodborne Diseases Active Surveillance Network (Food Net), the infection is more common in winter; in complicated infections may be useful antibiotics such as fluoroquinolones, doxycycline, trimetroprim-sulfamethoxazole or aminoglycosides. This study examined the occurrence of *Yersinia enterocolitica* in meat products samples.

Materials and methods

One hundred and twenty raw meat sample were examined:

– 40 samples of beef;
– 40 sample of chicken;
– 40 samples of ham.

25 g of the sample were inoculated into 10-fold volume of phosphate-buffered saline containing sorbitol and bile salts. It was kept at 4°C for 2 weeks. After this period, a loopful of the cold-enriched sample was plated on SS [Salmonella Shigella] agar. The plates were incubated at 30°C for 24-48 h. On SS agar, the colonies of *Yersinia enterocolitica*, were minute, non-lactose fermenting, smooth, circular and translucent, without the production of H_2S. Colonies non-lactose fermenting (NLF), were selected and subjected to detailed biochemical characterization by Api 20 E System and following biochemical tests (Lipase, β-D-Glucosidase, Pyrazinamidase).
Lipase test: after growth of culture on agar media containing egg yolk, a positive reaction is indicated by iridescent, pearl-like colony surrounded by precipitation ring and outer clearing zone.

β-Glucosidase test: emulsify culture in physiologic saline to Mc Farland Turbidity Standard No 3 and add 0.75 of culture to 0.25 ml of test medium. Incubate at 30° overnight. A yellow color indicates a positive reaction.
Pyrizinamidase test: after growth of culture on slanted pyrazinamidase agar at RT, flood 1 ml of 1% freshly prepared ferrous ammonium sulfate over slant. Development of pink color within 15 min is positive test indicating presence of pyrazinoic acid formed by pyrazinamidase test.

Results

Was isolated a strain of *Yersinia enterocolitica* biotype 1 A non agglutinable (NAG) from a sample of raw beef. The colonies flat, NLF, without production of H_2S, showed positive reactions for Urease, Ornithine decarboxylase, Amygdaline, Sorbitol, Mannitol, Indole and negative reactions for Lysine decarboxylase, Arginina dihydrolase and Phenylalanine deaminase (test Api 20 E System). The strain exibit Lipase activity; and showed a positive reaction to β-D-Glucosidase test and to Pyrazinamidase test. All the biochemical reactions are given in Table I. The strain identified as belonging to the genus *Yersinia* was sent to Italian National Health Institute for confirmation and serotyping.

Tab. I. Biochemical reaction of strain isolated.

ONPG	Negative
ADH	Negative
LDC	Negative
ODC	Positive
CIT	Negative
H_2 S	Negative
URE	Positive
TDA	Negative
IND	Positive
VP	Negative
GEL	Negative
GLU	Positive
MAN	Positive
INO	Negative
SOR	Positive
RHA	Negative
SAC	Positive
MEL	Negative
AMY	Positive
ARA	Negative
OXY	Negative
Lipase	Positive
β-Glucosidasi	Positive
Pyrazinamidase	Positive

Discussion and conclusions

Either in the past or late, many Authors related about pathogenicity of strain of *Yersinia enterocolitica* biotype 1 A. Singh et al. [6] reported results of the isolation of *Yersinia enterocolitica* from pediatric diarrhoeic patients and from ground waters, waste waters and river. All the *Yersinia enterocolitica* isolates from human and water belonged to biotype 1 A, some were non agglutinable (NAG). Singh et al. [7] studied also the production of *Yersinia* stable toxin in biotype 1 A strains of *Yersinia enterocolitica*. Singh et al. [8] concluded: it may be construed that *Yersinia enterocolitica* biotype 1 A isolates of clinical and swine origin have higher virulence potential than those from other sources. Morris et al. [9] related that *Yersinia enterocolitica* biotype 1 A strains were isolated from children with diarrhea. By Burnens et al. [10]: "*Despite being considered as non-pathogenic, biogroup 1 A isolates have constituted a sizeable fraction of strains from patients with gastroenteris in many reports*". Was indicated the existence of potentially pathogenic strains among biogroup 1 A of *Yersinia enterocolitica*. Sinha et al. [11] reported occurrence of *Yersinia enterocolitica* biotype 1 A in wastewater samples and refered that there has been a renewed interest about the pathogenicity of biotype 1 A. It has been shown that biotype 1 A may cause self-limiting enteritis without overt symptoms or even gastroenteritis similar to that caused by virulent biotypes. In several parts of world, significant number of *Yersinia enterocolitica* isolates from clinical cases of gastroenteritis belong to biotype 1. It was suggested recently that isolates belonging to biotype 1 A may be pathogenic by novel, as yet unknown mechanism. Sharma et al. [12] studied detection of β-lactamase in clinical and non-clinical strains of *Yersinia enterocolitica* biovar 1 A. Cavazzini et al. [13] studied biotypes and serotypes of *Yersinia enterocolitica* isolated from horticultural products and reported that in can be assumed that "environmental strains of *Yersinia enterocolitica*" may also acquire virulence and cause

infection in man. Tennant et al. [14] reported that there is growing epidemiological, clinical and experimental evidence to suggest that biotype 1 A strains are virulent and can cause gastrointestinal diseases. In other related articles Tennant et al. [14, 15] indicated that at least some strains of *Yersinia enterocolitica* biotype 1 A are able to cause gastrointestinal symptoms which resemble those caused by pYV-bearing strains (*Yersinia* virulence plasmid). Grant et al. [16] by their studies established that biotype 1 A *Yersinia enterocolitica* strains were able to escape from macrophages or epithelial cells without causing detectable cytolysis, suggesting that escape was achivied by a process resembling exocytosis. The observations that biotype 1 A *Yersinia enterocolitica* strains of clinical origin are significantly more resistant to killing by macrophages and significantly more likely to escape from host cells than are strains of non clinical origin suggest that these properties may account for the virulence of these bacteria. Paz et al. [17] analyzed virulence factors in a strain of *Yersinia enterocolitica* biotype 1 A and indicated that certain clinical isolates of *Yersinia enterocolitica* of the biotype 1 A ("avirulent"), could be the etiological agent of the illness through other mechanism of virulence, that would differ from those previously characterized in species of enteropathogenic *Yersinia*. Bhagat et al. [18] related that some biovar 1 A strain, have been reported to cause symptoms similar to that produced by isolate belonging to know pathogenic biovars. In conclusion, the presence of *Yersinia enterocolitica* biotype 1 A in raw meat products, represents a healthy risk for consumers; to prevent or control *yersiniosi* is suggested: through hand washing, clean and sanitize utensils after contact with raw meat, cook through meat and especially pork products. In USA the Center for Diseases Control and Prevention (CDC), conducts investigations of outbreaks of *yersiniosis* to control them and to learn more about how to prevent these infections. According with others Authors, Ramirez et al. [19], Hoffmann et al. [20], it can be assumed that further clinical studies are needed to assess the epidemiological importance, the occurrence and the possible etiological relevance of "*Yersinia enterocolitica* biotype 1 A: not as harmless as you think" [21].

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