

Yersinia enterocolitica

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15.1. INTRODUCTION

Yersinia enterocolitica has the dubious distinction of being termed the pathogenic bacterium of the 1980s. The new human pathogen was discovered 60 years ago in the United States by Schleifstein and Coleman, who named it *Bacterium enterocoliticum*. Little was known about this bacterium until the early 1960s when clinical and veterinary microbiologists started reporting the isolation of “new” isolates of bacteria similar to *B. enterocoliticum*. Consequently, these isolates were classified and named *Y. enterocolitica* (i.e., pertaining to the intestine and colon). The reclassification of this organism makes possible a more meaningful evaluation of the distribution of this species and its pathogenicity. Subsequent studies showed a ubiquitous distribution and a wide range of *Y. enterocolitica* strains in food and clinical samples.

15.2. NATURE OF ILLNESS

Yersinia enterocolitica is recognized as a foodborne pathogen, and the disease caused by this bacterium is called yersiniosis. Symptoms of yersiniosis include severe abdominal pain that suggests an appendicitis-like attack, as well as fever, diarrhea, headache, and vomiting. The very young and very old are most susceptible to *Y. enterocolitica* infection. The incubation period for yersiniosis is 24–36 h or longer. The duration of illness is usually 1–3 days. A large number of food-associated outbreaks of yersiniosis have been reported. In developed countries, *Y. enterocolitica* can be isolated from 1–2% of all human cases of acute enteritis. According to the U.S. Centers for Disease Control and Prevention, although there have been an estimated 96,000 cases of yersiniosis in North America, there have been no reported deaths.

15.3. CHARACTERISTICS OF AGENT

Yersinia enterocolitica belongs to the Enterobacteriaceae family. It is a gram-negative, oxidase-negative, catalase-positive, nitrate-reductase-positive, cold-tolerant facultative anaerobic rod $0.5\text{--}0.8 \times 1\text{--}3 \mu\text{m}$ in size that exhibits significant pleomorphism. The organism is not motile when grown at 37°C but motile at $22\text{--}25^\circ\text{C}$, with relatively few, peritrichous flagella when it is grown at less than 30°C . In addition, the bacterium is urease positive, and it ferments mannitol and produces gas from glucose. *Yersinia enterocolitica* differs from most members of the family Enterobacteriaceae by virtue of its slower growth at 37°C .

Yersinia enterocolitica has been called a cold-tolerant pathogen. It can grow at temperatures as low as 0°C . However, the bacterium can grow at temperatures as high as 44°C , with the optimum temperature being $32\text{--}34^\circ\text{C}$. The bacterium is inactivated at 50°C . The pH range for growth is pH 4.5–8.5, with an optimum of pH 7–8. The organism can grow in the presence of 0.5–5% of NaCl. *Yersinia enterocolitica* can survive in frozen conditions for 12 weeks.

Human pathogenic strains of *Y. enterocolitica* are endowed with a number of properties that confer virulence on the organism. Several of these strains exhibit a marked temperature-dependent expression of genes that are correlated with the presence of a 70- to 75-kbp plasmid that is directly involved with the virulence of the organism. A number of plasmid-mediated phenotypic characteristics including colony morphology, low-calcium response (LcR), Congo red (CR) uptake, crystal violet (CV) binding, autoagglutination (AA), serum resistance, tissue culture detachment, and hydrophobicity (HP) have been applied to the determination of virulence in strains of *Y. enterocolitica*. At a low level of calcium ions, the virulence plasmid also encodes for "Yops" (i.e., a set of proteins secreted by plasmid-bearing virulent strains), which are important virulence factors. The delivery of Yops into the host cell subverts or modulates normal host cell signal transduction and cytoskeletal functions. Another plasmid-encoded, calcium-independent, outer membrane protein, YadA, on the bacterial surface mediates cellular attachment and entry. These physiological traits associated with the virulence plasmid are expressed only at 37°C . However, growth at 37°C also fosters the loss of the virulence plasmid and the concomitant disappearance of the associated virulence characteristics. Elements encoded by the chromosome are also necessary for virulence. Pathogenic *Y. enterocolitica* share two chromosomal loci, *inv* (i.e., the invasion loci that mediates the penetration of host cells) and *ail* (i.e., the attachment-invasion loci that mediates bacterial attachment to host cells), which are involved in the first step of pathogenesis.

15.4. EPIDEMIOLOGY

Transmission of *Y. enterocolitica* is by the fecal–oral route, facilitated by ingesting fecally contaminated food or water. Pigs are the principal reservoir for virulent strains, and the organisms are often isolated from the oral cavity and gastrointestinal tract of apparently healthy animals. *Yersinia enterocolitica* colonize the tongue and tonsil areas of the pigs. Common food vehicles in outbreaks of yersiniosis are meat (particularly pork), milk, dairy products, powdered milk, cheese, tofu, and raw vegetables. Since *Yersinia* can grow at low temperatures, even refrigerated foods are potential vehicles for the growth and dissemination of these organisms. The majority of food isolates differ in biochemical (based on biochemical reactions, termed biovars) and serological (based on lipopolysaccharide surface O antigens, termed O serovars) characteristics from “typical” clinical strains and are usually called “nonpathogenic” or “environmental” *Yersinia* strains.

Outbreaks of yersiniosis are uncommon considering the widespread occurrence of *Y. enterocolitica* in the environment, its ability to colonize and persist within animals, and its ability to grow at refrigerated temperatures. During the mid 1970s, two outbreaks of yersiniosis were reported. The first outbreak was caused by the consumption of raw milk by 138 Canadian schoolchildren, but the organism was not recovered from the suspected source. The second and most highly publicized outbreak, which occurred in 1976, involved 220 grade school children in a small community in New York. The source of infection was chocolate-flavored milk that was culture positive. Thirty-six children were hospitalized with apparent acute appendicitis. Before it could be established that the patients were suffering from *Yersinia*-mediated pseudoappendicitis, 16 of the children had already undergone emergency appendectomies. A 1981 outbreak affected 35% (159 persons) of 455 individuals at a diet camp in New York. Five of seven patients hospitalized underwent appendectomies. The source of infection was reconstituted powdered milk and/or chow mein contaminated by an infected food handler. In the same year, an outbreak was reported among 50 individuals in Washington State involving tofu and spring water. In a 1982 outbreak, 172 cases of infection occurred in Arkansas, Mississippi, and Tennessee due to consumption of pasteurized milk that may have been contaminated with pig manure during transport. Water was the putative source of infection for an individual in New York State in 1974 and for a small outbreak in Ontario, Canada, in 1986. That same year, a Pennsylvania outbreak caused by bean sprouts and well water affected 16 individuals. An outbreak in 1989 affecting 15 infants and children in Atlanta, Georgia, was transmitted from raw pork chitterlings by the food handlers. More recently, an outbreak in 1992 was reported in Los Angeles County, California, involving seven persons. The source of the outbreak remains unknown. No other yersiniosis outbreaks have been reported in the 1990s in the United States. The paucity of yersiniosis outbreaks in the 1990s as compared to the 1980s has no clear explanation.

15.5. DETECTION OF ORGANISM

15.5.1. Virulence Determinants

The plasmid-associated virulence determinants have been used to differentiate between virulent and avirulent strains of *Y. enterocolitica*. Thus, these virulence determinants provide a rapid, reliable, and simple method for isolation and detection of plasmid-bearing virulent *Y. enterocolitica* (YEP⁺) strains from foods. The main disadvantage of the use of these plasmid-borne virulence determinants is the instability of the virulence plasmid. Incubation of strains at 37°C for isolation fosters the loss of the virulence plasmid resulting in plasmidless avirulent (YEP⁻) strains; however, the plasmid-associated phenotypes are only expressed at 37°C. Because of the instability of the virulence plasmid at 37°C, it is difficult to isolate YEP⁺ strains after initial detection. As a consequence, detection has been hampered in clinical, regulatory, and quality control laboratories that employ an incubation temperature of 37°C for isolation/detection of the organism.

15.5.2. Congo Red Binding

Congo red binding has been used to screen *Y. enterocolitica* strains for virulence. When YEP⁺ and YEP⁻ strains were cultivated at 37°C for 24 h on a CR-

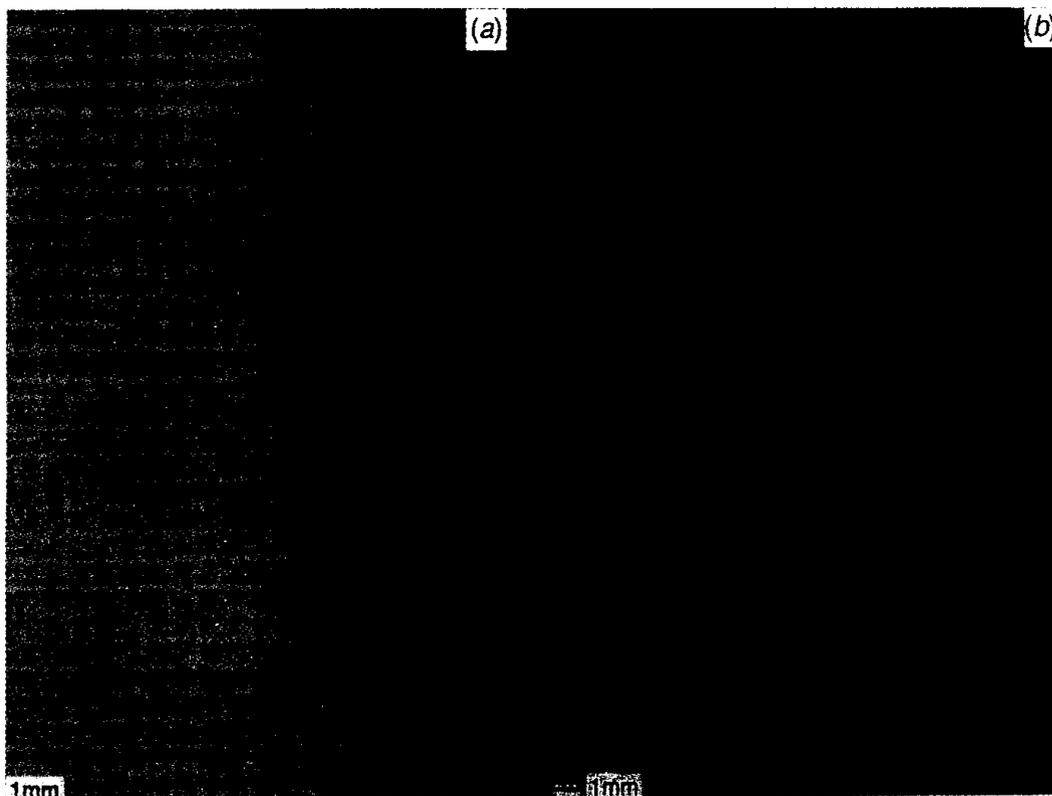


Figure 15.1. CR binding of colonies of *Y. enterocolitica* cells grown on CR-BHO for 24 h at 37°C: (a) YEP⁺ cells showing pinpoint red colonies; (b) YEP⁻ cells showing large white or light orange colonies. The concentration of CR used in the binding assay was 75 µg/mL.

containing, low-calcium brain heart infusion agarose (CR-BHO) medium, two types of readily discernible colonies were observed. The YEP⁺ cells absorbed CR and formed red pinpoint colonies (CR⁺) (Fig. 15.1a). The YEP⁻ cells failed to bind the dye and formed much larger white or light orange colonies (CR⁻) (Fig. 15.1b). The size and colony morphologies of YEP⁺ strains on CR-BHO also showed a LcR. The CR binding test was correlated with the presence of the virulence plasmid, with a number of virulence-associated properties and with mouse virulence, for a wide variety of pathogenic serotypes of *Y. enterocolitica* (Table 15.1). Thus, the binding of CR by YEP⁺ strains consistently and efficiently allows differentiation of virulent and avirulent strains of *Y. enterocolitica*. In an investigation of a *Yersinia* outbreak in Los Angeles County, California, in 1992, the CR binding technique detected 1 YEP⁺ isolate per 300 colonies recovered on CR-BHO from each of the seven patients. These data highlight the sensitivity of the CR binding technique for detection of YEP⁺ cells in clinical samples that contained predominantly YEP⁻ cells due to incubation of samples at 37°C during the initial isolation of the organism.

Since incubation at 37°C causes the loss of the virulence plasmid, an additional advantage of the CR binding technique is that it can be used to isolate *Y. enterocolitica* cells carrying the virulence plasmid. The ability of the CR binding technique for recovery of YEP⁺ cells varied from 5 to 95%, indicating strain variation in the stability of the virulence plasmid. The YEP⁺ strains showed all of the expected plasmid-associated properties, including virulence in a mouse (Table 15.1). By using the CR binding recovery technique mentioned above, Food and Drug Administration (FDA) investigators recovered and enhanced the level of plasmid carriage of YEP⁺ strains from 0.3% to over 92% from clinical samples obtained during a 1992 outbreak of yersiniosis in Los Angeles County, California. Thus, the recovery technique is useful to isolate and enrich for viable YEP⁺ cells, even if they are present at very low levels among a mixture of cells.

15.6. ISOLATION OF PATHOGENIC YEP⁺ STRAINS FROM FOODS

The increasing incidence of *Y. enterocolitica* infections and the role of foods in some outbreaks of yersiniosis has led to the development of a wide variety of methods for the isolation of this bacterium from foods. The unstable nature of the virulence plasmid during passage at 37°C complicates the isolation of YEP⁺ strains by causing the overgrowth of virulent cells with plasmidless derivatives and can eventually lead to a completely avirulent culture. Since the population of *Y. enterocolitica* in foods is usually low and since the natural microflora suppresses the growth of this organism, isolation methods usually involve enrichment followed by plating onto selective media.

Several approaches have been taken to enrich and isolate *Y. enterocolitica* from food. One method employs prolonged enrichment for 2–4 weeks at refrigeration temperatures to take advantage of the psychrotrophic nature of *Y.*

TABLE 15.1. Correlation among CR Binding Technique, Virulence, and Virulence-Associated Properties of Original and Recovered Plasmid-Bearing Strains of *Y. enterocolitica*

Strains ^a	Serotype	CM ^b	CV: Binding	LCR ^d	CR ^e Binding	AA ^f	HP ^g	Plasmid (70-75 kbp)	Diarrhea in Mice ^h
GER	O:3	+	+	+	+	+	+	+	+
GER-RE	O:3	+	+	+	+	+	+	+	+
GER-C	O:3	-	-	-	-	-	-	-	-
EWMS	O:13	+	+	+	+	+	+	+	+
EWMS-RE	O:13	+	+	+	+	+	+	+	+
EWMS-C	O:13	-	-	-	-	-	-	-	-
PT18-1	O:5:0:27	+	+	+	+	+	+	+	+
PT18-1-RE	O:5:0:27	+	+	+	+	+	+	+	+
PT18-1-C	O:5:0:27	-	-	-	-	-	-	-	-
O:TAC	O:TACOMA	+	+	+	+	+	+	+	+
O:TAC-RE	O:TACOMA	+	+	+	+	+	+	+	+
O:TAC-C	O:TACOMA	-	-	-	-	-	-	-	-
WA	O:8	+	+	+	+	+	+	+	+
WA-RE	O:8	+	+	+	+	+	+	+	+
WA-C	O:8	-	-	-	-	-	-	-	-

^a Strains are from the FDA. Recovered strains are designated as RE. Plasmidless avirulent YEP⁻ strains are designated as C (cured).

^b CM: Colony morphology. In a calcium-adequate brain heart infusion agar (BHA) medium, YEP⁺ cells appeared as small colonies (diameter 1.13 mm) as compared to larger YEP⁻ colonies (diameter 2.4 mm).

^c CV binding: Crystal violet binding. YEP⁺ cells appeared as small dark violet colonies on BHA.

^d LcR: Low-calcium response. Calcium-dependent growth at 37°C. YEP⁺ cells appeared as pin point colonies of diameter 0.36 mm compared to the larger YEP⁻ colonies of diameter 1.37 mm on CR-BHO.

^e CR binding: Congo red binding. YEP⁺ cells appeared as red pinpoint colonies on CR-BHO.

^f AA: Autoagglutination.

^g HP: Hydrophobicity.

^h Fecal material consistency was liquid; diarrhea was observed starting on days 3 and 4 postinfection.

enterocolitica and to suppress the growth of any background flora. Due to the extended time period needed for this method, efforts have been made to devise selective enrichment techniques employing shorter incubation times and higher temperature, thus making them more practical for routine use. However, high levels of indigenous microorganisms can overgrow and mask the presence of YEP⁺ and nonpathogenic *Y. enterocolitica* strains. Enrichment media containing selective agents such as Irgasan, ticarcillin, and potassium chlorate are effective for enhancing recovery of a wide spectrum of *Y. enterocolitica* strains from meat samples. However, no single enrichment procedure is adequate for recovery of a broad spectrum of pathogenic *Y. enterocolitica* from foods. Since there is no specific plating medium for the isolation of YEP⁺ strains, cefsulodin-irgasan-novobiocin (CIN) and MacConkey (MAC) agars are commonly used to isolate presumptive *Y. enterocolitica* from foods. The initial isolation of presumptive *Y. enterocolitica* from enriched samples on CIN and MAC agars adds an extra plating step, and the picking of presumptive *Y. enterocolitica* requires skilled recognition and handling of the colonies. The unstable nature of the virulence plasmid further complicates the detection of YEP⁺ strains since isolation steps may lead to plasmid loss and the loss of associated phenotypic characteristics for colony differentiation. Moreover, the presumptive *Y. enterocolitica* colonies isolated should be confirmed as YEP⁺ strains. Biochemical reactions, serotyping, biotyping, and virulence testing are essential for differentiation among YEP⁺, YEP⁻, environmental *Yersinia* strains, and other *Yersinia*-like presumptive organisms. Biochemical tests using commercially available systems such as analytical profile index (API) 20E test strips, give similar reactions among these organisms and are not conclusive. Serotyping of major O and H antigens differentiates between pathogenic and environmental *Y. enterocolitica* but fails to discriminate between YEP⁺ and YEP⁻ strains. Likewise, biotyping does not confirm the presence of the virulence plasmid in YEP⁺ strains. Several plasmid-associated phenotypic virulence determinants including colony morphology, AA, serum resistance, tissue culture detachment, HP, LcR, and CV binding have been used to portend the virulence of *Yersinia* isolates. These methods require specific reagents and conditions and do not give definite results. In addition, most of these procedures are costly, time consuming, complex, and impractical for routine diagnostic use in field laboratories. Although virulence can be demonstrated effectively using laboratory animals, this test is not suitable for routine diagnostic use. Molecular techniques such as deoxyribonucleic acid (DNA) colony hybridization, DNA restriction fragment length polymorphisms, and the polymerase chain reaction (PCR) have also been successfully applied to the detection of virulent strains. However, these techniques are complex and time consuming. These methods detect only the presence of a specific gene, not the actual presence of the live organism. Although virulence is plasmid mediated in all strains examined, the plasmids involved differ in molecular weight. Thus, in epidemiological studies, it is not sufficient to search for plasmids of a particular molecular weight as an indicator of *Y. enterocolitica* virulence. Unfortunately, methods described in the literature for

the isolation of pathogenic *Y. enterocolitica* from foods do not treat confirmation of virulence in presumptive or known *Y. enterocolitica* isolates recovered from selective agars as an integral part of the detection method. The most rapid enrichment procedure available for the isolation of a wide spectrum of *Y. enterocolitica* serotypes does not include the identification of isolates as YEP⁺ strains.

An improved homogenization-based procedure for selective enrichment, identification and maintenance of various pathogenic YEP⁺ serotypes from pork samples was developed. This procedure is suitable for ground and liquid food samples wherein bacterial contamination is distributed throughout. In some cases, food slurries are used for enrichment, and the presence of food in the enrichment medium increases the background microflora and requires increased time for enrichment of YEP⁺ strains. The CR binding technique is also not applicable for both detection and isolation, because the appearance of a red pin point colony is masked by the background microflora. Hence, it is necessary to first isolate presumptive *Y. enterocolitica* on selective agar plates and then subsequently identify any YEP⁺ strains by the CR binding and LcR techniques. This approach takes 6 days to complete from sample enrichment through confirmation of YEP⁺ strains, and as few as 9 colony-forming units (CFU/g) of YEP⁺ strains of spiked ground pork can be recovered.

Recently, a method for simultaneous detection and isolation of pathogenic YEP⁺ serotypes from enriched swab samples of various foods was reported. This procedure is applicable to foods that have a physical surface for swabbing, and such surfaces are often the primary site of contamination. Since the actual food sample was not used and since there was a low level of competing microflora, the time for enrichment of YEP⁺ strains and their subsequent confirmation by the CR binding and LcR techniques was appreciably reduced (Fig. 15.2). This technique allowed for recovery of YEP⁺ serotypes from various foods spiked with as low as 0.5 CFU/cm² within 4 days.

The above-mentioned homogenated slurry and swabbing techniques were effective for recovery of YEP⁺ strains from naturally contaminated porcine tongues. The PCR assays validated these methods for detection of YEP⁺ strains (Fig. 15.3). The YEP⁺ serotypes isolated by these two procedures expressed plasmid-associated virulence characteristics and were positive in the mouse virulence test.

15.7. PREVENTION AND CONTROL

The patterns observed in foodborne *Y. enterocolitica* outbreaks indicate that postprocessing contamination is the main cause of illness. Specific prevention and control measures of foodborne yersiniosis include the following:

1. Special care should be taken during incision and removal of the intestines, tongue, pharynx, and tonsils of pigs to avoid cross-contamination.

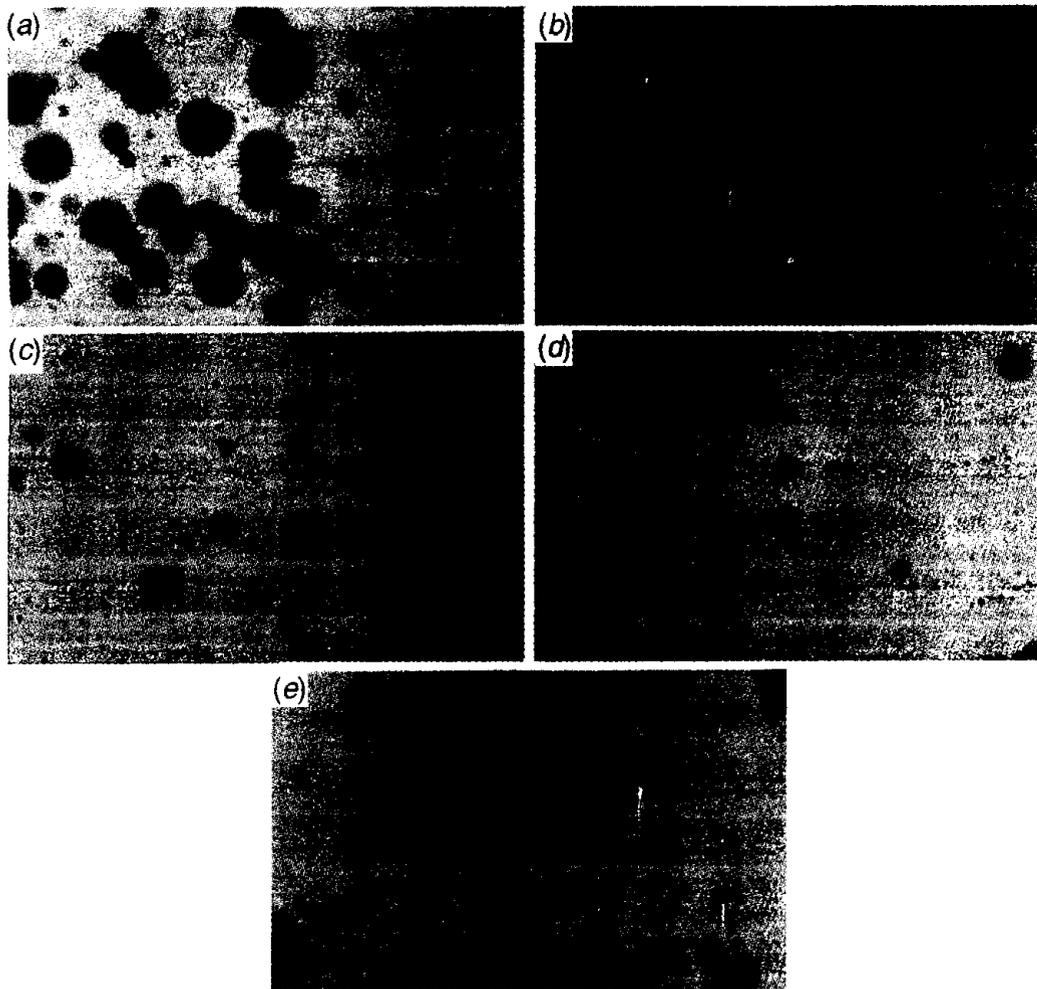


Figure 15.2. Recovery of YEP⁺ strains by swabbing technique as red pinpoint colonies on CR-BHO from artificially contaminated pork chops (a), ground pork (b), cheese (c), and zucchini (d) and naturally contaminated porcine tongue (e).

2. Meat products, particularly pork, should be handled with care; food utensils, equipment, and countertops should be thoroughly cleaned to prevent cross-contamination.
3. Refrigerated foods should be thoroughly cooked or heated at temperatures sufficient to kill the pathogen. Likewise, cooked and refrigerated foods should be heated to a steaming temperature to kill *Yersinia* before consumption.
4. Precautions for prevention of fecal–oral spread of the pathogen should be practiced. Water supplies should be free from animal and human fecal waste.
5. Hands should be washed with soap and hot water after handling raw foods, including pork, as well as before serving food and eating food.

YERSINIA ENTEROCOLITICA

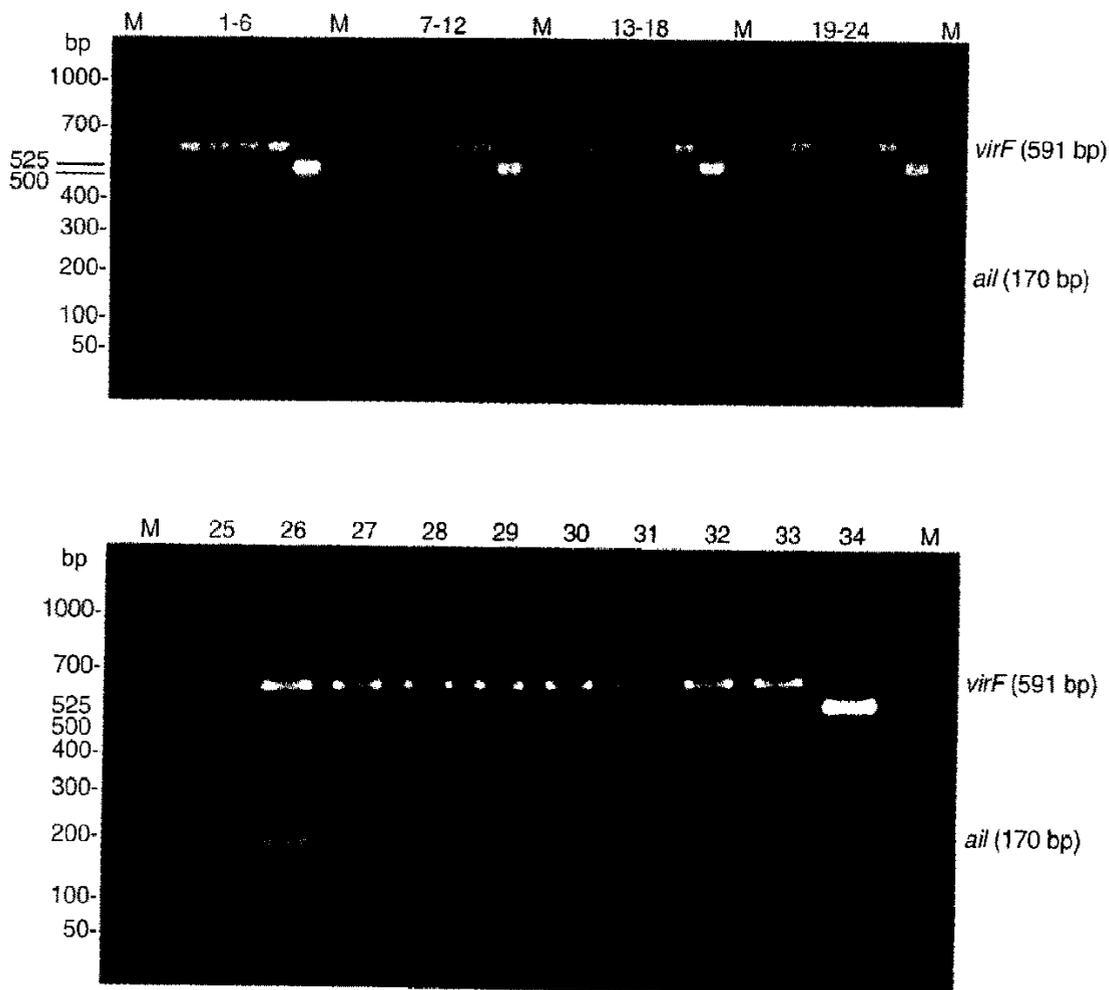


Figure 15.3. Confirmation of CR⁺ clones isolated by swabbing technique from artificially contaminated various foods and from naturally contaminated porcine tongue as YEP⁺ strains by multiplex PCR using chromosomal *ail* gene and *virF* gene from virulence plasmid. Lane M, 50- to 1000-bp ladder marker. Negative control with no template: lanes 1, 7, 13, 19, and 25. CR⁺ colony showing the presence of 170- and 591-bp products with mixture of both *ail* and *virF* primers from chromosome and virulence plasmid, respectively: pork chops (lanes 2–4), ground pork (lanes 8–10), cheese (lanes 14–16), zucchini (lanes 20–22), and porcine tongues (lanes 26–32). Positive control with purified DNA from YEP⁺ strain showing the presence of 170- and 591-bp products with mixture of both *ail* and *virF* primers from chromosome and virulence plasmid, respectively: lanes 5, 11, 17, 23, and 33. Positive control for PCR assay with λ as DNA template: lanes 6, 12, 18, 24, and 34.

In summary, these control measures require cooking and reheating to destroy YEP⁺ strains since they are sensitive to heat. Cleanliness of the kitchen area, proper personal hygiene, and hand washing are very important in preventing infection.

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