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Review

Conventional and molecular methods used in the detection and subtyping of *Yersinia enterocolitica* in food

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ABSTRACT

Yersinia enterocolitica is an important foodborne pathogen, but the prevalence in food is underestimated due to drawbacks in the detection methods. Problems arise from the low concentration of pathogenic strains present in food samples, similarities with other Enterobacteriaceae and *Y. enterocolitica*-like species and the heterogeneity of *Y. enterocolitica* as it comprises both pathogenic and non-pathogenic isolates. New rapid, cost-effective and more sensitive culture media and molecular techniques have been developed to overcome the drawbacks of conventional culture methods. Recent molecular subtyping methods have been applied to *Y. enterocolitica* strains to track infection sources and to investigate phylogenetic relationships between different *Yersinia* strains. Further application of modern subtyping tools such as WGS in a variety of bioserotypes, and comparison with other members of the genus will help to better understanding of the virulence determinants of pathogenic *Y. enterocolitica*, its mechanisms to cope in the host environments, and can contribute to the development of more specific detection and typing strategies.

Contents

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1. Introduction

Yersiniosis was the third most commonly reported zoonosis in the EU in 2013. Pigs are considered to be a major reservoir for *Yersinia*, and pork products are considered to be the most important source for pathogenic *Y. enterocolitica* infection in humans. *Y. enterocolitica* occurrence was also reported in member states of EU in bovine meat, cow's milk and poultry, as well as in vegetables consumed raw by humans. *Y. enterocolitica* was also detected in other domestic and wild animals (EFSA, 2015).

Human clinical infections with *Y. enterocolitica* ensue after ingestion of the microorganisms through contaminated food or water or through blood transfusion. Gastrointestinal symptoms range from self-limiting gastroenteritis to acute enteritis (particularly in children), mesenteric lymphadenitis and terminal ileitis mimicking appendicitis (children older than 5 years). In the case of immunosuppressed individuals and patients with hemochromatosis or being treated with desferrioxamine, infection with *Y. enterocolitica* can lead to septicaemia (Bottone, 1997).

Y. enterocolitica is a Gram-negative bacterium that belongs to the family Enterobacteriaceae. It has the unusual ability among pathogenic enterobacteria to grow well at refrigeration temperatures (psychotrophic). This organism can also withstand freezing and survive for extended periods of time in frozen food, even after repeated freezing and thawing. The ability to propagate at refrigeration temperature in vaccum packed or modified atmosphere foods aimed for a prolonged shelf-life is of considerable significance in food hygiene (Feng and Weagant, 1994; Fredriksson-Ahomaa, 2012; Nesbakken, 2006). *Y. enterocolitica* is a highly heterogeneous group of bacteria consisting of different subtypes (biotypes and serotypes). Biotyping and serotyping are both used to determine the strain pathogenicity (Kapperud, 1991), although virulence markers have also been found in non-pathogenic biotype 1A strains (Grant et al., 1998). In addition, the large number of organisms in the background microbiota, the presence of non-pathogenic *Yersinia* spp. (including non-pathogenic *Y. enterocolitica* strains) and the low concentration of pathogenic strains, especially in food samples complicate the isolation leading to an underestimated prevalence of pathogenic *Y. enterocolitica* in food (Fredriksson-Ahomaa and Korkeala, 2003; Bonardi et al., 2010). New conventional and molecular methods have been designed to overcome the challenges regarding detection and subtyping of *Y. enterocolitica* in food.

2. Conventional methods

2.1. Detection methods

A variety of cultural methods has been used by different researchers in the detection of *Yersinia enterocolitica.* Based on the available literature data the source of the specimen suspected for *Y. enterocolitica* infection can markedly affect the choice of the detection method. In clinical specimens – faeces or organ abscesses of infected individuals – where pathogenic strains of *Y. enterocolitica* are often the dominant bacteria, it is easier to isolate the microorganism by direct plating on conventional and selective enteric media. In food samples, direct plate isolation is not so efficient because the microorganism is present in low concentration amid the much larger numbers of complex background microbiota. In order to increase the number of pathogenic *Y. enterocolitica* cells in food samples enrichment in liquid media before plating on selective solid media is required (Fredriksson-Ahomaa and Korkeala, 2003). There are several culture methods, including some standardized reference methods, for the isolation of *Y. enterocolitica* from non-human samples, relying on standard enrichment and selective plating protocols (Table 1).

Conflicting results have been reported regarding the difference between selective and cold enrichment for the isolation of pathogenic *Y. enterocolitica* from food (de Boer and Seldam, 1987; Martinez et al., 2011; Van Damme et al., 2013a, 2013b; Wauters et al., 1988).

2.1.1. Cold enrichment

Due to the psychrotrophic nature of *Yersinia* strains, they can outgrow several mesophilic organisms also belonging to Enterobacteriaceae when cultivated at low temperatures. Using lower incubation temperatures allows *Y. enterocolitica* to achieve higher population density than many other bacteria. The incubation time depends on temperature: at 4 °C, incubation for 14–21 days is recommended, but it may be shortened to 3 days by increasing the temperature to 10 °C (Mills, 2004; Schiemann and Olson, 1984).

Different media have been used for the cold enrichment of *Yersinia*. Non- selective broths such as phosphate-buffered saline (PBS), or phosphate-buffered saline supplemented with 1% sorbitol and 0.15% bile salts (PSB) or tryptose soya broth (TSB) (Bonardi et al., 2010; Doyle and Hugdahl, 1983; Schiemann, 1982, 1983a). Instead of sorbitol, mannitol has also been used with PBS and bile salts (PMB) (Martinez et al., 2011; Van Damme et al., 2013a, 2013b).

Major disadvantages are the time consuming incubation period of 14 to 21 days that can also increase the recovery of non-pathogenic *Y. enterocolitica* 1A and *Y. enterocolitica* – like strains (Bonardi et al., 2010; de Boer, 1992; de Boer and Seldam, 1987) and other psychrotrophic bacteria, such as *Hafnia alvei*, which also multiply during enrichment and grow over the growth of the pathogenic strains (Fukushima and Gomyoda, 1986). Nevertheless, cold enrichment is suitable for food samples in which pathogenic *Yersinia enterocolitica* is present in low concentration.

2.1.2. Selective enrichment

Selective/secondary enrichment methods enhance the selectivity and shorten the incubation time. They include selective enrichment broths with different antimicrobial agents used as selective supplements. Various enrichment media including modified Rappaport broth (MRB), irgasan-ticarcillin-potassium chlorate medium (ITC), bile oxalate sorbose broth (BOS) and modified selenite medium have been developed. These media are highly selective for some strains of *Y. enterocolitica* but are also quite inhibitory for others. BOS is most suited for recovery of serotype O:8, while MRB and ITC are more suited for isolation of serotype O:3 strains. The most frequently used media are ITC broth and MRB (Feng and Weagant, 1994; Fredriksson-Ahomaa and Korkeala, 2003; Mills, 2004).

Wauters (1973) formulated the MRB containing magnesium chloride, malachite green, and carbenicillin, in which the sample is incubated at 22 °C for 2 days or longer. MRB is often used without carbenicillin, because it may inhibit the growth of certain strains of *Y. enterocolitica* O:3 (Schiemann, 1982). Wauters et al. (1988) also developed ITC an enrichment broth derived from the MRB. It has been reported that ITC enhances the isolation rate of *Y. enterocolitica* O:3, but not that of O:9 from meat samples (De Zutter et al., 1994). MRB and ITC broths are inoculated either directly from sample homogenates or from cold-enriched cultures at ratios of 1 volume inoculum to 100 volumes broth. Incubation of