

## OCCURRENCE OF *YERSINIA ENTEROCOLITICA* IN RAW PORK

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### ABSTRACT

In the present study, 100 pork samples including 50 each of ground pork and pig tonsils collected from the local retail market were examined by culture method for the presence of *Yersinia enterocolitica*. Isolation of *Yersinia enterocolitica* from pork samples included direct plating and broth enrichment followed by alkali treatment prior to inoculation on selective agar medium plates. Out of the 100 pork samples, the samples positive by culture method were 18 (18%) including 8 (16%) of ground pork and 10 (20%) of pig tonsil. Of the 100 pork samples, 4 (4%) were positive by direct plating method, 19 (19%) and 17 (17%) by 2 and 5 days broth enrichment methods, respectively. Isolation rate of *Y. enterocolitica* from pork sample was higher with 2 and 5 days broth enrichment than direct plating.

**Key words:** *Yersinia enterocolitica*, ground pork, tonsils, broth enrichment, direct plating

*Yersinia enterocolitica* is an important emerging food-borne enteric pathogen that causes acute gastroenteritis, enterocolitis and mesenteric lymphadenitis as well as a variety of extra intestinal disorders in humans. Acute yersiniosis due to *Y. enterocolitica* in animals is characterized by enteritis and enlargement of lymph nodes and spleen, whereas, chronic infections may cause granulomatous nodules and localized abscesses affecting various organs, typically liver and lungs (Zhang *et al.*, 2008). It has been frequently isolated from animals, food and environment (Fredriksson-Ahomaa *et al.*, 2006; Subha *et al.*, 2009; Virtanen *et al.*, 2012; Paixao *et al.*, 2013; Pugazhenthii *et al.*, 2013). A number of outbreaks of *Yersinia* infection in human beings in countries like Finland, Japan, America, Brazil, Sweden, India and China have been reported (Wang *et al.*, 2009). Case control studies indicate that majority of outbreaks are due to consumption of uncooked pork as well as contaminated water, milk and vegetables (Fredriksson-Ahomaa *et al.*, 2006). In India, a food borne outbreak of gastroenteritis in Tamil Nadu was attributed to *Y. enterocolitica* (Abraham *et al.*, 1997). In countries where *Y. enterocolitica* is a significant food borne pathogen, estimated carrier rate ranges approximately 35% to 70% in swine herds and 4.5% to 100% in individual pigs (Robinson-Browne, 2007). It is well established that pigs are the main reservoir for *Y. enterocolitica* and pork is therefore likely to be the most important vehicle for its transmission to humans, directly or indirectly. Nielsen *et al.* (1996) reported that pigs are infected early in life and maintain infection in the tonsils

for longer periods, perhaps for life. In India, isolation of *Y. enterocolitica* has been reported from different foods of animal origin including pork, chicken meat, and milk (Subha *et al.*, 2009; Saikia and Joshi, 2010; Arora, 2010; Pugazhenthii *et al.*, 2013; Anju *et al.*, 2014).

### MATERIALS AND METHODS

**Sampling:** One hundred pork samples comprising 50 each of ground pork and pig tonsils were collected from the local retail market in sterile sample containers with all aseptic precautions and were transported to the laboratory under cold condition immediately after collection. All the samples were immediately processed for isolation of *Y. enterocolitica*.

**Isolation Protocol:** *Y. enterocolitica* organisms were isolated from pork samples as per the method described by ISO (International Organization of Standardization) (ISO 10273, 2003). A total of 12 g pork sample was blended aseptically for 4 min in a homogenizer with 108 ml Peptone Sorbitol Bile Broth (PSB, Hi-media). From this initial homogenate, testing was carried out in parallel by direct plating as well as enrichment plating.

**Direct Plating:** Ten ml PSB broth was transferred to a sterile test tube and 0.1 ml thereof was spread plated over Salmonella-Shigella-desoxycholate-calcium chloride (SSDC, Hi-media) and cefsulodin-irgasan-novobiocin (CIN) agar (YSA, *Yersinia* selective agar base and *Yersinia* selective supplement, Hi-media) plates. All agar plates were incubated at 28°C for 24 h and examined for appearance of characteristic red 'bull eye' colonies of *Y. enterocolitica* on CIN and colourless colonies on SSDC agar.

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**Enrichment Plating:** Ten ml of the same initial homogenate was transferred to 90 ml irgasan-ticarcillin-potassium chlorate (ITC) broth (ITC broth base supplemented with ticarcillin and KClO<sub>3</sub>, Hi-media) and incubated at 28°C for 2 days. After enrichment, 10 µl was streaked on to a SSDC agar plate. The remaining PSB homogenate was incubated at 28°C. After 2 days and 5 days, 10µl was streaked on CIN agar. In parallel to that, the enriched PSB culture was plated on CIN agar after alkali treatment for which 0.5 ml of enriched PSB culture was transferred to 4.5 ml of 0.5% KOH solution and mixed for 20 seconds. All agar plates were incubated at 28°C for 24 hours and examined for appearance of characteristic red ‘bull eye’ colonies of *Y. enterocolitica* on CIN agar plates.

**Identification of Isolates:** Standard strain was received from IMTECH and revived as per procedure recommended and biochemically identified. Standard strain as well as presumptive *Y. enterocolitica* isolates having characteristic red ‘bull eye’ colonies with light periphery on CIN and colourless colonies on SSDC agar plates were purified on tryptone soya agar (TSA, Hi-media) and examined initially for their morphology and Gram’s staining reaction followed by catalase, oxidase, indole, methyl red, Voges Proskauer, urease, Simmon’s citrate, Kligler’s Iron Agar (KIA), amino acid decarboxylation and sugar fermentation pattern according to Bergey’s Manual of Determinative Bacteriology (Holt *et al.*, 1994). Standard methods of biochemical examination were used as described by Cowan *et al.* (1974).

## RESULTS AND DISCUSSION

The cultural examination of 100 pork samples resulted in isolation of 18 (18%) *Y. enterocolitica* isolates (Table 1). The colonies of *Y. enterocolitica* on CIN agar appeared as red “bull eye” with light yellowish periphery and colourless colonies on SSDC agar. More number of samples from pig tonsils 10 (20%) were found positive as compared to 8 (16%) of ground pork. Of the 100 pork samples, 4 (4%) were positive by direct plating method, 19 (19%) and 17 (17%) by 2 and 5 days broth enrichment methods (Table 2). Isolation rate of *Y.*

*enterocolitica* from pork samples was higher with 2 days and 5 days broth enrichment than direct plating (Table 2). All the isolates (n=18) of *Y. enterocolitica* revealed similar biochemical characters (Table 3). In addition, four isolates initially suspected as *Yersinia* were confirmed as *Y. intermedia* (three) and other *Yersinia* spp. (one) on further biochemical characterization (Table 3).

*Y. enterocolitica*, an important food borne gastrointestinal agent is regarded as an emerging pathogen worldwide. In contrast to most other common bacterial enteropathogens, *Y. enterocolitica* is able to multiply at low temperatures of 4°C. This psychrophilic nature is responsible for the survival of the bacterium at refrigerated temperature in food. Pigs are regarded as primary reservoir of human pathogenic *Y. enterocolitica* and pork is estimated to constitute the major source of human cases. Pigs asymptotically carry the pathogen in their oral cavity and pharynx (mainly tonsil and tongue), lymph nodes and intestine. From these infected tissues, pathogens may contaminate the carcass during slaughter and dressing procedure (Nesbakken *et al.*, 2003).

The results of the present study revealed pig tonsils to be more contaminated (10 isolates; 20%) as compared to ground pork (eight isolates; 16%). Likewise, earlier investigators have reported higher rate of prevalence of *Y. enterocolitica* upto 42 - 62% and 20% in pig tonsils and tongue, respectively while low incidence of 1 - 25% was reported in ground pork (De Boer and Nouws, 1991; Fredriksson-Ahomaa *et al.*, 2009). Arora (2010) in a study also reported higher isolation of *Y. enterocolitica* in pig tonsil (34.74%) as compared to ground pork (4.35%). A number of studies in different parts of the world have reported varied prevalence of *Y. enterocolitica* in pork samples (Hudson *et al.*, 2008; Subha *et al.*, 2009; Fondrevez *et al.*, 2010; Divya and Varadaraj, 2011; Van Damme *et al.*, 2013; Paixao *et al.*, 2013; Anju *et al.*, 2014). Subha *et al.* (2009) examined 150 (75 pork and 75 milk) samples collected in and around Namakkal, Tamilnadu for presence of *Y. enterocolitica* and reported 29.3% of pork and 10.6% of milk samples as positive. Divya and Varadaraj (2011) reported incidence pattern of 1.7% for *Y. enterocolitica*

**Table 1**

**Isolation of *Y. enterocolitica* from pork by culture method**

Nature of sample examined (no.)	No. positive by culture technique (%)
Pig tonsil (50)	10 (20)
Ground pork (50)	08 (16)
Total (100)	18 (18)

**Table 2**

**Comparison of different isolation methods for the recovery of *Y. enterocolitica* in pork samples**

Isolation method	No. of positive pork samples (%)
Direct plating	04 (4%)
2 days enrichment	19 (19%)
5 days enrichment	17 (17%)

**Table 3**  
**Biochemical characterization of *Yersinia* spp. isolated from pork sample**

Biochemical parameters	<i>Yersinia</i> spp. identified			
	<i>Y. enterocolitica</i>	Std. strain of <i>Y. enterocolitica</i>	<i>Y. intermedia</i>	Other <i>Yersinia</i> spp.
Catalase	+	+	+	+
Oxidase	-	-	-	-
Indole	+	+	+	+
MR	+	+	+	+
VP	-	-	-	-
Citrate	-	-	-	-
Urease	+	+	+	+
KIA	A/A	A/A	A/A	A/A
Arginine	-	-	-	-
Lysine	-	-	-	-
Ornithine	+	+	+	+
Fructose	+	+	+	+
Mannose	+	+	+	-
Adonitol	-	-	-	-
Maltose	+	+	+	-
Mannitol	+	+	+	-
Sucrose	+	+	+	-
Raffinose	-	-	+	-
Rhamnose	-	-	+	-
Melibiose	-	-	+	-
Cellulobiose	+	+	+	+
Dulcitol	-	-	-	-
Inositol	-	-	-	-

MR=Methyl red; A/A=Alkaline slant/acidic butt; VP=Voges Prausker; KIA=Kligler's iron agar; +=positive; -=negative; Std=Standard

and 2.5% for *Y. intermedia* in an analysis of 120 traditional fast foods collected from local market of Mysore, Southern India. Anju *et al.* (2014) examined 210 pig samples collected in and around Trissur and Eranakulum, Kerala by multiplex PCR and found one sample positive for *Y. enterocolitica*.

The occurrence of *Y. intermedia* in this study is interesting as this organism has not been much reported earlier from pork in India. However, Atobla *et al.* (2012) reported its isolation from pig tonsils from Abidjan. Generally *Y. intermedia* are considered as non pathogenic as they lack classical virulence markers like *yst* and *ail* gene. Divya and Varadaraj (2011) reported isolation of *Y. intermedia* from fast foods in India and the *Y. intermedia* isolates have been found to give amplification product for phospholipase gene (*ypl*) which codes for phospholipase– A, known to be a virulence factor, which indicates about possible pathogenic potential of this species.

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