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Enterobacter spp. Lactose Negative Findings and Their Implications on Food Safety

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Abstract: This study provides *Enterobacter* spp. lactose negative strains characterization susceptible of being wrongly identified as *Salmonella* spp. having into account its meaning for what concerns on food safety. More than five hundred feed samples for farm animal feeding were microbiologically analyzed according the Spanish Normative for feed, in which different media are accepted for detecting *Salmonella* spp. after performing preenrichement steps. In this study SS agar was chosen for differential isolation. Results show that 55% of those colonies supposed to be *Salmonella* spp. belonged to the genus *Enterobacter* when performing confirmation by means of API20E method and TSI/LIA tests, indicating that several strains of the genus *Enterobacter* have lost their condition of lactose positive microorganisms and becoming lactose negative, inducing confusion when trying to identify *Salmonella* spp. by traditional and reference methods.

Key words: *Enterobacter* spp., lactose negative, food safety, coliform, *Salmonella* spp.

INTRODUCTION

Salmonella spp. strains detection from colonies growth on selective media, after performing suitable pre-enrichments, may be interfered by the growth of some other colonies that might be quite similar to them inducing, therefore, to a wrong identification resulting as false positives. In this case, samples and whole food entries may be rejected unnecessarily, due to the fact that current legislation does not allow presence of this genus.

The main aim of this study was to characterize bacterial genus susceptible of being wrongly identified as *Salmonella* spp. and to assess which broth selective medium allows recovering with a higher efficiency *Salmonella* strains, inhibiting those ones that may induce us to mistake (Pascual and Calderón, 2000; Cowan and Steel, 1993).

MATERIALS AND METHODS

Samples were collected from September of 2003 until January of 2005 from eight different farms (Table 1) in Catalonia (Spain).

Five hundred forty six feed samples of feed for different animal species, where microbiologically analyzed focusing on the following parameters: Total *Enterobacteriaceae,* coliforms and *Escherichia coli* detection and counts; *Staphylococcus aureus* detection and counts; total anaerobic bacteria and *Clostridium perfringens* detection and counts; fungi detection and counts and presence/absence detection of genus *Salmonella* strains.

Table 1: Distribution of the samples collected

Origin of samples	No. of samples
Farm 1	73
Farm 2	56
Farm 3	81
Farm 4	71
Farm 5	69
Farm 6	78
Farm 7	60
Farm 8	58
Total of samples	546

The applied methodology was performed according to the procedures described in some reference handbooks for this kind of products analysis (Pascual and Calderón, 2000).

With the aim of detecting *Salmonella* strains, the procedures were as briefly follows:

Non-selective pre-enrichment: (25 g sample plus 225 mL lactose broth). Incubation parameters set at 37°C during 18 h.

Selective enrichment: (10 mL pre-enrichment broth plus 100 mL selenite cystine broth). Incubation parameters set at 37°C during 24 h.

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Table 2: Identification of those strains considered as *Salmonella* spp.

Origin of samples	No. of samples	Samples presumably positive to *Salmonella* spp.	Stains identified as *Enterobacter* spp.	Strains identified as *Salmonella* spp.
Farm 1	73	14	9	5
Farm 2	56	8	2	6
Farm 3	81	18	11	7
Farm 4	71	19	10	9
Farm 5	69	12	5	7
Farm 6	78	16	9	7
Farm 7	60	9	5	4
Farm 8	58	11	7	4
Total	546	107	58	49

Differential isolation: On solid selective medium (Agar SS plates). Incubation parameters set at 37°C during 24 h.

A second part of this study was about performing all these analyses at the same time modifying the selective enrichment medium, accepted into alimentary analyses procedures as well. Selenite cystine broth was replaced by Rappaport Vassiliadis (RVS) broth. 0.1 mL of the pre-enriched culture were inoculated in 10 mL RVS. This time, incubation was at 42°C during 24 h.

In all cases, isolated strains in SS medium from the feed samples were identified using API20E micromethod and at the same time, tubes containing Three Sugar Iron Agar (TSI) and Lysine Descarboxylase Agar (LIA) were also seeded with each of the colonies grown (Cowan and Steel, 1993).

At the same time, strains that did not show capacity for fermenting lactose in any of the media used in this study, were also identified.

RESULTS AND DISCUSSION

After isolating those colonies that grew on SS Agar with a quite similar appearance with a *Salmonella typhimurium* positive control, by means of API20E micromethod, 55% were identified as strains belonging to different species of the genus *Enterobacter*. Combinating biochemical tests just like TSI and LIA, the same results were obtained, although no species level was reach.

By means of the analytic procedure in which selective pre-enrichment was performed with RVS, colonies identified from Agar SS plates were the same than with the previous procedure (Selective enrichment with selenite cystine broth). Its identification using both API20E method and TSI/LIA combination fit 100%.

Lactose negative strains isolated from McConkey Agar, were identified using TSI and LIA (apart from Gram staining and microscopic characteristics) and its results proved that those strains belonged to the genus *Enterobacter*, but were strains unable to produce lactose fermentation (De Boer, 1998; Finney *et al*., 2003).

According to the results obtained (Table 2) it may indicate that we can never state *a priori* that *Salmonella* spp. strains were found. The fact of having detected up to 55% of genus *Enterobacter* strains, among those likely to be *Salmonella,* is a clear indicator of the great mistake we would have made if the genus to which they belong were not reconfirmed.

It must be said that after growth on SS Agar of the same strains with independence of the used selective enrichment broth medium, we can assess that any of media tested produce total inhibition of the rest of the microbiota but *Salmonella* spp. Therefore, recommendation of confirming its identification become a highly important in both modifications of the procedure.

It is also remarkable to suggest that a revision of all the main concepts for alimentary safety is very necessary. Among these concepts, we emphasize coliforms and its implication as food fecal contamination indexes. This idea arises from the finding of several lactose negative strains that belong to *Enterobacter* genus (as other studies point), traditionally considered as positive lactose and that is why it is included in coliform group. A change in this lactose fermentative capacity in *Enterobacter* strains, means having lower counts of colonies belonging to this genus.

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