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A comparison of standard cultural methods for the detection of foodborne *Salmonella* species including three new chromogenic plating media

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Abstract

In this study the draft of the horizontal method for the detection of *Salmonella* species from human food and animal feed (ISO 6579:2002) was compared to the European gold standard (DIN EN 12824:1998), including the three new chromogenic plating media AES *Salmonella* Agar Plate (ASAP), Oxoid *Salmonella* Chromogen Media (OSCM) and Miller–Mallinson agar (MM). First the growth and appearance of 36 bacterial type strains (*Salmonella* and other 21 species) on ASAP, OSCM and MM were compared to those on the three traditional agars Brilliant Green Agar according to Edel and Kampelmacher (BGA), Xylose Lysine Deoxycholate Agar (XLD) and Xylose Lysine Tergitol 4 Agar (XLT4). Only on MM agar, did all of 36 tested type strains produce typical colonies, especially strains of *S.* Senftenberg, *Salmonella arizonae*, *S.* Dublin and *S.* Derby. Artificial inoculation experiments using raw pork ground meat ($n=92$) were subsequently conducted. A shortened incubation time of 24 h in RVS broth yielded a *Salmonella* species recovery of 100% from spiked meat samples. Finally, 286 naturally contaminated raw porcine and bovine minced meat samples and raw poultry meat samples were investigated. Forty-three strains from a total of 39 *Salmonella*-positive samples were found.

S. Typhimurium ($n=21$), with DT 104 L, DT 012 and RDNC being the most prevalent subtypes isolated. D-tartrate-positive *S.* Paratyphi B ($n=2$) and *S.* Saint-Paul ($n=3$) were also recovered. They were cultured from poultry meat and were multi-resistant against antibiotics including nalidixic acid.

Rappaport Vassiliadis broth with soypeptone (RVS) yielded the highest recovery of *Salmonella* spp. (97,4%) compared to Tetrathionate broth with Novobiocin according to Muller and Kauffman (MKTTn, 94,9%) and Selenite Cystine broth (SC, 38,5%). However, no significant difference was obtained by comparing the ISO 6579:2002 draft to the gold standard.

Keywords: *Salmonella*; ISO 6579; Chromogenic media; Ground meat

1. Introduction

Infections with non-typhoid *Salmonella* are one of the most commonly recorded cause of gastroenteritis in humans in the western industrial countries. The great majority of human cases of salmonellosis are due to the consumption of contaminated foods of animal origin.

Enormous efforts in the areas of human and animal disease control as well as food hygiene has resulted in a visible reduction of food borne salmonellosis worldwide. Nevertheless, *Salmonella* will still be a risk to human health in the future (Anonymous, 2005, 2006).

The gold standard for the detection of *Salmonella* from food relies on a nonselective preenrichment, followed by a selective enrichment step, isolation on selective agar media and a preliminary biochemical and serological confirmation. This conventional cultural method is very time consuming and expensive thus its protocol is always revised by the standardization committees. Selenite cystine (SC) broth has been replaced

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by Rappaport Vassiliadis broth with soypeptone (RVS) within the ISO as well as the AOAC protocol (Van der Zee, 2003).

The International Dairy Federation (IDF) refused the controversely discussed ISO 6579:2000 draft and referred to its own method ISO 6785:2001 IDF 93. This standard procedure for the detection of *Salmonella* from milk and milk products is similar to the protocol EN 12824:1998 (Becker et al., 2003).

The aim of our study was to compare the draft of the horizontal method for the detection of *Salmonella* species from human food and animal feed (ISO 6579:2002) with the European gold standard (DIN EN 12824:1998) on the basis of raw porcine, bovine and poultry meat samples (spiked $n=92$; naturally $n=286$). Three new chromogenic plating media have been studied, because most of the earlier studies investigated clinical stool samples. Therefore, chromogenic media had a higher specificity but a lower sensitivity (Rambach, 1990; Dusch and Altwegg, 1993; Gaillot et al., 1999; Perez et al., 2003).

2. Materials and methods

2.1. Bacterial type strains

As summarized in Table 1 a total of 36 bacterial type strains were selected to assess the growth and appearance on six different plating media. This included non-typhoid *Salmonella* strains ($n=27$), different Enterobacteriaceae ($n=8$) and *Pseudomonas aeruginosa* ($n=1$).

All type strains were cultured in Brain Heart Infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) under required conditions. The purity of the cultures was confirmed by streaking onto Plate count (PC) agar (Merck KGaA, Darmstadt, Germany). Subsequently the six different plating media: Brilliant Green Agar (BGA) according to Edel and Kampelmacher (Oxoid, Wesel, Germany); Xylose Lysine Deoxycholate (XLD) Agar (Sifin, Berlin, Germany); Xylose Lysine Tergitol 4 (XLT4) Agar (Merck KGaA, Darmstadt, Germany).

Miller–Mallinson (MM) Agar (Miller and Mallinson, 2000.); AES *Salmonella* Agar Plate (ASAP) Agar (AES Laboratoire, Combourg, France); and Oxoid *Salmonella* Chromogen Media (OSCM) Agar (Oxoid, Wesel, Germany) were inoculated by using standard multiple loop techniques.

2.2. Artificial inoculation of raw pork ground meat

Artificial spiking experiments were conducted using 1–10 colony-forming units ($n=25$), 11–50 CFU ($n=41$) and 51–200 CFU ($n=26$) concentrations of *S.* Typhimurium 164/93 BgVV in 25 g initial weight of raw ground pork meat samples ($n=92$). Prior to an oculation, all samples were initially confirmed as *Salmonella*-negative by gold standard. Artificial inoculation of other types of meats was beyond the scope of this study.

2.3. Naturally contaminated raw meat samples

A total of 286 meat samples comprising porcine and bovine minced meat ($n=206$) and poultry meat samples ($n=80$) including marinated poultry meat and poultry edible offal were examined for naturally occuring *Salmonella* contamination. All meat samples used in this study were bought at various local German grocery stores during the one-year trial period. Cultural enrichment investigation was performed as described below.

Table 1
Stock cultures ($n=36$) used

No.	Serotype or species	Identification
1	*Salmonella arizonae*	AES[a] 8.1.
2	*Salmonella* Bovismorbificans	IFTN[b] H 217
3	*Salmonella* Derby	BgVV[c] 1454/61
4	*Salmonella* Dublin	x-O 162/98[d]
5	*Salmonella* Dublin	x-O 163/98[d]
6	*Salmonella* Enteritidis	ATCC[e]13076
7	*Salmonella* Enteritidis	BgVV 164/93
8	*Salmonella* Enteritidis	IFTN W 28/8
9	*Salmonella* Give	IFTN W 37/8
10	*Salmonella* Goldcoast	IFTN H 9
11	*Salmonella* Hadar	IFTN W 30/1
12	*Salmonella* Infantis	IFTN H 21
13	*Salmonella* I-Rauhform	IFTN H 59
14	*Salmonella* Livingstone	IFTN H 19
15	*Salmonella* London	IFTN H 74
16	*Salmonella* Manhattan	IFTN W 33/4
17	*Salmonella* Newport	IFTN W 30/11
18	*Salmonella* Ohio	IFTN H 103
19	*Salmonella* Oranienburg	ATCC 3592
20	*Salmonella* Panama	IFTN H 100
21	*Salmonella* Paratyphi B	IFTN W 30/11
22	*Salmonella* Paratyphi B	IFTN W 35/11
23	*Salmonella* Senftenberg	DSM[f]10062
24	*Salmonella* Thompson	IFTN SV 4/7
25	*Salmonella* Typhimurium O:5	IFTN H 273
26	*Salmonella* Typhimurium	BgVV 2260/93
27	*Salmonella* Virchow	BgVV 174
28	*Citrobacter freundii*	AES 1.2.
29	*Citrobacter freundii*	IFTN M 50b
30	*E. coli*	ATCC 25922
31	*Enterobacter aerogenes*	BgVV 1799/89
32	*Hafnia alvei*	NCTC[g]8105
33	*Proteus mirabilis*	NCTC 11938
34	*Proteus morganii*	BgVV 696/84
35	*Shigella sonnei*	BgVV 7887/89
36	*Pseudomonas aeruginosa*	ATCC 15442

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[e] American Type Culture Collection (ATCC), Virginia, USA.
[f] Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), Braunschweig, Germany.
[g] National Collection of Type Cultures (NCTC), London, UK.

2.4. Preparation of culture media

Buffered peptone water (BPW) enrichment (Merck KGaA, Darmstadt, Germany), Selenite Cystine (SC) broth (Oxoid, Wesel, Germany), Rappaport Vassiliadis broth (RVS) with soypeptone (Merck KGaA, Darmstadt, Germany) and Tetrathionate broth (MKTTn) with Novobiocin according to Muller and Kauffman