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Demonstration of earlier detection of *Salmonella* species from stool samples by using chromogenic media.

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Introduction

Background: Salmonellosis is a worldwide public health issue and non-typhoid species are one of the most common causative agents of gastroenteritis in the western world.[1] Typhoidal and Paratyphoidal salmonellae cause systemic syndromes characterised by sustained bacteraemia.[2] Although the number of cases is under reported and therefore the incidence rates are underestimated,[3] worldwide up to 1.3 billion non-typhoidal and an estimated 20 million typhoidal cases of *Salmonella* infection are reported annually.[4,5]

Purpose of the Study: The isolation of salmonellae involves culturing faecal samples directly onto one or more selective culture media, with and without enrichment in Selenite broth. Xylose Lysine Deoxycholate (XLD) agar remains the UK standard selective medium and relies upon the absence of fermentation of lactose and sucrose with or without the production of hydrogen sulphide (H_2S) by salmonellae.[6] However, this medium is not reliable for some serotypes, is prone to a false positives and overgrowth with commensals e.g. *E. coli*.[7] In recent years, chromogenic media have been made available for the rapid detection of pathogens in the clinical laboratory.[8] The aim of the current study was to assess three selective media for the isolation and discrimination of *Salmonella* spp. in stool samples from patients with diarrhoea.

Materials and Methods

Two chromogenic agar media, *Brilliance* Salmonella (Oxoid, Basingstoke, UK) and ASAP medium (bioMérieux / AES, Marcy-l'Étoile, France), were compared with XLD (bioMérieux). The three pre-prepared media were evaluated with 280 stool samples from patients suffering from diarrhoea. Approximately 0.5g or 0.5 ml of sample was suspended in 0.5 ml of saline (0.85 %). The three media were inoculated with 50 µL aliquots of the stool suspension, the remainder of which was inoculated into selenite F broth (bioMérieux). All plates and broths underwent aerobic incubation at 37 ± 0.5 °C for 18 h. After incubation, selenite broths were subcultured onto the same three media, which were incubated in the same conditions for 18 h. All plates were interpreted after 18 h of incubation and any suspect colonies resembling *Salmonella* were identified by Maldi-TOF mass spectrometry (Bruker, Coventry, UK).

References

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Results and Conclusions

Salmonella spp. produced purple colonies on *Brilliance* Salmonella and ASAP medium, and black or colourless colonies on XLD. On both chromogenic media commensal bacteria were invariably non-coloured.

Table: The sensitivity and specificity of three culture media for the isolation of Salmonella spp.

Medium		Positives	True Positives	False positives	Sensitivity (%)	Specificity (%)
XLD	- Direct	15	9	6	31	98
	- enrichment	38	28	10	97	96
BRILLIANCE	- Direct	23	13	10	45	96
	- enrichment	39	29	10	100	96
ASAP	- Direct	20	14	6	48	98
	- enrichment	35	29	6	100	98

Twenty nine isolates of *Salmonella* were recovered using a combination of all three media and all of these were recovered on *Brilliance* Salmonella and ASAP medium, as opposed to 28 on XLD, following enrichment in selenite broth. Although there was little difference in *Salmonella* recovery after enrichment, there were differences in performance after direct culture. ASAP medium recovered significantly more isolates of *Salmonella* spp. than XLD medium (McNemar's test; $P = 0.04$) whereas ASAP isolated only one additional isolate when compared with *Brilliance* Salmonella. The least number of false positive colonies were recovered on ASAP medium (n = 12) compared with *Brilliance* Salmonella (n = 20) and XLD (n = 16). Data from this study suggests that the use of chromogenic agars can result in earlier detection of *Salmonella* species from some samples, although enrichment in selenite broth remains essential.

Figure: *Salmonella* sp. as black colonies on XLD (a) and purple colonies on *Brilliance* Salmonella (b) and ASAP medium (c) isolated from direct culture.