ABC chromogenic agar: a cost-effective alternative to standard enteric media for *Salmonella* spp. isolation from routine stool samples

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ABSTRACT

Salmonellosis is the second most common cause of bacterial gastroenteritis, yet the yield from routine stool culture is low. Commonly used selective enteric media have poor specificities for salmonella identification, resulting in a high laboratory workload. A special chromogenic medium, ABC agar, is a promising alternative but its cost-effectiveness has not been evaluated in diagnostic laboratories. A collaborative study is therefore undertaken in two district general hospitals laboratories. Routine stool samples (n=866) were subcultured onto ABC agar half plates after selective enrichment in selenite broth. Similarly, 246 and 620 stool samples were subcultured onto desoxycholate lactose sucrose (DCLS) and xylose lactose desoxycholate (XLD) whole agar plates, respectively. *Salmonella* spp. were isolated from only 14 (1.6%) of stool samples tested. Specificity was significantly higher for ABC (98%) than DCLS (67%) or XLD (78%) agars. Welcan workload units (ABC 4.8, XLD and DCLS both 7.3) and costs per specimen (ABC £1.26, DCLS £3.81 and XLD £1.83) were similarly lower with ABC agar. The results indicate that ABC chromogenic agar offers improvements in specificity, workload and costs over conventional enteric media for *Salmonella* spp. isolation.

KEY WORDS: Agar.
Chromogenic compounds.
Salmonella.

Introduction

Salmonellosis is the second most common cause of bacterial gastroenteritis in England and Wales, although the annual number of reported isolates continues to decline.[1] The diagnostic yield from routine stool culture is therefore relatively low. In a recent large community study performed by the Public Health Laboratory Service (PHLS), only 146 salmonella (5%) were isolated from 2893 stool samples examined.[2]

Guidelines produced by the Association of Clinical Pathologists (ACP) in 1990 for the examination of faecal bacterial pathogens relied on traditional culture media, such as desoxycholate-citrate agar (DCA) and xylosine-lysine-desoxycholate (XLD) agar, for the isolation of salmonella.[3] Although the ACP has not updated its guidance, similar methods were recommended by the PHLS in 1998.[4] These conventional media rely on simple biochemical tests (e.g., production of hydrogen sulphide, non-fermentation of lactose, sucrose or xylose) to distinguish salmonella from other members of the Enterobacteriacae. However, as these biochemical features are not specific for salmonella, currently recommended selective media can result in a high laboratory workload.

In order to improve specificity, there has been considerable interest in the use of chromogenic media for the selective isolation of salmonella from routine stool samples. A small study comparing CHROMagar salmonella (CAS) medium with Hektoen enteric agar demonstrated improved specificity (88.9% versus 78.5%, respectively).[5] A further study with CAS, XLD and salmonella-shigella (SS) agars produced similar results (CAS specificity 83% versus 55% for XLD and SS).[6] A very recent study of four commercial chromogenic agars (CAS, COMPASS Salmonella agar, SM ID agar and ABC medium) again demonstrated improved specificity when compared with Hektoen agar.[7]

In 1999, Perry *et al*. described a special chromogenic medium, ABC (alpha-beta chromogenic) agar, which incorporates two substrates (3, 4-cyclohexenoesculetin-β-D-galactoside and 5-bromo-4-chloro-3 indolyl-α-D-galactopyranoside) to facilitate the selective isolation of salmonella. ABC agar exploits the fact that salmonella can be distinguished from other members of the Enterobacteriacae by the detection of α-galactosidase activity in the absence of β-galactosidase activity, resulting in green colonies. Enterobacteriacae typically produce black colonies (e.g., *Escherichia coli*) or colourless colonies (e.g., *Proteus* spp.) (Figure 1). ABC's improved specificity has been confirmed by comparison with DCA (90.5% versus 26.9%) and Hektoen (99.1% versus 85.6%) agars[7].

However, diagnostic laboratories in district general hospitals (DGHs) may be wary about introducing a chromogenic agar for routine salmonella isolation because of the cost implications. Therefore, this study evaluates the cost-effectiveness of ABC agar versus some conventional selective media in two DGHs. Furthermore, to assist the evaluation, a retrospective review of salmonella isolation rates from routine stool culture is undertaken.

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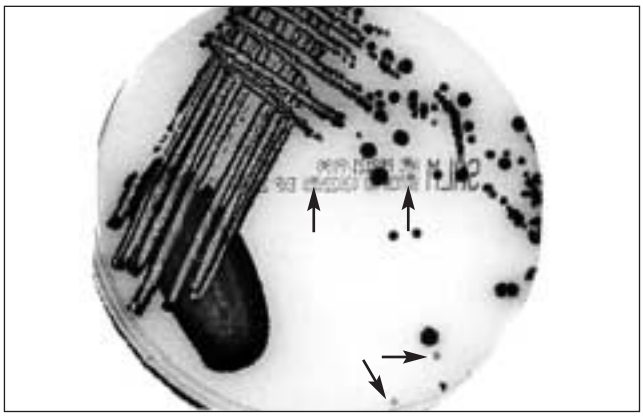

Fig 1. Mixed culture of *Salmonella enteritidis* (arrowed, appear green) with *Escherichia coli* (black colonies) on ABC agar.

Materials and methods

The evaluation involved a collaborative study between the microbiology departments of two typically-sized DGHs (hospitals A and B), serving a combined population of approximately 500,000. The objectives of the study were to assess the sensitivity, specificity, costs and workload associated with processing routine stool samples using ABC agar (LabM, Bury, UK), in comparison with the commonly used selective desoxycholate-lactose-sucrose (DCLS) and XLD agars (Oxoid Ltd., UK) for salmonella isolation.

Costs per specimen was determined for each agar, based on consumable prices and staff time taken to follow-up suspect colonies from the various media. Workload was assessed by calculating Welcan units for individual agars (reading of plates and supplementary tests). Statistical analysis was by χ^2 test.

A retrospective review of salmonella isolation rates was undertaken by analysing archived data stored in each department's computer system between 1995 and 2001. Data from another local DGH (Conquest Hospital, Hastings) was also included in this review.

Prior to the commencement of the evaluation, staff at hospital A had already become familiar with the colonial appearance of salmonella on ABC agar during a six-month pilot study. In this period, whole ABC plates were not found to be necessary as the isolation of suspect salmonella colonies on half plates proved satisfactory with chromogenic agar.

Laboratory protocols

Over a three-month study period in 2001, all routine stool samples were cultured on ABC agar and either DCLS or XLD agars, depending on local methods. A pea-sized sample of stool was placed in 10 mL selenite F broth and incubated for 18–24 h at 37°C aerobically. Subsequently, a sterile swab was used to inoculate DCLS or XLD whole plates and ABC half plates, which were incubated for 18–24 h at 37°C aerobically. After incubation, all plates were examined for characteristic colonies and each colony type was subjected to primary identification procedures.

Hospital A inoculated a urea broth (E & O Laboratories), which was incubated aerobically for 4 h at 37°C. Any urea-negative isolates were then inoculated onto a triple sugar iron (TSI) agar slant and incubated for 18–24 hours at 37°C aerobically. If the organism gave a characteristic TSI reaction for salmonella (TSI red slant, yellow butt, H_2S positive and gas production) then further identification was undertaken using the API 20E system (bioMérieux, France).

Hospital B also inoculated suspect colony types into a urea broth (Oxoid), which was incubated aerobically for 4 h at 37°C. Urea-negative isolates were inoculated into a sulphide/indole/motility (SIM) tube (Oxoid) and onto a TSI agar slant, which were incubated for 18–24 hours at 37°C aerobically. Any isolate that gave characteristic reactions for salmonella (indole-negative, motile-positive, TSI reactions as noted above) was further identified using the API 20E system and underwent standard O and H agglutination tests. All presumptive salmonella isolates were sent to the Division of Enteric Pathogens, Central PHLS, London, for confirmation.

In summary, a total of 866 stool samples were examined. All were subcultured onto ABC agar; 246 samples were subcultured onto DCLS by hospital A only; and 620 samples were plated onto XLD by both hospitals A and B.

Results

Salmonella were isolated from 14 (1.6%) stool samples and all were confirmed by the PHLS. An analysis of the sensitivity and specificity of ABC compared to the combined use of XLD and DCLS agars is shown in Tables 1 and 2. In hospital A, one *Salmonella* sp. strain was detected by ABC agar only. All other salmonella were identified by ABC, DCLS and XLD agars, the individual sensitivities being 100%, 67% and 100%, respectively.

Comparative specificities for ABC agar were different at the two hospital sites (hospital A 100%, hospital B 97% [overall 98%]). This was probably due to hospital A's prior experience with this medium and staff familiarity with the typical green colour of suspect salmonella colonies.

All suspect colonies on ABC agar were correctly identified as *Salmonella* spp. by hospital A. However, in the first six weeks of the study, hospital B followed up 12 suspect colonies (from 183 stool samples) which were subsequently identified as *Proteus* spp. Although *Proteus* spp. should produce colourless colonies on ABC agar, these were probably misinterpreted because of the lack of prior experience with this medium. In the second phase of the study, when hospital B examined a further 244 stools, only two further suspect colonies from ABC agar were identified as *Proteus* spp.

The specificity for the combined use of XLD and DCLS agars proved inferior to ABC, and were 73% and 77% for hospitals A and B, respectively (Table 2). Overall, individual specificities for ABC, XLD/DCLS and combined ABC/DCLS agars were 78%, 67% and 75%, respectively.

The difference in Welcan units between XLD/DCLS and ABC media was 2197 (Table 3). The cost per stool sample using ABC half plates and whole plates of XLD and DCLS media was £1.26, £1.88 and £3.81, respectively, reflecting the extra work entailed in false-negative follow-up required by the last two media.

The results of a retrospective review of rates of salmonellosis from the three DGHs are presented in Table 4. During the period 1995 to 2001, the rate of salmonella isolation varied between 3.3% and 1%. However, this difference was not statistically significant ($P>0.05$).

Table 1. Sensitivity for ABC medium compared with combined XLD/DCLS for salmonella isolation after enrichment.

	Hospital A			Hospital B		
Medium	Colonies with true-positive results	Colonies with false-negative results	Sensitivity (%)	Colonies with true-positive results	Colonies with false-negative results	Sensitivity(%)
ABC	7	0	100	7	0	100
XLD/DCLS	6	1	86	7	0	100

Table 2. Specificity for ABC medium compared with combined XLD/DCLS for salmonella isolation after enrichment.

	Hospital A			Hospital B		
Medium	Colonies with true-negative results	Colonies with false-positive results	Specificity (%)	Colonies with true-negative results	Colonies with false-positive results	Specificity (%)
ABC	422	0	100	413	14	97
XLD/DCLS	307	115	73	331	99	77

Table 3. Summary of workload for XLD/DCLS and ABC media using Welcan units for salmonella isolation

	Workload (Welcan units)		
	Supplementary tests required	Reading of plates	Totals
XLD/DCLS	4452	1892	6344
ABC	2861	1286	4147
Differences	1591	606	2197

Table 4. Numbers of salmonella identified from routine stool samples in three district general hospitals, 1995–2001.

Year	Total stool samples processed	Number of salmonella-positive stool samples (%)
1995	11,364	314 (2.8)
1996	13,327	392 (2.9)
1997	13,672	456 (3.3)
1998	14,753	286 (1.9)
1999	14,570	242 (1.7)
2000	10,777	112 (1.0)
2001	10,441	157 (1.5)
Totals	88,904	1959 (2.2)

Discussion

This study demonstrated the high sensitivity and superior specificity of ABC agar over XLD and DCLS agars for the detection of salmonella from routine stool samples, following selenite enrichment. The high specificity of ABC agar has also been demonstrated in previous studies.[7-10] However, arguments against the routine use of ABC agar in diagnostic laboratories have pointed to the occasional *Salmonella* sp. that lack α-galactosidase activity, the sensitivity following direct plating, and high costs.

In an initial evaluation of ABC agar, Perry *et al*. detected three atypical strains of salmonella from 1032 stock salmonella cultures: two strains produced colourless colonies, due to lack of α-galactosidase (*S. braenderup* and *S. saint paul*), and one strain produced a black colony due to β-galactosidase production (*S. arizonae*).[8] However, none of these species are commonly associated with human salmonellosis.

In a more recent study, in which 63 salmonella were isolated from 1000 routine stool samples, nine isolates lacked α-galactosidase (six *S. typhimurium* and an *S. enteritidis*, *S. virchow* and salmonella group B).[9] However, the problem of α-galactosidase-negative salmonella was not noted in the present study nor in other similar ones,[7,10] and may reflect geographical differences.

Concern has also been expressed about the poor specificity of ABC agar for salmonella identification following direct plating culture.[10] However, selective enrichment broth is considered the 'gold standard'[11,12] and a delay in detecting salmonella by 24 h is unlikely to affect clinical management in most cases. It has therefore been argued that direct plating for salmonella need only be performed in an outbreak situation.[10]

The overall diagnostic yield of salmonella from routine stool culture in the three DGHs studied was lower than the recent national PHLS study.[2] However, the overall incidence (2.2%) is comparable with similar local evaluations in the UK and Australia.[10] The incidence of nosocomial salmonellosis will be even lower than that found in the community. Consequently, as faecal bacteriology is both labour-intensive and expensive, it is recommended that patients who develop diarrhoea more than three days after admission should not be routinely cultured for salmonella.[13]

This approach places considerable reliance on clinicians to advise the diagnostic laboratory accordingly and could result in the failure to detect nosocomial salmonella outbreaks.[14,15] Hence, if surveillance for hospital-acquired salmonellosis is to continue, an enteric medium of proven high specificity and cost-effectiveness must be chosen. This study demonstrated that ABC agar can significantly reduce laboratory workload and is cheaper than either DCLS or XLD media.

As a result of the high specificity of ABC agar, laboratories can rely on half-plate cultures, provided that staff have had suitable training in the use of this medium. This was highlighted during the present study by hospital B's experience with ABC agar, where the number of false-positive suspect colonies fell substantially after the first six weeks of the evaluation.

Highly selective media exist for the isolation of *Campylobacter* spp. and *E. coli* O157, and similar developments are seen in the identification of uropathogens,[16] *Staphylococcus aureus*[17] and *Candida* spp.[18] However, laboratories continue to rely on traditional culture media with poor specificity for the isolation of *Salmonella* spp. and *Shigella* spp. Development of ABC medium has opened a window of opportunity for diagnostic laboratories to reduce their enteric workloads, allowing the more effective use of staff time.

As there are a number of different manufacturers of chromogenic media for salmonella isolation, further comparative, prospective study is indicated. Isolation rates for *Shigella* spp. are even lower than those for salmonella from routine stool culture (approximately 0.1% of all stool samples in both laboratories); hence, a future aim for manufacturers of chromogenic agar must be to devise a suitable selective medium for combined shigella and salmonella isolation. □

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