Use of Cephalexin-Aztreonam-Arabinose Agar for Selective Isolation of *Enterococcus faecium*

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Cephalexin-aztreonam-arabinose agar (CAA), a new selective agar, was examined in comparison with nalidixic acid-colistin agar for the differentiation of *Enterococcus faecium* from other enterococci and the ability to isolate the organism from feces. Two hundred sixteen enterococcus isolates and a variety of gram-positive and gram-negative control strains were inoculated onto both media. All control strains of *E. faecium* were easily differentiated from *Enterococcus faecalis* and *Enterococcus durans* on the basis of arabinose fermentation on CAA. Differentiation of *E. faecium* from other enterococci or *Streptococcus bovis* was not possible on nalidixic acid-colistin agar. Increased isolation of *E. faecium* was demonstrated on CAA when both media were compared for the isolation of the organism from feces. CAA has been shown to possess excellent differential and selective features allowing the simple and effective isolation of *E. faecium* from heavily contaminated sites.

During the past decade enterococci have emerged as important nosocomial pathogens (4, 9). In recent years an increasing number of reports have implicated *Enterococcus faecium* as a cause of epidemiological problems (1, 6). The emerging importance of this organism is due to its inherent resistance to commonly used antimicrobial agents (5) and its ability to spread in nosocomial outbreaks throughout the hospital environment. Of major concern is the increasing number of reports of *E. faecium* strains resistant to the glycopeptide vancomycin, previously regarded as the mainstay of treatment of serious infections with this organism (2). It is of vital importance that diagnostic laboratories monitor the presence of this important nosocomial pathogen, particularly when vancomycin-resistant strains are encountered.

While enterococci are easily cultivated on commonly used laboratory media, the isolation of *E. faecium* from mixed bacterial populations can be problematic. This is particularly true when *E. faecium* is mixed with other enterococci, notably *Enterococcus faecalis*, since the colonial appearances of these related species are similar. This problem is intensified during epidemiological surveillance when heavily contaminated specimens such as feces may need to be screened (9). Because of this, several authors have employed media containing antibiotics to reduce the bacterial load and select for the relatively antibiotic-resistant enterococci (9, 10). We describe below a novel medium which allows for both the selective isolation and presumptive identification of *E. faecium*. We investigated the performance of the medium when challenged with a battery of control strains and the ability of the medium to recover *E. faecium* from feces, a site examined for colonization during nosocomial outbreaks (9).

MATERIALS AND METHODS

Two hundred sixteen isolates of enterococci previously identified by the method of Facklam and Collins (3), comprising one hundred twenty strains of *E. faecalis*, eighty-two strains of *E. faecium*, five strains of *Enterococcus avium*, four strains of

Enterococcus durans, three strains of *Enterococcus gallinarum*, and two strains of *Enterococcus raffinosus*, were used.

Control strains of enterococci used were *E. faecium* NCTC 7171, *E. faecalis* NCTC 755, *E. durans* NCTC 8307, *E. gallinarum* NCTC 11428, *E. raffinosus* NCTC 12192, *Enterococcus casseliflavus* NCTC 12341, and *Enterococcus mundtii* NCTC 12343. Other control strains used were *Streptococcus bovis* NCTC 8177, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 10662, *Staphylococcus aureus* NCTC 6571, and *Staphylococcus epidermidis* NCTC 11047.

Cephalexin-aztreonam-arabinose agar (CAA) was prepared by adding 40 g of Columbia agar base (Unipath, Basingstoke, United Kingdom), 10 g of arabinose (Sigma Chemical Co., Poole, United Kingdom), and 3.6 ml of phenol red (2%; BDH, Lutterworth, United Kingdom) to 1 liter of deionized water. The medium was mixed, and the pH was adjusted to 7.8. The agar was autoclaved at 114°C for 20 min. Fresh sterile solutions of aztreonam (Bristol-Myers Squibb, Hounslow, United Kingdom) and cephalexin (Sigma) were added to give final concentrations of 75 and 50 mg/liter, respectively.

Plates were stored for up to 3 weeks at 4°C. Each batch of medium was tested with the control strains of *E. faecium*, *E. faecalis*, *E. coli*, *P. aeruginosa*, and *S. aureus* listed above.

Nalidixic acid-colistin (NC) agar was prepared by the addition of 50 mg of nalidixic acid (Sanofi Winthrop, Guilford, United Kingdom) per liter and 10 mg of colistin sulfomethate sodium (Pharmax, Bexley, United Kingdom) per liter to 1 liter of molten Columbia agar base containing 5% horse blood (Gibco, Paisley, United Kingdom) cooled to 50°C. Plates were tested with the control organisms listed above.

Isolates were applied to the surface of CAA, NC agar, and Columbia blood agar plates containing 5% horse blood at an inoculum of 10^5 organisms per ml with a Denley multipoint instrument. The plates were incubated at 37°C in air for 24 h and examined for growth and fermentation of arabinose. A change in the color of the medium surrounding the colony, from red to yellow, indicated arabinose fermentation.

A total of 84 stool samples submitted from all patients in the Nephrology Unit of Freeman Hospital, collected over a period of 2 weeks, were examined for the presence of *E. faecium*. A small portion of the sample was inoculated onto NC agar and CAA. All plates were incubated aerobically at 37°C and

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TABLE 1. Growth on NC agar and fermentation of arabinose
on CAA by 216 enterococcus test strains[a]

Organism	No. of strains	Growth on:		Arabinose fermentation
		NC agar	CAA	
E. faecalis	120	+	+	−
E. faecium	82	+	+	+
E. avium	5	+	−	NA
E. durans	4	+	+	−
E. gallinarum	3	+	±	±
E. raffinosus	2	+	+	+

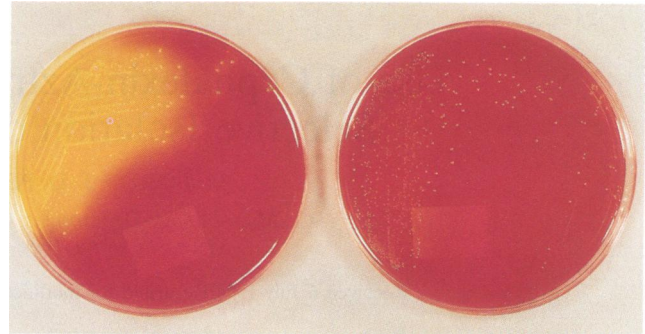[a] +, growth; ±, poor growth; −, no growth; NA, not applicable.

FIG. 1. Growth of *E. faecium* colonies (left) showing arabinose fermentation and *E. faecalis* colonies (right) showing no fermentation. Plates are shown following growth for 24 h at 37°C.

examined for the presence of *E. faecium* following 24 h of incubation.

All arabinose-fermenting colonies isolated on CAA and all suspect enterococci isolated on NC were subcultured and identified by the method of Facklam and Collins (3).

RESULTS

Table 1 compares the growth of 216 test strains of enterococci on NC agar and CAA and shows their ability to ferment arabinose on CAA. All 82 *E. faecium* strains grew on both media. Fermentation of arabinose was demonstrated by all *E. faecium* strains on CAA. All *E. faecalis* and *E. durans* strains also grew on both media, but arabinose fermentation on CAA was absent. Of the other enterococci, *E. raffinosus* and *E. mundtii* grew on both media and fermented arabinose on CAA. The control strains of *E. casseliflavus* and *E. gallinarum* grew poorly on CAA and appeared to only weakly ferment the carbohydrate. Five *E. avium* strains failed to grow on CAA. On NC agar all enterococcal strains grew well. All enterococcal control strains behaved as per test isolates.

All gram-negative control strains failed to grow on either medium. In the gram-positive control strains, some differences were observed. On CAA no growth was observed with the control strains of *S. aureus* and *S. epidermidis* because of cephalexin sensitivity. On NC agar these strains grew poorly because of partial inhibition by the nalidixic acid. The main difference between the two media was the lack of growth shown by the control strain of *S. bovis*. This organism fails to grow on CAA because of its sensitivity to cephalexin.

E. faecium was isolated from 84 stool samples inoculated onto CAA. Twelve samples produced no growth. All *E. faecium* strains were arabinose fermenting and were white. In contrast, *E. faecalis* appeared as clear colonies because of a lack of arabinose fermentation (Fig. 1). Of the 34 *E. faecium* isolates, 24 were isolated in mixed growth with non-arabinose-fermenting organisms and the remaining 10 were isolated in pure growth. Occasional plates showed the growth of small numbers of staphylococci. No staphylococci exhibited arabinose fermentation.

A total of 106 typically grey enterococcal type colonies were removed from 80 NC agar plates on which growth occurred. *E. faecium* was isolated from 26 stool samples. The remaining colonies were identified as *E. faecalis* (*n* = 64), *S. bovis* (*n* = 14), and *E. durans* (*n* = 2). No *E. faecium* that was not detected on CAA was isolated on NC agar. In addition, a number of plates grew staphylococci and lactobacilli which were not observed on CAA presumably because of cephalexin sensitivity.

DISCUSSION

A new selective agar, CAA, was compared with blood agar supplemented with nalidixic acid and colistin for the differentiation of *E. faecium* from other enterococci and selectivity in isolating the organism from feces. Although both media were comparable in their selective abilities, an important distinction was the lack of growth of *S. bovis* on CAA. This organism has been found to occur in 23.8% of stool samples (7).

Using CAA, we observed that *E. faecium*, *E. raffinosus*, *E. mundtii*, *E. gallinarum*, and *E. casseliflavus* were able to ferment arabinose and produce yellow colonies, although the last two organisms demonstrated poor growth. Excluding *E. faecium*, the other arabinose-fermenting enterococci have been isolated infrequently from clinical sites (8).

We have shown that *E. faecium* was isolated from an additional eight feces samples with CAA, and this superior isolation rate is largely due to the differential characteristics of the medium which distinguishes *E. faecium* from *E. faecalis*. Moreover, the failure to recover *E. faecium* from feces samples plated onto NC agar was due in most cases to the presence of only small amounts of *E. faecium* in association with predominant amounts of *E. faecalis* or *S. bovis*.

In addition to the increased isolation rate of *E. faecium*, a major advantage of CAA proved to be a substantial reduction in the workload required for screening the feces samples. Numerous suspect colonies required removal from NC agar to screen for *E. faecium*, resulting in the requirement for large amounts of media for biochemical and/or susceptibility testing. In contrast, when reading CAA plates, the absence of any arabinose-fermenting colonies excludes the presence of *E. faecium*. Following the isolation of arabinose-fermenting colonies, identification of *E. faecium* can be confirmed by simple tests (3). In this study all yellow colonies isolated on CAA from feces samples were confirmed as *E. faecium*.

We believe that this is the first report of the use of a differential agar specifically designed for *E. faecium*. While other enterococci may be involved in nosocomial infection, the combination of differential and selective features enables simple and effective screening of both feces and environmental samples for the presence of this important nosocomial pathogen when its involvement has been established. The use of this medium also results in a significant reduction in time and resources when compared with conventional approaches for the isolation of *E. faecium* from feces, a site often examined for colonization of *E. faecium* during nosocomial outbreaks (9).

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