Use of Cefoperazone MacConkey Agar for Selective Isolation of *Laribacter hongkongensis*

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A new selective medium, cefoperazone MacConkey agar (CMA), was developed for primary isolation of *Laribacter hongkongensis* from stool. Its performance in quantitative recovery and in a clinical evaluation of 4,741 human diarrheal stool specimens was superior to that of charcoal cefoperazone deoxycholate agar. In addition, with CMA, *Arcobacter butzleri* was unexpectedly isolated from the stools of six patients.

Based on the cefoperazone susceptibilities of *L. hongkongensis*, in this study we developed a new agar medium, cefoperazone MacConkey agar (CMA), which was modified from MacConkey agar by adding 32 µg of cefoperazone/ml. We compared five different isolation media—MacConkey agar, CMA, CMA-8 (MacConkey agar with 8 µg of cefoperazone/ml), CCDA, and CCDA-8 (CCDA with 8 µg of cefoperazone/ml)—on their ability to recover *L. hongkongensis* and suppress the enteric bacteria. An evaluation that compared the efficacies of CMA and CCDA on clinical specimens was also carried out.

Seven clinical isolates of *L. hongkongensis* were used for cefoperazone susceptibility tests and quantitative assessment of various media (16; Woo et al., submitted). Four standard strains of aerobic enteric bacteria were also used for evaluating the suppressive abilities of the media (Table 1). The MICs of cefoperazone were determined by the macrodilution broth method according to NCCLS guidelines (12). All agar media were prepared according to the manufacturer’s (Oxoid Ltd., Basingstoke, United Kingdom) instructions, with modifications of cefoperazone (Sigma, St. Louis, Mo.) concentrations.

MacConkey agar, CMA, CMA-8, CCDA, and CCDA-8 were quantitatively tested for their ability to support the growth of *L. hongkongensis* and inhibit the growth of four standard enteric bacteria. Working suspensions were prepared by emulsifying cultures of each isolate in 0.01 M phosphate-buffered saline and adjusted visually to a 0.5 McFarland standard. Six 10-fold dilutions were made, and 100 µl of the last four dilutions was inoculated evenly onto each medium in triplicate. All plates were incubated at 37°C in ambient air, with an additional set of CCDA at 42°C under a microaerophilic environment. The log$_{10}$ mean colony counts were determined at both 24 and 48 h.

Based on the results of quantitative assessment, CMA was compared to CCDA on 4,741 consecutively freshly collected stool specimens from patients with community-acquired diarrhea in four regional hospitals in Hong Kong during a 6-month period (July-December 2002). All specimens were directly cultured onto both agars. The CMA plates were incubated at 37°C in ambient air, and the CCDA plates were incubated at 42°C under microaerophilic incubation, a standard procedure for *Campylobacter* (6). All plates were examined for the presence of *L. hongkongensis* at 24 and 48 h, with all suspected isolates identified by phenotypic tests and 16S rRNA gene sequencing (11, 15, 16; Woo et al., submitted).

The MICs of cefoperazone on all seven isolates of *L. hongkongensis* were higher than 256 µg/ml. With the exception of CCDA under microaerophilic incubation, where colonies at 24 h were too small to be recognized, colony counts for each isolate on each medium at 24 and 48 h were the same. The log$_{10}$ mean colony counts of each isolate on each medium at 48 h are shown in Table 1. Apart from that of HKU1, which did not grow on CCDA or CCDA-8, the colony counts of the *L. hongkongensis* strains on MacConkey agar, CMA, CMA-8, CCDA, and CCDA-8 incubated in ambient air were comparable to those obtained with the blood agar control. However, counts on CCDA under microaerophilic incubation were significantly reduced ($P < 0.01$). Moreover, colonies on CCDA
under microaerophilic incubation at 48 h were still of pinpoint size and were difficult to pick up. The four standard aerobic enteric bacterial strains failed to grow on CMA, CMA-8, CCDA, or CCDA-8.

The isolation rate of \textit{L. hongkongensis} from human diarrheal stool specimens was higher on CMA incubated in ambient air than on CCDA under microaerophilic incubation. From the total of 4,741 specimens, \textit{L. hongkongensis} was isolated in pure culture on CMA from 22 specimens from 22 patients but on CCDA it was isolated from only 7 specimens from 7 of the 22 patients ($P < 0.05$). However, the colonies on CCDA were very small and difficult to recognize, in contrast to the large, lactose-negative colonies on CMA. In addition, six strains of \textit{L. hongkongensis}-like organisms subsequently identified as \textit{Arcobacter butzleri} by 16S rRNA gene sequencing were unexpectedly present in specimens from six patients and were isolated in pure culture on CMA but not on CCDA. These strains shared similar biochemical characteristics with \textit{L. hongkongensis}, except that they were negative for urease and arginine dihydrolase. All 22 strains of \textit{L. hongkongensis} and 6 strains of \textit{A. butzleri} were isolated sporadically from the specimens of 28 unrelated patients from among the 4,741 clinical specimens.

The historical failure to recognize \textit{L. hongkongensis} from human stools is likely due to a combination of misidentification and the lack of an optimal selective medium. All six initial \textit{L. hongkongensis} strains from stools were recovered on CCDA under microaerophilic incubation. They were at first mistaken for \textit{Campylobacter} species but were all found to grow in an aerobic environment after aerotolerance testing. In most clinical laboratories, these strains would have been discarded as nonpathogens or wrongly reported as \textit{Campylobacter}.

CMA was developed because \textit{L. hongkongensis} strains were highly resistant to cefoperazone and grew well on MacConkey agar as large lactose-negative colonies. CMA and CMA-8 were superior in supporting the growth of all seven \textit{L. hongkongensis} strains. To suppress more cefoperazone-resistant enteric flora that may be encountered in clinical stool specimens, CMA would probably be more advantageous than CMA-8. A clinical evaluation also showed that CMA incubated in ambient air was superior to CCDA under microaerophilic incubation in terms of both isolation rate and colony size. All 22 isolates of \textit{L. hongkongensis} were recovered on pure culture on CMA, suggesting that CMA also effectively inhibits normal gut flora.

The unexpected isolation of \textit{A. butzleri} on CMA suggests that the medium may also be potentially useful in isolating \textit{Arcobacter}, which has also been associated with diarrhea and invasive disease by entry through the gut (9, 11, 14, 15). Owing to the lack of a specific selective medium for isolation of \textit{Arcobacter} in fecal specimens, its role as a diarrheal pathogen remains undetermined (5, 7). CMA may serve as a selective medium for both \textit{L. hongkongensis} and \textit{Arcobacter} and assist in defining their causative roles in infectious diarrhea.

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\textbf{REFERENCES}


\begin{table}
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\begin{tabular}{|l|c|c|c|c|c|c|c|}
\hline
Bacterial strain & Mean log_{10} colony count at 48 h \\
\hline
& Blood agar & MacConkey agar & CMA & CMA-8 & CCDA & CCDA-8 & CCDA (microaerophilic incubation) \\
\hline
\textit{L. hongkongensis} strain 1 (HKU1) & 7.97 & 7.95 & 7.94 & 7.95 & No colony & No colony & No colony \\
\textit{L. hongkongensis} strain 2 (HLHK2) & 7.91 & 7.90 & 7.91 & 7.89 & 7.88 & 7.90 & 5.85 \\
\textit{L. hongkongensis} strain 3 (HLHK3) & 7.99 & 7.98 & 8.00 & 7.97 & 7.97 & 7.97 & 5.77 \\
\textit{L. hongkongensis} strain 4 (HLHK4) & 8.02 & 8.03 & 8.00 & 7.99 & 7.99 & 7.96 & 5.84 \\
\textit{L. hongkongensis} strain 5 (HLHK5) & 7.98 & 8.00 & 7.97 & 7.99 & 7.96 & 7.97 & 5.86 \\
\textit{L. hongkongensis} strain 6 (HLHK6) & 8.05 & 8.04 & 8.03 & 8.03 & 8.00 & 8.01 & 5.90 \\
\textit{L. hongkongensis} strain 7 (HLHK7) & 7.95 & 7.96 & 7.97 & 7.95 & 7.98 & 7.96 & 5.81 \\
\textit{Enterococcus faecalis} ATCC 29212 & 8.03 & 8.01 & No colony & No colony & No colony & No colony & No colony \\
\textit{Escherichia coli} ATCC 25922 & 7.96 & 7.99 & No colony & No colony & No colony & No colony & No colony \\
\textit{Klebsiella pneumoniae} ATCC 13883 & 7.94 & 7.97 & No colony & No colony & No colony & No colony & No colony \\
\textit{Proteus mirabilis} ATCC 7002 & 7.99 & 7.97 & No colony & No colony & No colony & No colony & No colony \\
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\end{tabular}
\caption{Mean colony counts for seven \textit{L. hongkongensis} strains and four standard strains of enteric bacteria on various media.}
\end{table}


