IMPROVEMENT OF CAMPYLOBACTER ISOLATION FROM STOOL SPECIMENS AS REEVALUATION BY TWO CULTURE METHODS AND TWO ENRICHMENT BROTHS

Pitarangsi C, Srijan A, Wongstitwilairoong B, Piyapong S, Mason CJ and Bodhitdatta L

Two different studies were conducted in order to improve the isolation of Campylobacter from stool specimens. An assessment of the efficacy of two culture methods, a non-selective blood agar with 0.65 µm membrane filtration technique, and a selective agar, the modified Charcoal-Cefoperazone Deoxycholate Agar (mCCDA) and a comparison between Doyles and Preston enrichment broths for enhancement of the growth of Campylobacter was conducted on 477 human stool specimens. Of 102 Campylobacter isolates from 89 positive specimens, the evaluation of mCCDA demonstrated that more Campylobacter (57 of C. jejuni and 9 of C. coli) could be isolated from this medium much successfully than the filtration method (50 of C. jejuni and 10 of C. coli), whereas the latter was the only method to isolate all of the catalase-negative Campylobacter (11 of C. upsaliensis). The second study was conducted to evaluate the yields from Preston and Doyles enrichment broth in the enhancement step. Campylobacters (C. jejuni and C. coli) enhanced by Preston broth then cultured on blood agar (filtration method) and mCCDA were isolated 62.7% (64/102), 70.6% (72/102) respectively. Doyles enrichment broth cultured were 55.9% (57/102), 64.7% (66/102) respectively. Finally, combination of filtration method on BAP and mCCDA media and enhancement in Preston enrichment broth was found to be the most suitable for the isolation of Campylobacter from stool specimens.

103rd General Meeting of the American Society for Microbiology. Washington, DC, 18-22 May 2003.

THE STRUCTURE OF A MULTIPLE ANTIBIOTIC RESISTANCE PLASMID FROM CAMPYLOBACTER JEJUNI

Nirdnoy W, Mason C and Guerry P

Background: Due to high rates of both antibiotic resistance and plasmids in Campylobacter jejuni isolates from Thailand, we hypothesized that plasmids play important roles in antibiotic resistance. Methods: A plasmid of Campylobacter jejuni isolated from a diarrhea case of US military deployed to Thailand during Cobra Gold Exercise in 1999 was sequenced. Such clinical isolate, Campylobacter jejuni strain CG 8245, was resistant to ciprofloxacin, nalidixic acid, cephalothin, ampicillin, gentamycin, streptomycin, sulfisoxazole, bactrim and tetracycline. Results: Sequence analysis of a 40 kb plasmid in this strain revealed the presence of a block of genes encoding inactivating enzymes for kanamycin-, streptomycin-, streptothricin-, and erythromycin adjacent to a gene encoding a protein that showed 51% sequence identity to a putative transposase from Helicobacter pylori. The antibiotic inactivating enzymes included: (1) the aminoglycoside 3-phosphotransferase or aph, previously found on a plasmid in Campylobacter coli (100% amino acid identity); (2) an erythromycin resistance methylase (ermB) from Staphylococcus intermedius (100% amino acid identity); (3) streptomycin
adenyltransferase from *Staphylococcus intermedius* (100% sequence identity); and (4) spectinomycin adenyltransferase from a plasmid in *Enterococcus faecalis* (55% amino acid identity). **Conclusion:** This molecular structure, which is reminiscent of multiple drug resistance plasmids in the *Enterobacteriaceae*, suggests that transposons may have facilitated the spread of antibiotic resistance from gram positive organisms into *Campylobacter jejuni* strains.

103rd General Meeting of the American Society for Microbiology. Washington, DC, 18-22 May 2003.

**EVALUATION OF COLONY-BORN ROOF RATS (*RATTUS RATTUS*) AS A SUITABLE ANIMAL MODEL FOR EXPERIMENTAL INFECTION WITH HEPATITIS E VIRUS**


Hepatitis E is an important cause of morbidity and mortality in developing countries, and although the mortality rate of hepatitis E is low in the general population, it averages 20 percent among third-trimester pregnant women. During the last decade various animal models have been examined for experimental HEV infection including non-human primates, swine, rat, mice and lamb. Consistent and reproducible experimental hepatitis E has been confirmed only in cynomolgus macaques, rhesus macaques, and tamarins but the studies were limited by the small numbers of animals used. An alternative species that is easier to handle and that could be used in larger numbers is desired for defining the pathogenesis of hepatitis E and to evaluate candidate vaccines. Although the ecology of HEV remains to be determined, antibodies to HEV have been detected in wild caught rodents. To evaluate whether colony-born Thai roof rat (*Rattus rattus*) is a suitable model for experimental infection with HEV, 7 rats were inoculated with the human isolate of HEV (Nepal), 3 with the rat isolate of HEV (Nepal) and 4 with PBS placebo. The inoculum was given intravenously through the dorsal metatarsal vein. Stool was collected daily post inoculation to detect fecal shedding and blood was collected on days 22 and 35 post inoculation to test for anti-HEV antibodies, viremia and liver enzymes. **Remarks and Conclusions:** Roof rats could be infected as evidenced by detection of HEV RNA in feces in 1 out of 2 animals receiving the rat isolate and 5 out of 7 animals receiving the human isolate. Seroconversion was seen in both rats receiving the rat isolate and one rat receiving the human isolate. However, viremia and histopathological evidence of acute viral hepatitis were absent and evidence of hepatitis by enzyme elevation was only seen in 2 animals. These results are probably due to the fact that the animals were euthanized early before antibody or histological response to HEV or that some strains of HEV are better adapted to wild rodents than laboratory bred ones.