# BACTERIAL CAUSES OF AIDS-ASSOCIATED DIARRHEA IN THAILAND

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**Abstract.** The incidence of bacterial diarrhea in AIDS patients has increased steadily and has led to enormous medical and public health problems. In this study, the clinical data together with 350 rectal swab samples each from AIDS patients with diarrhea (APD) and non-AIDS patients with diarrhea (NAPD), were collected and examined for bacterial enteropathogens at the Bamrasnaradura Infectious Diseases Hospital (BIDH), Nonthaburi, Thailand from May to December 1996. Patients were matched by age and sex. The majority of these patients were male (79%, 554/700), aged between 15 and 34 years (70.9%). The study found that the isolation rates of bacterial enteropathogens causing diarrhea in APD (18%, 62/350) were considerably lower than those in NAPD (43%, 152/350) ($p<0.05$). The infection rate with *Salmonella* group B (19.7%, 12/61) in APD was found to be significantly higher than that in NAPD (14.3%, 2/14) ($p<0.05$). *Vibrio parahaemolyticus* (53.3%, 81/152), *Plesiomonas shigelloides* (27%, 41/152), *Aeromonas* spp (19.1%, 29/152) and *V. cholerae* O1 (15.1%, 23/152), were more frequently detected in NAPD than in APD ($p<0.05$). Only nine *Escherichia coli* strains were isolated from APD, of which six were enteroinvasive *E. coli*, two enterotoxigenic *E. coli* and one enterohemorrhagic *E. coli* (non O157) possessing both *vt*1 and *vt*2. No *V. cholerae* strains were detected in APD. The least effective antibiotics were ampicillin, tetracycline and cotrimoxazole. Antibiotic resistant patterns of the isolated organisms were similar from both groups. The results from this study might be useful in Thailand in the diagnosis and management of clinical cases of bacterial diarrhea, especially APD.

## INTRODUCTION

The number of people infected with the human immunodeficiency virus (HIV) has increased in the past decade. Worldwide, approximately 8.4 million infections were notified in 1996, of which 1.7 million were in children (WHO, 1996). In the same period, 52,997 cases of AIDS and a further 22,209 cases of symptomatic HIV infection were recorded in Thailand (Division of Epidemiology, 1996). HIV transmission occurs primarily through heterosexual contact (98%) (AIDS Division, 1996). Aside from the pathology directly attributable to the HIV, patients are also more susceptible to secondary infections by a large number of prokaryotic and eukaryotic opportunistic pathogens. Patients may have several such infections concurrently, resulting in rapidly changing clinical conditions that may pose diagnostic and therapeutic problems (Modigliani *et al*, 1985; Goodgame, 1996).

Diarrhea is a very common clinical symptom in HIV infections, hence, chronic diarrhea associated with a progression of HIV infection has been included in the clinical criteria for defining a case of AIDS. The incidence of diarrhea in AIDS patients appears to vary geographically. It has been reported that 95%

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of AIDS patients in Africa develop diarrhea, as compared with 50-60% of those in the USA (Gelb and Miller, 1982; Simmonds *et al*, 1992), and secondary infections may be found in up to 85% of cases (Smith *et al*, 1988; Laughon *et al*, 1988). Thus, diarrhea may be a clinical symptom of HIV enteropathy. Furthermore a specific opportunistic protozoan associated with diarrhea, and which has only been found in AIDS patients, *Enterocytozoon bieneusi* has recently been recognised. A number of studies have focused on bacterial causes of diarrhea in AIDS patients. *Salmonella*, *Campylobacter* spp have been found to be associated with 3.9-25.0% and 2.3-11.0% of cases, respectively. Other bacterial pathogens incriminated included *Clostridium difficile* (2.0-7.1% of cases), *Shigella* spp (3.0-4.9%) and *Vibrio parahaemolyticus* (4.0-4.3%) (Smith *et al*, 1988; Laughon *et al*, 1988; Quinn *et al*, 1983; Whimbey *et al*, 1983; Antony *et al*, 1988; Guerrant and Bobak, 1991; Blanshard and Gazzard, 1995).

The bacterial enteropathogens causing diarrhea among AIDS patients in Thailand have not been studied extensively. This gap in knowledge hinders the optimum care and management of Thai AIDS patients with diarrhea (APD). In this study, bacterial enteropathogens in APD were identified and compared to those in non- AIDS patients with diarrhea (NAPD). The study included a comparison of basic clinical findings in the two patient groups together with a comparison of antimicrobial susceptibility patterns of isolates and the presence of certain bacterial virulence factors in isolates from both patient groups.

## MATERIALS AND METHODS

### Study population

The study was conducted at Bamrasnaradura Infectious Diseases Hospital (BIDH) at Nonthaburi (50 km northwest of Bangkok, Thailand) between May and December 1996. Seven hundred patients among those attending the hospital out-patient department (OPD) or admitted to the Medical or Pediatric wards were included in this study. Sample size analysis indicated that 350 APD were required by using the formula: $n = 1/\Delta^2 [Z_\alpha\sqrt{2\ \overline{PQ}} + Z_\beta\sqrt{P_1Q_1 + P_2Q_2}]^2$ (Khan and Sempos, 1989). The probability of an opportunity for detecting bacterial enteropathogens in APD ($P_1$) was equal to not detecting these infections in APD ($Q_1$). These patients were defined as persons who were infected with HIV as diagnosed by clinical laboratory methods and the further progress of various clinical manifestations as mentioned in the criteria of the 4th Revision of Thailand AIDS Definition 1993 (Division of Epidemiology, 1993). Diarrhic symptoms were diagnosed using the criteria described by WHO (1982). An equal number of diarrheal patients without AIDS were also recruited according to age and sex matched criteria. NAPD were determined by using the same questionnaires of the self-selective format used by the Thai Red Cross Society, designed to screen all blood donors for identifying possible HIV positive individuals. The validity of the discrimination for the AIDS group using this questionnaire, ranged from 98.9 to 99.3% (the Thai Red Cross Society, 1992-1994). In this study, an individual who gave a positive answer for even one of all the questions asked, was excluded.

A detailed medical history was taken from all patients using a standard questionnaire. Written consent in this study was obtained from all patients over 21 years of age and from their parents or guardians in the case of minors.

### Bacteriology

A total of 700 rectal swab specimens were collected. These samples were saved in Cary-Blair transport medium and cultured promptly on MacConkey (MC), *Salmonella-Shigella* (SS) agar and thiosulfate citrate bile salt sucrose medium (TCBS, Eiken, Japan) before and after enrichment in Selenite F (SF) broth and alkaline peptone water (APW) containing 2% NaCl. All suspected enteropathogenic strains were further biochemically identified as *Salmonella*, *Shigella* spp, *Escherichia coli*, *Vibrio* spp, *Aeromonas* spp, and *Plesiomonas shigelloides*

using standard methods (Hickman-Brenner *et al*, 1988; Balows *et al*, 1991).

For isolation of *Campylobacter* spp, samples of fecal suspension before and after enrichment in Doyle's broth, were placed onto a 0.45 μM sterile Millipore membrane filter (Millipore Corp, Bedford, MA) on pre-dried blood agar (BA) plates. The BA plates were incubated at 37ºC under microaerophilic conditions for 48 hours. After removing the filter membrane as described previously (Fongsiri *et al*, 1989), *Campylobacter* isolates were biochemically identified using standard procedures as described by Penner (1991).

All culture media except the specified media, were purchased from Difco laboratories (Difco, Detroit, Michigan). All the identified enteropathogenic strains were stored in cryobank vials (Nunc; Denmark) and then kept at -85ºC until studied further.

**Serotyping**

*Salmonella*, *Shigella* and *E. coli* were provisionally serotyped using slide agglutination techniques with specific antisera and following the methods of Edwards and Ewing (1986). Isolates were then sent to the WHO National *Salmonella* and *Shigella* Typing Center, Thai National Institute of Health (TNIH), Nonthaburi for confirmation. Serotyping of *V. parahaemolyticus* was done by a slide agglutination test with commercial O and K antisera (Denka Seiken, Tokyo) as described previously (Suthienkul *et al*, 1995).

**Modified Elek test**

The modified Elek test was performed to detect thermostable direct hemolysin (TDH) of isolated *V. parahaemolyticus* strains following the method described by Honda *et al* (1980).

**Detection of virulence factors by PCR**

The PCR primers used in this study are listed in Table 1 (Ito and Ratchtrachenchai, 1996; Nishibuchi and Kaper, 1985; Nishibuchi *et al*, 1989; Wright *et al*, 1992). All oligonucleotide primers were synthesized at the BioScience Service Institute of Science and Technology Development, Bangkok, Thailand. The oligonucleotide primers of enterotoxigenic, enteroinvasive, and enterohemorrhagic *E. coli* (ETEC, EIEC, EHEC, respectively) were obtained commercially (Vertex Company, Canada).

Table 1
PCR primers and annealing temperature of enteropathogens.

| Enteropathogens | Target gene | PCR primers' sequences (5′-3′) | Product size (bp) | Annealing temperature | References |
|---|---|---|---|---|---|
| *Escherichia coli* | | multiplex | | 45ºC | Ito and Ratchtrachenchai, 1996 |
| EIEC | *Inv*E | ATATCTCTATTTCAATCGCGT<br>GATGGCGAGAAATTATATCCG | 382 | | |
| EHEC | *vt*1/2 | TTTACGATAGACTTCTCGAC<br>CACATATAAATTATTTCGCTC | 228 | | |
| ETEC | *l*th/p | AGCAGGTTTCCCACCGATCACCA<br>CGTGCTCAGATTCTGGGTCTC | 132 | | |
| | *st*Ia/Ib | TTTATTTCTGTATTGTCTT<br>CAATTACAACACAGTTCACAG | 171 | | |
| *V. parahaemolyticus* | *tdh* | GTACCGATATTTTGCAAA<br>ATGTTGAACTGTACTTGA | 382 | 48ºC | Nishibuchi and Kaper, 1985 |
| | *trh* | CTCTACTTTGCTTTCAGT<br>TACCGTTAGATAGTCGCTTA | 276 | 48ºC | Nishibuchi *et al*, 1989 |
| *V. cholerae* O1 | *ctx*A | CGGGCAGATTCTAGACCTCCTG<br>CGATGATCTTGGAGCATTCCCAC | 564 | 60ºC | Wright *et al*, 1992 |

The template DNA of *E. coli, V. parahaemolyticus* and *V. cholerae* O1 isolates were extracted by suspending a loopful of an overnight culture in 100 μl of sterile deionized water in a microcentrifuge tube. The cell suspension was then kept at 100ºC for 10 minutes, the supernatant of target DNA was collected after centrifugation for 1 minute and kept at -20ºC until used.

The amplification conditions of their target DNA were done according to the procedures described by Ito and Ratchtrachenchai (1996) for ETEC, EIEC, EHEC; by Suthienkul *et al* (1995) for the *tdh* and *trh* of *V. parahaemolyticus*; and by Wright *et al* (1992) for the *ctxA* of *V. cholerae* O1. The PCR mixture was subjected to a thermalcycler (Model 480, Perkin-Elmer Corporation, USA). All the PCR products (6-10 μl of each) were analyzed using 1.2% agarose gel electrophoresis. *E. coli* isolates were analysed using a 2% agarose gel. The DNA bands were visualized under an UV transilluminator (Fotodyne, Hartland, USA) after staining with ethidium bromide solution (0.5 μg/ml).

**Antimicrobial susceptibility assay**

All isolated enteropathogens were tested for their susceptibility to 7 antibiotics: ampicillin (Am,10 μg), gentamicin (Gm,10 μg), nalidixic acid (NA, 30 μg), nitrofurantoin (F/M, 300 μg), norfloxacin (Nor, 10 μg), tetracycline (Tc, 30 μg) and cotrimoxazole (Sxt, 1.25/23.75 μg) using the disc diffusion method of Bauer *et al* (1966). All antimicrobial discs were purchased from BBL (Cockeysville, MD, USA).

**Data analysis**

Statistical analyses were performed using SPSS PC version 7.5 (SPSS Inc, Chicago, IL, USA) and EpiInfo. Statistic significance of the differences between the 2 groups was tested with chi-square test and Fisher's exact test (when expected values were less than 5).

## RESULTS

**Patients' profiles**

Table 2 shows the demographic data of the study population. The majority of the APD were male (79%, 554/700). The sex ratio between male and female was 3.8:1, and the ages of the patients were between 15 and 34 years (80%). Pediatric AIDS cases (< 5 years of age) made up 8% of the patients. The mean age of the APD was 30 ± 12.3 years. Sixty-one percent of APD were admitted to the hospital as in-patients, while 98% of the NAPD had acute diarrhea and were treated as out-patients. The mean period of hospitalization for the APD was 18.6 ± 1.4 days. The majority of the APD (65%, 229/350) reported having diarrhea of more than 1 month. In contrast, 93.7% (328/350) of the non-AIDS patients reported having had diarrhea for less than 1 week when seen at the hospital. Fever was not a significant finding in cases with diarrhea. In this study, fever was present in 4% (26/700) of patients when first admitted to the hospital or seen at the OPD. Other symptoms and signs found in both groups of patients were similar but with different frequencies including; anorexia, vomiting, abdominal pain, nausea, abdominal distention, and abdominal cramps. Weight loss was absent in the non-AIDS group but was present in APD (60.6%, 212/350). The stool types were similar but differed in frequency in the two groups. Types of stool included those that were: loose, loose-mucoid, watery and mucoid-visible-blood.

**Isolation rates and types of enteropathogens**

Enteropathogens were isolated from 213 (30.4%) of 700 cases. Of these positive specimens, 61 (17.4%) were from AIDS cases and 152 (43.4%) were from non-AIDS groups. The isolation of bacterial enteropathogens from APD was significantly lower than those from NAPD ($p<0.05$). Single enteropathogens 53/61 (86.9%) and multiple enteropathogens 8/61(13.1%) were noted from positive specimens in AIDS patients. Similarly, single enteropathogens from the positive specimens of non-AIDS patients were also isolated in a high percentage, 67.8% (103/152) of cases, and multiple enteropathogens in 32.2% (49/152) of cases.

Types of organisms and isolation rates are

Table 2
Demographic data of the study population.

| Characteristics | No. (%) of patients | | Total (%) (n=700) |
|---|---|---|---|
| | AIDS (n=350) | non-AIDS (n=350) | |
| **Sex**[a] | | | |
| Male | 277 (79.0) | 277 (79.0) | 554 (79.0) |
| Female | 73 (21.0) | 73 (21.0) | 146 (21.0) |
| **Age**[a] | | | |
| < 5 | 29 (8.3) | 29 (8.3) | 58 (8.3) |
| 5-14 | 1 (0.3) | 1 (0.3) | 2 (0.3) |
| 15-24 | 32 (9.1) | 32 (9.1) | 64 (9.1) |
| 25-34 | 164 (46.9) | 164 (46.9) | 328 (46.9) |
| 35-44 | 84 (24.0) | 84 (24.0) | 168 (24.0) |
| 45-54 | 28 (8.0) | 28 (8.0) | 56 (8.0) |
| 55-64 | 10 (2.9) | 10 (2.9) | 20 (2.9) |
| >65 | 2 (0.6) | 2 (0.6) | 4 (0.3) |
| **Department** | | | |
| OPD | 138 (39.4) | 343 (98.0) | 481 (68.7) |
| Admission | 212 (60.6) | 7 (2.0) | 219 (31.3) |
| **Duration of diarrhea (days)** | | | |
| 2-7 | 30 (8.6) | 328 (93.7) | 358 (51.1) |
| 8-14 | 26 (7.4) | 8 (2.3) | 34 (4.9) |
| 15-30 | 65 (18.6) | 12 (3.4) | 77 (11.0) |
| > 30 | 229 (65.4) | 2 (0.6) | 231 (33.0) |
| **Fever** | | | |
| No fever | 306 (87.4) | 301 (86.0) | 607 (86.7) |
| Low grade fever (< 38.5ºC) | 29 (8.3) | 38 (10.9) | 67 (9.6) |
| High grade fever (> 38.5ºC) | 15 (4.3) | 11 (3.1) | 26 (3.7) |
| **Symptoms and signs** | | | |
| Anorexia | 264 (75.4) | 9 (2.6) | 273 (39.0) |
| Weight loss | 212 (60.6) | 0 (0.0) | 212 (30.3) |
| Vomiting | 119 (34.0) | 147 (42.0) | 266 (38.0) |
| Abdominal pain | 92 (26.3) | 298 (85.1) | 390 (55.7) |
| Nausea | 83 (23.7) | 126 (36.0) | 209 (29.9) |
| Abdominal distention | 81 (23.1) | 66 (18.9) | 147 (21.0) |
| Tenesmus | 9 (2.6) | 22 (6.3) | 31 (4.4) |
| **Characteristics of stool** | | | |
| Loose | 183 (52.3) | 205 (58.6) | 388 (55.4) |
| Loose-mucoid | 151 (43.1) | 96 (27.4) | 247 (35.3) |
| Watery | 8 (2.3) | 34 (9.7) | 42 (6.0) |
| Mucoid-visible blood | 8 (2.3) | 15 (4.3) | 23 (3.3) |

[a]Age and sex of the non-AIDS patients were matched to the AIDS group.

shown in Table 3. *Salmonella* spp (32.8%, 20/61) were the commonest enteropathogens isolated from APD followed by *P. shigelloides* 27.8% (17/61), *Aeromonas* spp 26.2% (16/61), *Vibrio* spp 14.7% (9/61), *E. coli* 14.7% (9/61) and *Shigella* spp 11.4% (7/61). *Salmonella* group B (60%, 12/20) was the most common serogroup of all *Salmonella* isolates in APD and was significantly higher than those in non-AIDS group 14.3% (2/14) ($p<0.05$). The isolation rates of *P. shigelloides* and *Aeromonas* spp in NAPD were significantly higher than those in ADP ($p<0.05$). Four species of *Aeromonas* viz *A. caviae, A. hydrophila, A. sobria* and *A.*

*veronii* were isolated in 31.3%, 31.3%, 25%, and 12.5% of APD, respectively, and all were not significantly higher than those in NADP. When considering the isolation rates of vibrio organisms in both groups, *V. parahaemolyticus* was the most common vibrio isolated (55.6% in APD and 69.8% in the NAPD). *V. parahaemolyticus*, *V. cholerae* O1, *Aeromonas* spp and *P. shigelloides* were found in significantly lower numbers in APD than those in NAPD ($p < 0.05$). *E. coli* (EIEC, ETEC and EHEC), *Shigella* spp (*S. flexneri, S. boydii* and *S. sonnei*) were encountered at low and similar rates in both groups. Futhermore, *C. coli* was only detected in a male NAPD.

Table 4 shows the serogroups of *Salmonella*, *Shigella* and *E. coli* isolated from APD and NAPD. All 23 isolates of *V. cholerae* O1 detected from NAPD belonged to serogroup Ogawa. Among 32 *V. parahaemolyticus* isolates tested, (54 isolates were lost), eighteen different serogroups were detected. These included : O4:K4 and O7:K5 which were detected from APD only, while O3:K6(7/32), was the most common serogroup, followed by O1:KUT,

Table 3
Comparison of the isolation rates of enteropathogens between AIDS and non-AIDS patients with diarrhea.

| Bacterial enteropathogens[c] | No. (%) of positive patients for enteropathogens | | p-value |
|---|---|---|---|
| | AIDS (n=350) | non-AIDS (n=350) | |
| Total of positive cases of bacterial diarrhea | 61 (17.4) | 152 (43.4) | <0.05 |
| *Salmonella* | 20 (5.7) | 14 (4.0) | 0.373[a] |
| - *Salmonella* group B | 12 (60.0) | 2 (14.3) | 0.007[a] |
| - *Salmonella* group C | 5 (25.0) | 3 (21.4) | 0.725[b] |
| - *Salmonella* group E | 2 (10.0) | 5 (35.7) | 0.451[b] |
| - *Salmonella* group D | 1 (5.0) | 3 (21.4) | 0.624[b] |
| - *Salmonella* group H | - | 1 (7.1) | 1.000[b] |
| *Plesiomonas shigelloides* | 17 (4.9) | 41 (11.7) | 0.001[a] |
| *Aeromonas* spp | 16 (4.6) | 29 (8.3) | 0.003[a] |
| - *A. caviae* | 5 (31.3) | 11 (37.9) | 0.129[a] |
| - *A. hydrophila* | 5 (31.3) | 6 (20.7) | 0.761[a] |
| - *A. sobria* | 4 (25.0) | 5 (17.2) | 1.000[b] |
| - *A. veronii* | 2 (12.5) | 3 (10.3) | 1.000[b] |
| - *A. trota* | - | 4 (13.8) | 0.124[b] |
| *Vibrio* spp | 9 (2.6) | 116 (33.1) | 0.000[a] |
| - *V. parahaemolyticus* | 5 (55.6) | 81 (69.8) | 0.000[a] |
| - *V. cholerae* O1 (Ogawa) | - | 23 (19.8) | 0.000[a] |
| - *V. cholerae* non O1 | 2 (22.2) | 8 (6.9) | 0.153[b] |
| - *V. alginolyticus* | 2 (22.2) | 4 (3.4) | 0.686[b] |
| *Escherichia coli* | 9 (2.6) | 8 (2.3) | 0.816[a] |
| - Enteroinvasive *E. coli* | 6 (66.7) | 2 (25.0) | 0.286[b] |
| - Enterotoxigenic *E. coli* | 2 (22.2) | 6 (75.0) | 0.286[b] |
| - Enterohemorrhagic *E. coli* | 1 (11.1) | - | 1.000[b] |
| *Shigella* spp | 7 (2.0) | 7 (2.0) | 1.000[a] |
| - *S. flexneri* | 3 (42.9) | 3 (42.9) | 1.000[b] |
| - *S. sonnei* | 3 (42.9) | 3 (42.9) | 1.000[b] |
| - *S. boydii* | 1 (14.3) | 1 (14.3) | 1.000[b] |

[a]Chi-square test; [b]Fisher's exact test
[c]Only one isolate of *Campylobacter coli* was detected from a male non-AIDS diarrheal patient.

Table 4
Serotypes of *Salmonella, Shigella*, isolated from fecal specimens of AIDS and non-AIDS diarrheal patients at BIDH from May to December 1996.

| Serotypes | No.(%) of specimens | | Total (%) |
|---|---|---|---|
| | AIDS | Non-AIDS | |
| *Salmonella* total | 20 (59.0) | 14 (41.0) | 34 (100.0) |
| *Salmonella* Group B | 12 (86.0) | 2 (14.0) | 14 (41.0) |
| *S. typhimurium* | 5 (100.0) | - | 5 (36.0) |
| *S. agona* | 2 (100.0) | - | 2 (14.0) |
| *S. derby* | 1 (50.0) | 1 (50.0) | 2 (14.0) |
| *Salmonella* subspecies I.4,5,12: i: - | 4 (80.0) | 1 (20.0) | 5 (36.0) |
| *Salmonella* Group C | 5 (62.0) | 3 (38.0) | 8 (24.0) |
| *S. thompson* | 2 (67.0) | 1 (33.0) | 3 (38.0) |
| *S. hadar* | 1 (100.0) | - | 1 (13.0) |
| *S. newport* | 1 (100.0) | - | 1 (13.0) |
| *S. virchow* | 1 (100.0) | - | 1 (13.0) |
| *Salmonella* subspecies I.8,20:-:Z6 | - | 1 (100.0) | 1 (13.0) |
| *Salmonella* subspecies I.6,7:6:- | - | 1 (100.0) | 1 (13.0) |
| *Salmonella* Group D - *S. enteritidis* | 1 (25.0) | 3 (75.0) | 4 (12.0) |
| *Salmonella* Group E | 2 (29.0) | 5 (71.0) | 7 (21.0) |
| *S. weltevreden* | 1 (33.0) | 2 (67.0) | 3 (42.9) |
| *S. lexington* | - | 2 (100.0) | 2 (28.6) |
| *S. anatum* | 1 (50.0) | 1 (50.0) | 2 (28.6) |
| *Salmonella* Group H - *S. cerro* | - | 1 (100.0) | 1 (3.0) |
| *Shigella* total | 7 (50.0) | 7 (50.0) | 14 (100.0) |
| *S. flexneri* 2a | 1 (100.0) | - | 1 (17.0) |
| *S. flexneri* var x | 1 (100.0) | - | 1 (17.0) |
| *S. flexneri* untypeable | 1 (25.0) | 3 (75.0) | 4 (67.0) |
| *S. boydii* type 12 | 1 (50.0) | 1 (50.0) | 2 (14.0) |
| *S. sonnei* | 3 (50.0) | 3 (50.0) | 6 (43.0) |
| *Escherichia coli* total | 9 (53.0) | 8 (47.0) | 17 (100.0) |
| Enterotoxigenic *E. coli* total | 2 (25.0) | 6 (75.0) | 8 (47.0) |
| O 8 | 1 (100.0) | - | 1 (12.5) |
| poly 6+ mono- | 1 (100.0) | - | 1 (12.5) |
| O 148 | - | 2 (100.0) | 2 (25.0) |
| O 25 | - | 1 (100.0) | 1 (12.5) |
| O 128 | - | 1 (100.0) | 1 (12.5) |
| O 159 | - | 1 (100.0) | 1 (12.5) |
| poly 2+ mono- | - | 1 (100.0) | 1 (12.5) |
| Enteroinvasive *E. coli* total | 6 (75.0) | 2 (25.0) | 8 (47.0) |
| O 164 | 4 (80.0) | 1 (100.0) | 5 (62.5) |
| O 124 | 1 (100.0) | - | 1 (12.5) |
| O 28ac | 1 (100.0) | - | 1 (12.5) |
| O 152 | - | 1 (100.0) | 1 (12.5) |
| Enterohemorrhagic *E. coli* -O 8 | 1 (100.0) | - | 1 (6.0) |

O3:KUT, O4:K4, O4:K8, O4:K9, O8:K12, O1:K1, O1:K4, O1:K56, O3:K4, O4:K12, O5:K15, O5:K47, O5:K61, O5:KUT, O7:K52 and O12:KUT in NAPD.

**Virulence factors**

ETEC positive for *st*Ia/Ib were identified in 6 patients, one from AIDS group and 5 from non-AIDS group. In addition, only one ETEC

Table 5
Antibiotic resistant pattern of enteropathogens isolated from fecal specimens of AIDS and non-AIDS diarrheal patients at BIDH, May to December 1996.

| Antibiotics | No. (%) of antibiotic resistance of enteropathogens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Salmonella* | | *Shigella* | | *Vibrio* | | *Aeromonas* | | *Plesiomonas* | | EC[a] | |
| | APD[b] n=20 | NAPD[c] n=14 | APD n=7 | NAPD n=7 | APD n=9 | NAPD n=116 | APD n=16 | NAPD n=29 | APD n=17 | NAPD n=41 | APD n=9 | NAPD n=8 |
| Ampicillin | 11 (55) | 7 (50) | 7 (100) | 4 (57) | 9 (100) | 85 (73) | 16 (100) | 26 (90) | 9 (53) | 35 (76) | - | - |
| Cotrimoxazole | 8 (40) | 1 (7) | 1 (14) | 4 (57) | 4 (44) | 7 (6) | 7 (44) | 4 (14) | 8 (47) | 12 (29) | 8 (89) | 8 (100) |
| Gentamicin | 1 (5) | 1 (7) | - | - | 1 (11) | - | - | 1 (3) | - | - | 8 (89) | - |
| Nalidixic acid | 2 (10) | 2 (14) | - | - | - | 5 (4) | 7 (44) | 3 (10) | 2 (12) | 6 (15) | - | - |
| Nitrofurantoin | 1 (5) | 1 (7) | - | - | - | 17 (15) | - | 5 (17) | - | 3 (7) | 1 (11) | - |
| Norfloxacin | 1 (5) | 1 (7) | 3 (43) | - | - | - | 1 (6) | 1 (3) | 1 (6) | 1 (2) | - | - |
| Tetracycline | 9 (45) | 4 (29) | 1 (14) | 7 (100) | 3 (33) | 15 (13) | 7 (44) | 9 (31) | 5 (29) | 20 (49) | 8 (89) | 8 (100) |

[a]*Escherichia coli* including ETEC, EIEC and EHEC.
[b]AIDS patients with diarrhea.
[c]Non-AIDS patients with diarrhea.

isolate was found to be positive for *lt*h/p from each group. Six of the eight isolates of EIEC were from AIDS and the rest from non-AIDS patients. One isolate of EHEC in APD was found positive for *vt*1/2. Subsequently, 32 isolates of *V. parahaemolyticus* were further examined for hemolysin gene. PCR analysis indicated that 84.5% (27/32) possessed only *tdh*, 9% (3/32) had both *tdh* and *trh*, and 3% (1/32) harbored only *trh*. In addition, 84.5% (27/32) of *V. parahaemolyticus* isolates were positive for the production of TDH by the modified Elek test. All *V. cholerae* isolates were positive for *ctx*A.

**Antimicrobial susceptibility test of the isolates**

The antimicrobial resistance patterns of salmonellae, and shigellae, were similar in both groups (Table 5). Salmonellae isolated from APD had high rates of resistance to ampicillin (55%), tetracycline (45%), cotrimoxazole (40%) and nalidixic acid (10%); while those isolated from NAPD were also resistant to ampicillin 50%, tetracycline 29%, nalidixic acid 14% and cotrimoxazole 7%. Isolates from both groups were resistant at much lower rates to other antibiotics such as gentamicin, nitrofurantoin and norfloxacin. Eight of nine *E. coli* isolates in AIDS patients were resistant to cotrimoxazole, gentamicin and tetracycline, while all 8 isolates from non-AIDS group were resistant to cotrimoxazole and tetracycline. The rest of enteropathogens from both patient groups had similar antibiotic susceptibility patterns. In addition, one isolate of *Campylobacter coli* detected from a male NAPD was sensitive to erythromycin by minimal inhibition concentration technique (1.0 μg/ml).

## DISCUSSION

Diarrhea is a major problem and the most commonly presenting symptom of patients infected with HIV. Diarrhea in AIDS patients is usually chronic, with protozoa being the commonnest etiological agents (Punpoowong *et al*, 1998; Beaugerie *et al*,1998; Wanke *et al*, 1999). However, bacterial enteric pathogens have emerged as significant causes of diarrhea in HIV-infected cases. The broad range of these etiological agents was reported in 1983-1992 as being the following: *Salmonella* (3-15%), *Campylobacter* (2-10.6%), *Clostridium difficile* (3-7%), *A. hydrophila* (2%), *Shigella* spp (1-5%), and *V. parahaemolyticus* (4-4.3%) (Laughon *et al*, 1988; Quinn *et al*, 1983; Dworkin *et al*, 1985; Ahmad *et al*, 1998; Mwachari *et al*, 1998). In the present study, the percentage of enteropathogens in both groups was in the same range of those mentioned above. The isolation rates of bacterial enteropathogens causing diarrhea in APD (17.4%) were significantly lower than those in NAPD (43.4%) ($p < 0.05$). In contrast, in the study of the same fecal specimens, the isolation rates of intestinal parasites causing diarrhea in APD (43.1%, 151/350) were significantly higher than those in NAPD (6.3%, 22/350) ($p < 0.001$) (Pimsuta, 1997). It was also found that the majority of the APD were infected with microsporidia (15.1%, 53/350), and *Cryptosporidium* spp (10.3%, 36/350), but were not found in NAPD ($p < 0.001$). The low prevalence of enteropathogenic bacteria observed in both groups might be the result of the limited amount of fecal material collected and the use of rectal swabs instead of stool samples. However, most of the AIDS patients (65.4%) were cases of chronic diarrhea and over 60% were hospitalized while the non-AIDS (93.7%) had acute diarrhea (Dworkin *et al*, 1985).

In this study, the isolation rates of the common bacterial etiological agents, differed between the APD and NAPD groups. The infection rates due to *Salmonella* group B (60%, 12/20) in APD were significantly higher than those in NAPD (14.3%, 2/14) ($p<0.05$). The high prevalence rate of infection in our study with *S. typhimurium* (25%, 5/20) in APD, is in the agreement with several studies in American AIDS patients (Smith *et al*, 1988; Glaser *et al*, 1985; Rene *et al*, 1989). The higher prevalence rates of *Salmonella* group B infection in APD than in NAPD suggested that patients with AIDS may be more susceptible to *Salmonella* group B infection. A defect in T-cell function or compromised cell-medi-

ated immunity making AIDS patients more high risk to *Salmonella* infections has been shown to contribute this condition (Sperber and Schlenpner, 1987).

In contrast, the isolation rates of some enteropathogens including *V. cholerae*, *V. parahaemolyticus*, *Aeromonas* spp and *P. shigelloides* in APD were less than those in NAPD ($p < 0.05$). There may be several explanations for this phenomenon: firstly, the low resistance of APD to infections, results in large quantities of antibiotics *eg* norfloxacin and trimethoprim-sulfamethoxazole being used for the treatment of diarrhea. This may result in the suppression of bacteria sensitive to these antibiotics. Secondly, several reports have indicated that histological changes occur in the mucosa of HIV patients resulting in their having rare bacterial pathogens causing their diarrhea (Greenson *et al*, 1991; Clayton *et al*, 1992). These alterations might be unfavorable to colonization by non-invasive pathogens. Thus, APD were found not susceptible to *V. cholerae* infection, but 23 of *V. cholerae* O1 strains positive for *ctx*A were isolated from NAPD. Similar results were observed in all prior reports on the cause of APD and that *V. cholerae* O1 was not isolated (Smith *et al*, 1988; Laughon *et al*, 1988; Antony *et al*, 1988; Guerrant and Bobak,1991; Blanshard and Gazzard, 1995). This suggested that *V. cholerae* O1 might be not an enteropathogen causing cholera in AIDS patients. However, further intensive studies of this phenomenon are needed. As previously mentioned in this study, the infection rates of *V. parahaemolyticus* in NAPD was significantly higher than those in APD but virulence factors and serogroups were not different, and were similar as in the study of Suthienkul *et al* (1995). These serogroups showed the same virulence factors as reported in the study of Suthienkul *et al* (1995), *ie* O3:K6 had *tdh* and *trh*, etc. The infection rates of pathogenic *E. coli* in both groups were not significantly different; the only different being in frequency. In this study, it was shown that the invasive *E. coli* : EIEC and EHEC (*vt*1/2) infections occurred mostly in APD, while non-invasive *E. coli* : ETEC (*st*Ia/Ib) infections were found in NAPD. It was known that *Campylobacter* spp were a common cause of diarrhea in AIDS patients (2.3-11%) in developed countries (Smith *et al*, 1988; Laughon *et al*, 1988; Antony *et al*, 1988; Guerrant and Bobak, 1991). In contrast, *Campylobacter coli* was isolated only from a 29 years old male NAPD, and not from any APD. A previous study in Thailand showed that *Campylobacter* infection mostly occurred in infancy and early childhood, and their frequency decreased with age (Taylor *et al*, 1988), 91% of the patients in this study were adults.

It is interesting to note that American homosexuals engaging in anal-oral sex are highly susceptible to *shigella*e infection (Blaser *et al*, 1983). Shigella spp have been isolated from feces of homosexual men with diarrhea in up to 3% of cases (Quinn *et al*, 1983), and as a cause of diarrhea in AIDS patients in 2-4.9% of cases (Laughon *et al*,1988; Whimbey *et al*, 1983; Ullich *et al*, 1992). In this study, isolation rates of *Shigella* in APD and NAPD in Thailand were similar (2%) to previous studies. It suggested that heterosexual AIDS patients in Thailand were not more susceptible to shigellae infection than NAPD.

Gastrointestinal symptoms such as anorexia, and weight loss most commonly occurred in APD and less often in NAPD ($p<0.05$), while abdominal pain, vomiting and abdominal cramping were the most common symptoms in NAPD ($p<0.05$). Similar results were reported by Dworkin *et al* (1985), Ullrich *et al* ( 1992), and Serwadda *et al* (1985).

Antibiotic resistance among members of the family Enterobacteriaceae has been recognized for a decade (Chun *et al*, 1981; Echeverria *et al*, 1978; Carlson *et al*, 1983; Murray, 1986). The antibiotic resistance patterns in this study were similar to the previous studies. Most salmonellae isolates from APD were resistant to ampicillin (55%), tetracycline (45%), and cotrimoxazole (40%). *Shigella* spp isolated from APD were mostly resistant to ampicillin (100%) and norfloxacin (43%). *Vibrio* and *Aeromonas* spp isolated from AIDS patients were resistant to ampicillin 100%. EIEC isolates were resistant to cotrimoxazole and

tetracycline (89-100%). Antimicrobial resistant patterns of enteric pathogens isolated from APD and NAPD were similar and not significantly different. Many enteropathogens, including *Shigella*, *Vibrio*, *Aeromonas*, and *P. shigelloides* in APD, were susceptible to nitrofurantoin; many enteropathogens including *Shigella* , *Vibrio*, *P. shigelloides*, and *E. coli* in non-AIDS groups, were all sensitive to gentamicin (Table 5). Although the eradication of a pathogen may be impossible by the use of antibiotics, most of these patients responded well to specific antimicrobial agents (Smith *et al*, 1988), thus emphasizing the importance of the correct laboratory diagnosis of the specific etiologic agents whenever possible.

We believe that this study is the first comprehensive study of bacterial diarrhea in AIDS and non-AIDS patients in Thailand. The information obtained in this study could we believe contribute to a better understanding of the causes, pathogenesis and management of bacterial diarrhea specifically in AIDS patients. Furthermore, detection of other potential causative agents together with their virulence factors causing diarrhea in AIDS and non-AIDS patients in negative culture samples, should perhaps be carried out.

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