Original Article

Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during 2002-2004

Palpasa Kansakar[1], Pankaj Baral[2], Sarala Malla[1], Gokarna Raj Ghimire[1]

[1]*National Public Health Laboratory, Kathmandu, Nepal*
[2]*Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal*

Abstract

Introduction: The prevalence and antimicrobial susceptibility patterns of the bacterial enteropathogens *Vibrio cholerae*, *Salmonella* species and *Shigella* species were investigated.

Methodology: A total of 877 stool samples were received for culture at the National Public Health Laboratory (NPHL), Kathmandu, Nepal, during January 2002 to December 2004, from diarrhoea patients attending Shukraraj Tropical Infectious Hospital and referral outpatients. All samples collected were processed for isolation and antibiotic susceptibility testing of *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp.

Results: Of the 877 stool samples, 148 (16.8%) were culture positive for one of the three bacterial enteropathogens investigated. Among them, *Vibrio cholerae*, *Shigella* spp. and *Salmonella* spp. accounted for 98/877 (11.1%), 41/877 (4.6%), 9/877 (1.02%) of the isolates respectively. A year-to-year variation was seen in the type of predominant organism, with *Shigella* spp. being the most prevalent in 2002 and 2003 and *Vibrio* spp. in 2004. In all three years, *Vibrio cholerae* were encountered only during the months of April to June while *Salmonella* spp. and *Shigella* spp. were isolated throughout the whole year. All *Vibrio cholerae* and *Salmonella* isolates were susceptible to ciprofloxacin. All *Shigella* isolates were susceptible to ceftriaxone. Ciprofloxacin resistance was observed among isolates of *Shigella dysenteriae* type-1 isolated after 2003.

Conclusion: *Vibrio cholerae, Salmonella* and *Shigella* infections are prevalent in Kathmandu, Nepal. A gradual increase in resistance to commonly used antimicrobials was seen among bacterial enteropathogens. Antimicrobial resistance surveillance is necessary to guide empirical treatment.

Key words: antimicrobial resistance; enteropathogens; *Salmonella; Shigella; Vibrio cholerae*

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Introduction

Infectious diarrhoeal diseases are responsible for considerable morbidity and mortality, especially in developing countries [1]. According to a 2009 World Health Organization (WHO) bulletin, diarrhoeal diseases account for an estimated annual 1.5 million deaths among children younger than five years old in the world, while in Nepal 1.05 % (37,000/3,535,000) mortality was reported among children younger than 5 years old [2]. Owing to the low socioeconomic status and poor hygienic conditions of the people in Nepal, intestinal parasitic and bacterial infections constitute a major cause of morbidity and mortality, contributing to several epidemics each year [3]. Gastroenteritis prevails throughout the year with epidemics mainly in the rainy season [4]. Among the bacterial enteric-pathogens, *Vibrio cholerae, Salmonella* spp. and *Shigella* spp. are of special concern because of the severity of the illness they cause and their association with various outbreaks [5].

Though the treatment of choice for acute diarrhoea is fluid and electrolyte replacement, antibacterial agents are often recommended for treatment of suspected shigellosis, invasive salmonellosis and cholera. Since most diarrhoeal diseases are treated empirically, it is important to know the susceptibility pattern of the prevalent pathogens [6]. The problem of antimicrobial resistance in bacterial pathogens causing diarrhoeal diseases continues to be alarming. Emergence and spread of antimicrobial resistance to newer and more potent agents used in treatment have been described for *Salmonella*, *Shigella* and *Vibrio cholerae* [7,8,9].

Information concerning enteric pathogens in each country is essential in terms of epidemiology, surveillance, and management of patients. Despite few studies on diarrhoeal diseases in Nepal, there is lack of

adequate information on bacterial enteric pathogens and their antimicrobial resistance trend over a longer time period in Kathmandu valley. Hence this study aimed for the first time to investigate the prevalence of common enteric bacterial pathogens, *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp., and their antimicrobial susceptibility profiles in Kathmandu over a period of three years.

Methodology

The study was conducted at the National Public Health Laboratory (NPHL) in Kathmandu, the national reference laboratory of Nepal that receives referral samples for various laboratory investigations from different health-care institutions as well as from self-referred patients. However, the majority of samples referred to NPHL come from the Shukraraj Tropical Infectious Hospital, an infectious disease hospital for adults located in close proximity to NPHL. A total of 877 stool culture samples were obtained from NPHL during a period of three years from January 2002 to December 2004. Patient's demographic data were recorded which included name, age/sex and date of specimen collection. As expected, the majority of samples were from adult diarrhoea patients from Shukraraj Tropical Infectious Hospital. The samples were processed for isolation of *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. Alkaline peptone water was used for the enrichment of *Vibrio cholerae*, whereas Gram-negative (GN) broth (BD Diagnostic system, Sparks, MD, USA) was used for the enrichment of *Samlonella* spp. and *Shigella* spp. Enrichments were subcultured on thisulfate-citrate bile sucrose (TCBS) (Oxoid Ltd, Basingstoke, England) for *V. cholerae* and Salmonella-Shigella (S-S) agar (Oxoid Ltd, Basingstoke, England) for *Salmonella* spp. and *Shigella* spp. Biochemical tests [IMViC, Triple sugar iron test, Oxidation/fermentation (O/F) test, Urease test, Nitrate reduction test] were used to identify *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. and serological strain typing was performed using commercially available antisera (Denka-Seiken, Tokyo, Japan).

The antimicrobial susceptibility testing of *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. were performed by Kirby Bauer's disc diffusion technique [10] using commercially available discs (Oxoid Limited, Basingstoke, England). The antibiotics tested for *Vibrio cholerae* were tetracycline (T, 30 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (Cip, 5 µg), erythromycin (E, 15 µg), cotrimoxazole (Sxt, 25 µg) and furazolidone (F, 100 µg). The antibiotics tested for *Shigella* spp. were ampicillin (Amp, 10 µg), ciprofloxacin (Cip, 5 µg), nalidixic acid (NA, 30 µg), cotrimoxazole (Sxt, 25 µg), mecillinam (Mel, 25 µg) and ceftriaxone (CRO, 5 µg), while ampicillin (Amp, 10 µg), ciprofloxacin (Cip, 5 µg), chloramphenicol (Chl, 30 µg), cotrimoxazole (Sxt, 25 µg), ceftriaxone (CRO, 5 µg) and nalidixic acid (NA, 30 µg) were tested for *Salmonella* spp.. In this study, the defining criterion for multidrug resistance (MDR) was resistance to ≥ 2 of the antimicrobial agents belonging to different structural classes [11,12].

Results

Prevalence of Vibrio cholerae, Shigella *spp. and* Salmonella *spp.*

Out of 877 stool specimens, 148 (16.8%) were culture positive for the bacterial entero-pathogens investigated in the study. Of the 148 culture positive stool specimens, 87 were from males and 61 were from females (data not shown). There was no significant association between gender and enteropathogenic bacterial infection ($p > 0.05$). Of the 148 culture positive specimens, 43/148 (29.05%) were from the age group of 20 to 29 years (Table 1). However, the yearly breakdown of enteric pathogens showed that the highest isolation rates of 6/28 (21.42%) and 5/29 (17.24%), in the years 2002 and 2003 respectively, were from children aged 0-9 years.

Vibrio cholerae, *Shigella* spp. and *Salmonella* spp. were isolated from 98, 41 and 9 out of 877 stool specimens respectively (Table 2). Mixed infections of these enteric pathogens were not detected. *Salmonella* spp. and *Shigella* spp. were encountered throughout the whole year whereas *Vibrio cholerae* were isolated only during the months of April to June across the 3 year period. The monthly distribution of these enteropathogens is shown in Table 3. A year-to-year variation was observed in the type of prevalent organism with *Shigella* being the most prevalent in 2002 (12/20; 60%) and in 2003 (8/15; 53.33%). In 2004, a large increase in the number of *Vibrio cholerae* isolates was observed compared to those in 2002 and 2004, indicating an outbreak of cholera (Table 2). All *Vibrio cholerae* isolates were identified as belonging to serogroup O1, El Tor biotype and Ogawa serotype. Out of 41 *Shigella* spp., *Shigella dysenteriae* was the most common (26/41; 63.41 %) followed by *Shigella flexneri* (9/41; 21.95 %), while *Shigella boydii and Shigella sonnei* accounted for 3/41 (7%) each. Of the 26 *Shigella dysenteriae* isolated, 6 (24.39%) were identified to be *Shigella dysenteriae* type-1. Only nine isolates of *Salmonella* were found during the study

Table 1. Age-wise distribution of total and enteropathogen positive

Age in yrs	2002		2003		2004		Total	
	No. of Stool samples	No. of Culture positive	No. of Stool samples	No. of Culture positive	No. of Stool samples	No. of Culture positive	No. of Stool samples	No. of Culture positive
<10	28	6	29	5	42	8	99	19
10-19	39	4	36	3	54	15	129	22
20-29	48	3	42	3	83	37	173	43
30-39	55	2	39	2	85	31	179	35
40-49	37	2	31	1	67	10	135	13
≥50	54	3	30	1	78	12	162	16

period of which five were *Salmonella* Typhi, three were *S.* Paratyphi A and one was *S.* Enteritidis.

Antimicrobial susceptibility profile

All *Vibrio cholerae* isolated were resistant to nalidixic acid, but remained susceptible to tetracycline and ciprofloxacin (Table 4). Resistance rates for furazolidone and erythromycin varied during the study period. Cotrimoxazole resistance gradually increased from 35% in 2002 to 100% in 2004 (Table 4). All the *Shigella* isolates in the study were susceptible to ceftriaxone (Table 4). Nalidixic acid resistance in *Shigella* isolates increased from 43% in 2002 to 55% in 2004. No ciprofloxacin resistance was observed among the *Shigella* spp. isolated in 2002, but in the years 2003 and 2004 ciprofloxacin resistance was seen among 20% and 24% of the isolates. Serological typing identified all the ciprofloxacin resistant *Shigella* isolates to be *Shigella dysenteriae* type-1. All nine *Salmonella* isolates were susceptible to ciprofloxacin and ceftriaxone (Table 4). Out of five *Salmonella* Typhi identified, three were MDR showing simultaneous resistance to ampicillin, chloramphenicol, cotrimoxazole and nalidixic acid.

Discussion

Intestinal enteropathogens which cause gastroenteritis are major public health problems in developing countries, especially among children and the elderly. The present study showed that the enteropathogenic bacteria were almost equally distributed in both the genders. The majority of the bacterial enteropathogens encountered were from patients aged 20 to 29 years. This result is in agreement with recent outbreaks of diarrhoeal diseases in the western part of Nepal, which has reported that adults aged 15 to 44 years were most affected with equal impact on males and females [13]. Another report from Kavrepalanchok district, a region near Kathmandu valley, also found the most common age group to be 11 to 20 years, followed by ages 21 to 30 years [14]. However, our results contrast those of other studies [6,15] which report diarrhoeal diseases to be prevalent in children. One of the major reasons for this difference is associated with the predominance of samples received from patients above nine years old. However, the yearly breakdown showed that the majority of isolates were from the children aged 0 to 9 years in the years 2002 and 2003. This overall higher distribution of entropathogens in the adult age group is due to the cholera outbreak in Kathmandu [16] in 2004, that led to a large number of adult patients seeking treatment at Sukraraj Tropical Hospital which referred stool samples for laboratory investigation to NPHL and resulted in the high number of *Vibrio cholerae* isolated in the current study.

Table 2. Distribution of *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. in 2002, 2003 and 2004

Year	Number of isolates			
	Total samples received (Culture positive)	*Vibrio cholerae*	*Shigella* spp	*Salmonella* spp.
2002	261 (20)	6	12	2
2003	207 (15)	3	8	4
2003	409 (113)	89	21	3
Total	877 (148)	98	41	9

Table 3. Monthly distribution of enteropathogenic bacteria in 3 years

Month	*Vibrio cholerae*			*Salmonella* spp.			*Shigella* spp.			Total
	2002	2003	2004	2002	2003	2004	2002	2003	2004	
Jan	-	-	-	-	-	-	1	-	-	1
Feb	-	-	-	-	1	-	-	3	1	4
Mar	-	-	-	1	-	-	1	2	1	5
April	-	-	8	-	1	-	-	-	2	12
May	2	2	27	-	-	1	-	-	6	38
Jun	-	-	32	-	1	-	-	1	5	39
July	-	-	20	-	-	-	-	1	4	25
Aug	2	-	1	-	1	-	1	-	1	6
Sep	1	-	1	1	-	-	5	-	2	10
Oct	-	-	-	-	-	1	3	1	-	5
Nov	-	-	-	-	-	1	-	-	1	2
Dec	-	-	-	-	-	-	1	-	-	1
Total	6	3	89	2	4	3	12	8	21	148

Cholera outbreaks in Asian countries have been caused by *V. cholerae* O1 biotype El Tor, specific strains of *V. cholerae* O1 biotype Classical and *V. cholerae* O139 (17). Without adequate appropriate therapy, severe cholera kills approximately half of the affected individuals [18]. In this study, *Vibrio cholerae* which contributed to the cholera epidemic in Kathmandu, Nepal, in 2004 [16] was isolated in the highest frequency (89/113; 78.76%) in 2004, followed by *Shigella* spp. (21/113; 18.5%) (Table 2). All the *Vibrio cholerae* isolates in our study were *Vibrio cholerae* O1 Ogawa biotype El Tor and were 100% susceptible to tetracycline and ciprofloxacin. Ciprofloxacin is widely used in the empirical treatment of cholera, but the emergence of ciprofloxacin resistance in *Vibrio cholerae* from different parts of the world has raised concern [19, 20]. Although no ciprofloxacin-resistant strains were encountered in this study, all the *Vibrio cholerae* isolates were resistant to another quinolone, nalidixic acid. Nalidixic acid resistance can be suggestive of impending ciprofloxacin resistance among *Vibrio cholerae* isolates in Nepal. Hence continuous monitoring is necessary to trace changes in susceptibility patterns and the emergence of resistance to new agents.

Shigellosis in developing countries are commonly caused by *Shigella dysenteriae* and *Shigella flexneri* species and their presence is associated with inadequate sanitation, while *Shigella sonnei* is more prevalent in developed countries [21]. In agreement with this notion, our results also showed that *Shigella dysenteriae* and *Shigella flexneri* were the most prevalent of the four *Shigella* species. Among *Shigella* infections, those caused by *Shigella dysenteriae* type-1 are of major concern because of their potential to cause outbreaks and their high mortality rates. In this regard, our finding that around one fourth of the *Shigella dysenteriae* isolates were *Shigella dysenteriae* type-1 is of special concern. Increasing antimicrobial resistance is also becoming a problem in the treatment of Shigellosis [8]. The majority of *Shigella* species in this study, especially *Shigella dysenteriae*, were also MDR. Widespread use of nalidixic acid as the first-line drug for treatment of shigellosis resulted in the emergence of nalidixic acid resistant strains. After the spread of nalidixic acid resistance, ciprofloxacin was recommended as the first-line treatment for treatment of shigellosis. Multiple antibiotic resistance has been reported among *Shigella* spp. and lately ciprofloxacin resistance has also been reported among *Shigella dysenteriae* type-1 isolates from various countries [21,22]. In this study, ciprofloxacin resistance was also encountered among *Shigella dysenteriae* type-1 strains isolated in the years 2003 and 2004. These ciprofloxacin-resistant strains were MDR, also showing co-resistance to ampicillin, chloramphenicol, cotrimoxazole and nalidixic acid, but were susceptible to ceftriaxone and mecillinam. Of the nine *Salmonella* spp. isolated in this study, three were MDR (showing co-resistance to ampicillin, chloramphenicol and cotrimoxazole) and were identified to be *Salmonella* Typhi.

Table 4. Antimicrobial resistance of *Vibrio cholerae*, *Shigella* spp. and *Salmonella* spp.

Antibiotics	**Number of resistant isolates/total isolates (% resistance)**						
	Vibrio cholerae			*Shigella* spp.			*Salmonella* spp.
	2002	2003	2004	2002	2003	2004	2002-2004
Tetracycline	0/6 (0)	0/3 (0)	0/89 (0)	NT	NT	NT	NT
Ampicillin	NT	NT	NT	8/12 (66.66)	5/8 (75)	15/21 (71.42)	3/9 (33.33)
Nalidixic acid	6/6 (100)	3/3 (100)	89/89 (100)	5/12 (41.66)	4/8 (50)	10/21 (47.62)	4/9 (44.44)
Mecillinam	NT	NT	NT	8/12 (66.66)	4/8 (50)	13/21 (61.9)	NT
Chloramphenicol	NT	NT	NT	NT	NT	NT	3/9 (33.33)
Ciprofloxacin	0/6 (0)	0/3 (0)	0/89 (0)	0/12 (0)	2/8 (25)	5/21 (23.8)	0/9 (0)
Erythromycin	2/6 (33.33)	3/3 (100)	2/89 (2.24)	NT	NT	NT	NT
Cotrimoxazole	2/6 (33.33)	3/3 (100)	89/89 (100)	10/12 (83.33)	6/8 (75)	14/21 (66.66)	3/9 (33.33)
Furazolidone	6/6 (100)	2/3 (66.66)	89/89 (100)	NT	NT	NT	NT
Ceftriaxone	NT	NT	NT	0/12 (0)	0/8 (0)	0/21 (0)	0/9 (0)

NT; Not tested

Similar patterns of multidrug resistance (5.8 % MDR isolates) were encountered in *Salmonella* Typhi by Tamang *et al*. (2007) in Nepal during 2004 to 2006 [23]. All the *Salmonella* isolates in our study remained susceptible to ciprofloxacin and ceftriaxone. Multidrug resistance has been reported in *Salmonella* since 1989 [24] and the spread of multidrug resistance in *Salmonella* species is one of the major therapeutic challenges in the treatment of such infections. The incidence rates of MDR *Salmonella* species were 26% in the United Kingdom and 17% in the United States, but infections have been detected in patients with a recent history of travel to Asian countries [25,26]. In India an even higher percentage of *Salmonella* Typhi reported in 1993 were multiple antibiotic resistant (64.5%) [27].

The identification and management of outbreaks of cholera, *Salmonella* infections and shigellosis in Nepal is still challenging due to limited laboratory facilities in both the government and private sectors, as well as a lack of awareness about diarrhoeal infections and the limited practice of pathogen-directed antimicrobial therapy [28]. In this context, the present study addresses some important issues about diarrhoeal infections and their most common aetiogical bacterial agents at the national reference laboratory of Nepal. In conclusion, our results showed that enteric bacterial infections caused by *Vibrio*, *Salmonella* and *Shigella* are prevalent in Kathmandu Valley. Considering the threat of emerging antimicrobial resistance among these enteric bacterial pathogens, it is important to continue surveillance on these organisms in terms of prevalence, clinical epidemiology, and antimicrobial susceptibility patterns obtained from different hospital and community settings throughout the country.

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References

1. Thapar N and Sanderson IR (2004) Diarrhoea in children: an interface between developing and developed countries. Lancet 363: 641-653.
2. World Health Organization. (2009) Why children are still dying and what can be done. WHO Bulletin Available: http://www.who.int/child_adolescent_health/documents/9789241598415/en/index.html. Accessed March 20, 2009.

3. Sherchand JB, Larsson S, Shrestha MP (1996) Intestinal parasites in children and adults with and without abdominal discomfort. Trop Gastroenterol 17: 15-22.
4. Shrestha KD (1995) Acute diarrhea cases in Nepal during outbreak season, 1994: An epidemiological review, J of Nepal Med Assos 33: 219-231.
5. International Centre for Diarrhoeal Disease Research, Bangladesh (2003) Antimicrobial resistance surveillance on some bacterial pathogens in Nepal: a technical cooperation. Annual report 2003, #31, pp 143-162.
6. Taneja N, Mohan B, Khurana S, Sharma M (2004) Antimicrobial resistance in selected bacterial enteropathogens in North India. Ind J Med Res 120: 39-43.
7. World Health Organization, WHO Bulletin, Drug-resistant *Salmonella*, Available http://www.who.int/ mediacentre/factsheets/fs139/en. Accessed March 20, 2009.
8. International Centre for Diarrhoeal Disease Research, Bangladesh (2004) Surveillance update. Health and Science Bulletin 2: 1-16.
9. Garg P, Chakraborty S, Basu I, Dutta S, Rajendaran K, Bhatt T, Yamasaki S, Bhattacharya S K, Takeda Y, Nair G B, Ramamurthy T (2000) Expanding multiple antibiotic resistance among clinical strains of *Vibrio cholerae* isolated from 1992-7 in Calcutta, India. Epidemiol Infection 124: 393-399.
10. Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol 45: 493-506.
11. Bartoloni A, Pallecchi L, Benedetti M, Fernandez C, Vallejos Y, Guzman E, Villagran AL, Mantella A, Lucchetti C, Bartalesi F, Strohmeyer M, Bechini A, Gamboa H, Rodríguez H, Falkenberg T, Kronvall G, Gotuzzo E, Paradisi F, Rossolini GM (2006) Multidrug-resistant Commensal *Escherichia coli* in Children, Peru and Bolivia. Emerg Infect Dis 12: 907-913.
12. Wright SW, Wrenn KD, Haynes M, Haas DW (2000) Prevalence and risk factors for multidrug resistant uropathogens in ED patients. Am J Emerg Med 182: 143-146.
13. Bhandari GP, Dixit SM, Ghimire U, Maskey MK (2009) Outbreak Investigation of Diarrheal Diseases in Jajarkot. J Nepal Health Res Counc 7: 66-68.
14. Tamang MD, Sharma N, Makaju RK, Sarma AN, Koju R, Nepali N, Mishra SK (2005) An outbreak of El Tor cholera in Kavre district, Nepal. Kathmandu Univ Med J 3: 138-142.
15. Rai K, Sherchand J, Bhatta DR (2004) Study of enteropathogens and its predisposing factors in gastroenteritis suspected children attending Kanti Children Hospital, Kathmandu, Nepal. J Nepal Assos Med Lab Sciences 6: 48-53.
16. Kansakar P, Malla S, Ghimire G (2005) Cholera outbreak in KTM valley in 2004: A review of National Public Health Laboratory Finding, J Nepal Assos Med Lab Sciences 7: 20-23.
17. Faruque SM, Albert MJ, Mekallanos JJ (1998) Epidemiology, genetics and ecology of toxigenic *Vibrio cholerae*. Microbiol Mol Biol Rev 62: 301-314.
18. Sack DA, Sack RB, Nair GB, Siddique AK (2004) Cholera. Lancet 363: 223-233.
19. Mukhopadhyay AK, Basu I, Bhattacharya SK, Bhattacharya MK, Nair GB (1998) Emergence of fluoroquinolone resistnace in strains of *Vibrio cholerae* isolated from Hospitalised patients with acute diarrhea in Calcutta, India. Antimicrob Agents Chemother 42: 206-207.
20. Pancharren C, Mekmullica J, Niwattanakanjana N, Chongrisawat V (2004) Hospital based epidemiology of childhood cholera: A 6-year review in a University Hospital in Bangkok, Thailand. J Med Assos Thai 87: 59-61.
21. Sire JM, Macondo EA, Claude JD, Siby T, Bahsoun I, Seck A, Garin B (2008) Antimicrobial resistance in *Shigella species* isolated in Dakar, Senegal (2004-2006). Jpn J Infect Dis 61: 307-308.
22. Dutta S, Ghosh A, Ghosh K, Dutta D, Bhattacharya S K, Nair G B, Yoshida S (2003) Newly emerged Multiple-Antibiotic-Resistant *Shigella dysenteriae* Type 1 Strains in and around Kolkata, India, are clonal. J Clin Microbiol 41: 5833–5834.
23. Tamang MD, Oh JY, Seol SY, Kang HY, Lee JC, Lee YC, Cho DT, Kim J (2007) Emergence of multidrug-resistant *Salmonella enterica* serovar Typhi associated with a class 1 integron carrying the dfrA7 gene cassette in Nepal. Int J Antimicrob Agents 30: 330-335.
24. Shanahan PM, Jesudason MV, Thomson CJ, Amyes SGB (1998) Molecular Analysis of and Identification of Antibiotic Resistance Genes in Clinical Isolates of *Salmonella* Typhi in India. J Clin Microbiol 36: 1595-1600.
25. Ackers ML, Puhr ND, Tauxe RV, Mintz ED (2000) Laboratory based surveillance of *Salmonella serotype* Typhi infections in the United States: antimicrobial resistance on the rise. JAMA 283: 2668-2673.
26. Thretfall EJ and Ward LR (2001) Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi, United Kingdom. Emerg Infect Dis 7: 448-450.
27. Pillai PK and Prakash K (1993) Current status of drug resistance and phage types of *Salmonella* Typhi in India. Indian J Med Res 97: 154-158.
28. Dixit SM and Maskey M (2010) Diarrheal outbreaks in Nepal: A scientific perspective. Republicaopinion Available: http://www.myrepublica.com/portal/index.php?action=news_details & news_id=15949**)** Accessed March 20, 2009.

Corresponding author

Ms. Palpasa Kansakar
Department of Microbiology
Faculty of Science, Mahidol University
272 Rama VI Road, Ratchathewi District
Bangkok, Thailand
Email:palpasa_k@hotmail.com

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