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Full Length Research Paper

In vitro susceptibility of some antimicrobials against bacteria enteropathogens

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To investigate the current resistant levels of enteric bacteria in southwestern China, broth dilution method was used to detect *in vitro* susceptibility of rifaximin, ciprofloxacin, gatifloxacin, azithromycin and trimethoprim/sulfamethoxazole (SMZ/TMP) against 101 enteropathogens isolated from cases of bacterial diarrhea of four teaching hospitals. For all the isolates, ciprofloxacin, gatifloxacin and SMZ/TMP had MIC_{90}s of 64, 32 and >128 mg/L, respectively, much higher than CLSI resistance cutoffs. Azithromycin also had very high MIC_{90} value of >128 mg/L. The MIC_{90} of rifaximin was 64 mg/L, much lower than the intestinal lumen concentration measured after administration of this agent. Diarrheagenic *Escherichia coli* were highly resistant to fluoroquinolones with resistant frequency of >40%. The *in vitro* activity of ciprofloxacin, gatifloxacin and azithromycin on *Shigella* spp was of concern with the MIC_{90}s of 64, 16 and 64 mg/L, respectively. The majority of *Salmonella* spp isolated retained susceptibility to the antimicrobials tested. Comparing with other regions or countries, resistance rates of enteropathogens were much higher in this area of China. Rifaximin is a promising treatment and chemophylaxis for nonsystemic bacteria diarrhea.

Key words: Antimicrobial, infectious diarrhea, enteropathogen, susceptibility.

INTRODUCTION

Infectious diarrhea is the most common infectious disease in China with an annual incidence of 8.36 hundred million patients (China CDC, 2008). Food or water contamination is one of the most important reasons. Bacterial agents are responsible for 80% of such cases (DuPont et al., 2001). Diarrheagenic *E. coli*, *Shigella* spp., *Salmonella* spp, and *Proteus* spp are the frequently isolated enteropathogens. Antimicrobial agents are often recommended for shortening duration, reduction of symptomatology and bacterial excretion in the environment as well as for prevention. Antibacterial agents currently often used include ciprofloxacin, gatifloxacin, azithromycin and SMZ/TMP. Recently, rifaximin has been recommended to be a promising choice for treatment of infectious diarrhea (Koo and DuPont, 2010). It is notable that the majority of antimicrobials can be easily purchased over-the-counter in China, which is also widely used in the veterinary population. Thus bacteria resistance has evolved rapidly in China. The aim of our investigation was to investigate on the *in vitro* susceptibility of these five antimicrobials against the enteropathogens isolated from four teaching hospitals in southwestern China and provide optimal selection of empirical antimicrobial administration for gastrointestinal infection.

MATERIALS AND METHODS

Sources of bacteria

The pathogens were isolated from the fresh stool samples of pre-treatment patients, ranging from 18 to 65 years old, with community-acquired diarrhea of four teaching hospitals located in southwestern China. And the samples were processed within 2 h after collection. These pathogens were identified according to the biochemical methods previously described (Flores et al., 1999). Oligonucleotide DNA probes and PCR assay were used for further determination of the pathotypes of Diarrheagenic *E. coli* (DEC) (Gunzburg et al., 1995; Schultsz et al., 1994; Sethabutr et al., 1994; Vargas et al., 1998; Nataro and Kaper, 1998). Diarrheagenic strains

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Table 1. Range, MIC_{50}, MIC_{90} (mg/L) of 5 antimicrobials against101 enteropathogens.

Antimicrobials	MIC_{50}	MIC_{90}	MICr
Rifaximin	32	64	0.5 to >128
Ciprofloxacin	0.5	64	<0.03 to >128
Gatifloxacin	0.25	32	<0.03 to 128
Azithromycin	16	>128	<0.03 to >128
SMZ/TMP	64	>128	<0.03 to >128

MIC_{50}: MIC for ≥50% of strains tested; MIC_{90}: MIC for ≥90% of strains tested; MICr: MIC range.

of *E. coli* can be divided into at least five different categories with corresponding distinct pathogenic schemes, including enterotoxigenic, enteroinvasive, enteropathogenic, enteroaggregative and enterohemorrhagic *E. coli*.

MIC assay

MICs of rifaximin, ciprofloxacin, gatifloxacin, azithromycin and SMZ/TMP were determined by broth dilution method at least triplicate using anti-bacterial determiner (SAKUMA, Japan). Final concentrations of these drugs ranged from <0.03 to >128 mg/L. Susceptibilities were determined to the 5 antimicrobials with break points set by CLSI 2009 (Clinical and Laboratory Standards Institute, 2009). MICs of the antimicrobials were determined at 24 h by scoring the lowest concentration at which no growth was observed. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcu aureus* ATCC25923 and *Enterococcus faecalis* ATCC29212 was used as a quality control strains. All the antimicrobials were gifted by the manufacturers in China.

Statistical methods

Chi-square (χ^2) was used to test the hypotheses, with *p* values of less than 0.05 considered to be significantly different.

RESULTS

A total of 101 pathogens were isolated including 37 DEC (36.63%, including 19 Enterotoxic *E. Coli* (ETEC), 14 Enteropathogenic *E. Coli* (EPEC) and 4 Enteroinvasive *E. Coli* (EIEC)), 28 *Shigella* strains (27.72%, including 14 *S. flexneri*, 8 *S. sonnei*, 4 *S. boydii* and 2 *S. dysenteriae*), 18 *Salmonella* spp (17.82%, 11 *Salmonella* causing enteric fever, 7 non-*typhi Salmonella* spp.), 9 *Proteus* spp (8.9%), 3 *S. aurueus*, 3 *Enterococcus* spp, 1 *Aeromonos* spp and 1 *pseudomonos* spp.

MIC_{50}, MIC_{90} and the range of MIC for five antimicrobials tested against all the isolated strains were listed in Table 1. All the tested antimicrobials had high MIC_{90}s values of equal to 32 to >128 mg/L. Gatifloxacin had the lowest MIC_{50} and MIC_{90} values of 0.25 and 32 mg/L, respectively. As a traditional antimicrobial for bacteria diarrhea, SMZ/TMP had the highest MIC_{50} and MIC_{90} values of 64 and >128 mg/L, respectively.

Tables 2 and 3 showed the susceptibility profile and resistance rates of the antimicrobials against specific enteropathogens. The MIC_{50} of rifaximin ranged between 16 and 32 mg/L and MIC_{90} ranged from 64 to >128 mg/L against the isolated gram-negative enteropathogens in this study. Ciprofloxacin and gatifloxacin had the MIC_{50} values of <0.03 to 1 mg/L and <0.03 to 2 mg/L, respectively. However, the MIC_{90}s for both drugs were higher than their resistant break points set by CLSI. Azithromycin had MIC_{50}s of 8 to 16 mg/L and MIC_{90}s of 32 to >128 mg/L for *Shigella* spp., ETEC, EPEC, *Salmonella* spp and *Proteus* spp. As for SMZ/TMP, it was highly resistant to *Shigella* spp, EPEC and *Proteus* spp with MIC_{50} and MIC_{90} values of >128 mg/L. But it was very active to *Salmonella* spp. (both MIC_{50} and MIC_{90} equaled to 0.5 mg/L). Since there are no evaluation criteria of CLSI for rifaximin and azithromycin, resistance levels of these bacteria on ciprofloxacin, gatifloxacin and SMZ/TMP were analyzed: the overall resistance level of ciprofloxacin for DEC was as high as to 45.95% (17/37), which was significantly higher than that for *Shigella* spp. (21.43%, and half of the resistant strains were *S. flexneri*) or *Salmonella* spp. (16.7%, all of the resistant strains were *Salmonella* causing enteric fever) (p<0.05). The overall resistance level of gatifloxacin for DEC was also as high as to 43.24% (16/37), significantly higher than that for *Shigella* spp. (14.29%) (p<0.05) The resistance levels of SMZ/TMP to DEC, *Shigella* spp and *Proteus* spp were 45.95% (16/37), 75% (21/28) and 100% (9/9) respectively, which were significantly higher than that to Salmonella spp. (16.7%).

Rifaximin was active against the isolated gram-positive strains including *S. aureus* and *E. faecalis* with MIC range of 0.25 and 0.5 to 1 mg/L, respectively. Gatifloxacin was 8 -fold more potent than ciprofloxacin against *S. aureus*. And both were resistant to *E. faecalis* with MIC value more than 8 mg/L. *S. aureus* was susceptible to either azithromycin or SMZ/TMP (MICr=0.25-5). Azithromycin did not show much activity against *E. faecalis* isolated (MICr>128 mg/L), but SMZ/TMP still maintained partial activity on the species.

Furthermore, there were 17 multidrug resistant (MDR) strains (resistant to three antimicrobials tested) among 37 DEC, accounting for 45.95%, followed by *Proteus* spp with 33.3% (3/9) MDR isolates. The incidence of MDR strains in *Shigella* spp and *Salmonella* spp was 14.29% (4/28) and 16.67% (3/18), respectively.

DISCUSSION

The majority of the isolated bacteria in our study were non-conditional enteropathogens, accounting for 83.17%, among which DEC ranked the first place with a rate of 36.63%, followed by *Shigella* spp and *Salmonella* spp. No C*ampylobacter jejuni*, a common enteropathogen in many South-east Asian countries, were isolated in this study. The ideal antimicrobials for management of bacterial diarrhea should be broad-spectrum, low

Table 2. Range, MIC_{50}, MIC_{90} (mg/L) and resistance frequency of enteropathogens (n≥9).

Enteropathogens	***Shigella* spp. n=28**	**ETEC n=19**	**EPEC n=14**	***Salmonella* spp n=18**	***Proteus* spp n=9**
Rifaximin					
MIC_{50}	32	32	32	32	16
MIC_{90}	64	64	>128	64	>128
MICr	4 to >128	8 to >128	4 to >128	4 to 64	2 to >128
Ciprofloxacin (cutoff 4mg/L)					
MIC_{50}	0.5	0.125	1	<0.03	0.5
MIC_{90}	64	128	>128	32	64
MICr	<0.03 to >128	<0.03 to >128	<0.03 to 128	<0.03 to 64	<0.03 to 32
R%	21.43	31.58	50	16.7	33.3
Gatifloxacin (cutoff 8mg/L)					
MIC_{50}	0.125	0.125	2	≤0.03	0.25
MIC_{90}	16	32	64	8	8
MICr	<0.03 to 64	<0.03 to 128	<0.03 to 64	<0.03~32	<0.03 to 16
R%	14.29	31.58	42.86	16.7	33.3
Azithromycin					
MIC_{50}	8	16	16	16	16
MIC_{90}	64	>128	>128	32	>128
MICr	2 to >128	4 to >128	8 to >128	<0.03~>128	2 to >128
SMZ/TMP (cutoff 8/152mg/L)					
MIC_{50}	>128	0.5	64	0.5	>128
MIC_{90}	>128	>128	>128	0.5	>128
MICr	0.06 to >128	0.06 to >128	0.125 to >128	<0.03~>128	8 to >128
R%	75	42.11	57.14	16.7	100

Table 3. MIC Range (mg/L) of isolated enteropathogens (n<9).

Enteropathogens	**EIEC n=4**	***Pseudomonas* spp n=1**	***Aeromonas* spp n=2**	***Staphylococcus aureus* n=3**	***Enterococcus faecalis* n=3**
Rifaximin					
MICr	32 to 64	32	2 to 8	0.25	0.5 to 1
Ciprofloxacin					
MICr	32 to 128	0.125	<0.03 to 8	0.25 to 8	32 to 64
Gatifloxacin					
MICr	8 to 16	1	<0.03 to 0.125	≤0.03 to 1	16
Azithromycin					
MICr	8 to >128	>128	1 to 8	1	>128
SMZ/TMP					
MICr	0.125 to >128	64	0.25 to 8	0.25 to 5	0.06 to >128

potential to develop resistance, poor absorption with limited values outside gastrointestinal system and good

tolerability. SMZ/TMP and fluoroquinolones are traditional and current antimicrobial treatment of choice for bacteria diarrhea, respectively (Thielman and Guerrant, 2004). But *in vitro* susceptibility results here revealed that the total MIC_{90} values of ciprofloxacin (64 mg/L) and gatifloaxin (32 mg/L) were far above the CLSI cutoffs. Azithromycin, a broad-spectrum azilide with high intracellular concentrations and a prolonged half-life, has been recommended as the treatment option for fluoroquinolone-resistant *shigella* and *salmonella* (Khan et al., 1997). However, our results suggested that its role as an alternative for fluoroquinolones was doubtable since the MIC_{90} value was above 128 mg/L. Rifaximin is the only rifamycin analogue. It is poorly absorbed and acts as a nonsystemic antibiotic by inhibition of bacterial RNA synthesis. Previous studies have showed that this drug was active against a variety of gram-negative and gram-positive strains (Koo and DuPont, 2010; Koo et al., 2009). The almost non-absorbing attribute greatly contributes to its extra high concentration in the intestinal lumen after normal-dose oral institution, which average 7961 ug/g (Jiang et al., 2000), much higher than the MIC_{90}s of 64 mg/L measured in this study.

The total resistant frequency of DEC to fluoroquinolones was 45.95% in our study, close to that to SMZ/TMP. The increased number of resistant-DEC would cause problems on empirical antimicrobial treatments due to most of the diarrhea illnesses are empirically antimicrobial instituted without stool cultures. There were 31.58% of the ETEC resistant to ciprofloxacin and gatifloxacin, and 50 and 42.86% of the EPEC resistant to the fluoroquinolones tested, respectively, which were significantly higher than those previously reported in other countries or regions (ranged from 1 to 27.8%) (Gomi et al., 2001; Ruiz et al., 2007; Ouyang-Latimer et al., 2011). And the MIC_{90} values of the tested fluoroquinolones were far higher than the CLSI cutoffs for resistance. High-level fluoroquinolone-resistant *E. coli* has been a very serious problem in China, with resistant rates much higher than those of other developing countries (Nguyen et al., 2005; Jafari et al., 2009; Pandey et al., 2011). Although presenting similarly high resistant rates, gatifloxacin, a novel generation fluoroquinolone, displayed relatively lower MIC values against ETEC, EPEC and EIEC compared to ciprofloxacin, which was likely attributed to differences in the additional fluoro-group and other substitutions in its chemical structure. However, it possibly would be an unwise policy to select fluoroquinolones as the first choice of drug to treat DEC-caused infectious diarrhea in China. Individual drug susceptibility test would be warranted for the selection of fluoroquinolones and SMZ/TMP as the treatment for diarrhea. Preliminary reports showed that the MIC_{90}s of azithromycin against ETEC and EAEC were less than 0.0156 to 32 mg/L (Gomi et al., 2001; Ouyang-Latimer et al., 2011), while our study suggested azithromycin also not an appropriate drug of empirical choice due to very high MIC values (MIC_{90}>128 mg/L) against DEC. The MIC range of Rifaximin was from 32 to 128 mg/L, although higher than those published data in other countries or regions (MICr=0.5~32 mg/L) (13-15), which still possessed the great ability of inhibiting the ETEC and EPEC due to high gut concentrations mentioned above. This was also confirmed by good efficacy of different clinical trials (DuPont et al., 2001; Taylor et al., 2006). Thus rifaximin is likely particularly useful in the area with high frequency of fluoroquinolone-resistant DEC (except EIEC due to its invasive attribution).

Of the isolated *Shigella* spp, *Shigella flexneri* were the predominant serotype like in other developing countries (Ahmed et al., 2000; Anh et al., 2001; Brooks et al., 2003) with an occurrence rate of 50%. *Shigella* is a highly infectious pathogen. Antimicrobials are necessary for those confirmed patients suffered from shigellosis. Fluoroquinolones are broad spectrum, with high fecal concentration and have favorable safety profile. Also the resistance of *Shigella* spp. is generally caused by plasmids while fluoroquinolones can eliminate plasmids. Therefore they are listed as the first line antimicrobial against shigellosis, but also widely used on the treatment of other infections.

The *in vitro* susceptibility results here indicated that the resistance frequency of ciprofloxacin was above 20%, and even the new generation fluoroquinolone-gatifloxacin – 14.29%. It is possibly due to cross resistance. Both MIC_{90} values are higher than the CLSI cutoffs. It is indeed a problem in this area of China compared with other regions or countries, where *Shigella* spp were still highly susceptible to fluoroquinolones with very low MIC_{90} values (Ouyang-Latimer et al., 2011). Therefore it is essential of the selective administration to decrease the resistance development of this agent. Azithromycin here, with MIC_{90} >128 mg/L, also may not be the treatment option for shigellosis caused by fluoroquinolone-resistant *shigella*. An early report showed that the MIC_{90} of azithromycin to *shigella* was only 0.5mg/L (Gomi et al., 2001). The most recent MIC_{90}s about ciprofloxacin and azithromycin on *Shigella* spp. were just 0.125 and 2mg/L, respectively (Ouyang-Latimer et al., 2011). SMZ/TMP cannot be used to treat shigellosis because poor *in vitro* activity. The efficacy of rifaximin for *Shigella* spp, the invasive enteric pathogen, is limited due to its non-absorbable attribution, which is supported by clinical trials (Ahmed et al., 2000). But the prophylactic use in certain population may deserve to have a try for preventing *Shigella* invading luminal wall.

All of the non-*typhi Salmonella* spp. in this study was highly sensitive to the antimicrobials tested. As for *Salmonella* causing enteric fever, 3 of the 11 isolates demonstrated resistance to ciprofloxacin and gatifloxacin. Gatifloxacin displayed lower MIC_{90} (8 mg/L) values compared with ciprofloxacin (32 mg/L). Although published data also suggested the better *in vitro* activity of gatifloxacin (Capoor et al., 2007), further evaluation is

required of the clinical response and the alternative usage should be cautious due to the possibility of cross resistance. Our findings did not consist with other group's results, which showed a much lower fluoroquinolone MIC_{90}s of less than 0.03 mg/L (Ouyang-Latimer et al., 2011). In this study, the MIC_{90} of azithromycin was 32 mg/L for all the isolated S*almonella* spp, and only one *Salmonella enterica* displayed a high MIC of >128 mg/L. This was higher than previous reports, but azithromycin has very high intra-macrophage concentration, which is very favorable to inhibit intracellular *Salmonella*. And it is especially valuable for pediatric patients for whom fluoroquinolones are contraindicated (Girgis et al., 1999). Thus *in vitro* MIC values may not truly reflect the intracellular concentrations of this agent. Rifaximin would be also suitable for prevention of *Salmonella* infection rather than treatment due to the invasive characteristics of this species. SMZ/TMP still retained highly *in vitro* activity for *Salmonella* spp., with the resistance frequency comparable to fluoroquinolones.

Proteus spp are conditional pathogens causing diarrhea. Three of the resistant P*roteus* spp isolated here were *P. vulgaris*, which are intrinsic resistant to a variety of antibacterials. The other *Proteus* spp still kept considerably high sensitivity to the antimicrobials tested except SMZ/TMP.

Taken together, high resistance levels to the enteropathogens compromises the therapeutic use of SMX/TMP. The new agent rifaximin, with low system absorption and little effect on the intestinal microflora, may be used to treat uncomplicated cases or considered as a chemoprophylaxis, leaving fluoroquinolones and/or azithromycin for use in more severe cases or when invasive pathogens are suspected but susceptibility tests should be conducted before administration.

This study was limited by its relatively small sample size, although these isolates were collected from four teaching hospitals, and may not necessarily be representative of the epidemiological strains of diarrhea in China. Also we did not evaluate the resistance level of cephalosporins against the enteropathogens. But to some degree our investigation offers data for comparison in different regions or countries. The antimicrobials tested here had significant higher MIC values compared to those of previous reported data, and almost half of the enteropathogens isolated were multidrug resistant. Therefore, these observations highlight the importance of continuing surveillance in China for resistance in enteric bacteria.

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REFERENCES

Ahmed AA, Osman H, Mansour AM, Musa HA, Ahmed AB, Karrar Z, Hassan HS (2000). Antimicrobial agent resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. Am. J. Trop. Med. Hyg., 63: 259-263.

Anh NT, Cam PD, Dalsgaard A (2001). Antimicrobial resistance of *Shigella* spp isolated from diarrheal patients between 1989 and 1998 in Vietnam. Southeast. Asian. J. Trop. Med. Public Health 32: 856-862.

Brooks JT, Shapiro RL, Kumar L, Wells JG, Phillips-Howard PA, Shi YP, Vulule JM, Hoekstra RM, Mintz E, Slutsker L (2003). Epidemiology of sporadic bloody diarrhea in rural Western Kenya. Am. J. Trop. Med. Hyg., 68: 671-677.

Capoor MR, Rawat D, Nair D, Hasan AS, Deb M, Aggarwal P, Pillai P (2007). *In vitro* activity of azithromycin, newer quinolones and cephalosporins in ciprofloxacin-resistant Salmonella causing enteric fever. J. Med. Microbiol., 56: 1490-1494.

Center for Public Health Surveillance and Information Service, China CDC (2008).National data of class A, B and C infectious diseases in October 2008. Disease Surveillance. 23 (11). DOI:10.3784/j.issn.1003-9961.2008.11.002

Clinical and Laboratory Standards Institute (2009). Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16. Clinical and Laboratory Standards Institute, Wayne, P.A.

DuPont HL, Jiang ZD, Ericsson CD, Adachi JA, Mathewson JJ, DuPont MW, Palazzini E, Riopel LM, Ashley D, Martinez-Sandoval F (2001). Rifaximin versus ciprofloxacin for the treatment of traveler's diarrhea: a randomized, double-blind clinical trial. Clin. Infect. Dis., 33: 1807-1815.

Flores AJ, Suárez HG, Heredia NM, Puc FM, Vivas RM (1999). Four biochemical tests for identification of probable enteroinvasive *Escherichia coli* strains. Rev. Latinoam. Microbiol., 41: 259-261.

Girgis NI, Butler T, Frenck RW, Sultan Y, Brown FM, Tribble D, Khakhria R (1999). Azithromycin versus ciprofloxacin for treatment of uncomplicated typhoid fever in a randomized trial in Egypt that included patients with multidrug resistance. Antimicrob. Agents. Chemother., 43: 1441-1444.

Gomi H, Jiang ZD, Adachi JA, Ashley D, Lowe B, Verenkar MP, Steffen R, DuPont HL (2001). *In vitro* antimicrobial susceptibility testing of bacterial enteropathogens causing traveler's diarrhea in four geographic regions. Antimicrob. Agents. Chemother., 45: 212-216.

Gunzburg ST, Tornieporth NG, Riley LW (1995). Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. J. Clin. Microbiol., 33: 1375-1377.

Jafari F, Hamidian M, Rezadehbashi M, Doyle M, Salmanzadeh-Ahrabi S, Derakhshan F, Reza Zali M (2009). Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and *Shigella* species associated with acute diarrhea in Tehran, Iran. Can. J. Infect. Dis. Med. Microbiol., 20: e56-e62.

Jiang ZD, Ke S, Palazzini E, Riopel L, Dupont H (2000). *In vitro* activity and fecal concentration of rifaximin after oral administration. Antimicrob. Agents. Chemother., 44: 2205-2206.

Khan WA, Seas C, Dhar U, Salam MA, Bennish ML (1997). Treatment of shigellosis: V. Comparison of azithromycin and ciprofloxacin. A double-blind, randomized, controlled trial. Ann. Intern. Med., 126: 697-703.

Koo HL, DuPont HL (2010). Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases. Curr. Opin. Gastroenterol., 26: 17-25.

Koo HL, Dupont HL, Huang DB (2009). The role of rifaximin in the treatment and chemoprophylaxis of travelers' diarrhea. Ther. Clin. Risk. Manage., 5: 841-848.

Nataro JP, Kaper JB (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Infect., 11:142-201.

Nguyen TV, Le PV, Le CH, Weintraub A (2005). Antibiotic resistance in diarrheagenic *Escherichia coli* and *Shigella* strains isolated from children in Hanoi, Vietnam. Antimicrob. Agents. Chemother.,

49: 816-819.
Ouyang-Latimer J, Jafri S, VanTassel A, Jiang ZD, Gurleen K, Rodriguez S, Nandy RK, Ramamurthy T, Chatterjee S, McKenzie R, Steffen R, DuPont HL (2011). *In vitro* antimicrobial susceptibility of bacterial enteropathogens isolated from international travelers to Mexico, Guatemala, and India from 2006 to 2008. Antimicrob. Agents. Chemother., 55: 874-878.
Pandey P, Bodhidatta L, Lewis M, Murphy H, Shlim DR, Cave W, Rajah R, Springer M, Batchelor T, Sornsakrin S, Mason CJ (2011). Travelers' diarrhea in Nepal: an update on the pathogens and antibiotic resistance. J. Travel. Med., 18: 102-108.
Ruiz J, Mensa L, O'Callaghan C, Pons MJ, González A , Vila J, Gascón J (2007). *In vitro* antimicrobial activity of rifaximin against enteropathogens causing traveler's diarrhea. Diagn. Microbiol. Infect. Dis., 59: 473-475.
Schultsz C, Pool GJ, van Ketel R, de Wever B, Speelman P, Dankert J (1994). Detection of enterotoxigenic *Escherichia coli* in stool samples by using nonradioactively labeled oligonucleotide DNA probes and PCR. J. Clin. Microbiol., 32: 2393-2397.
Sethabutr O, Echeverria P, Hoge CW, Bodhidatta L, Pitarangsi C (1994). Detection of Shigella and enteroinvasive *Escherichia coli* by PCR in the stools of patients with dysentery in Thailand. J. Diarrhoeal. Dis. Res., 12: 265-269.
Taylor DN, Bourgeois AL, Ericsson CD, Steffen R, Jiang ZD, Halpern J, Haake R, Dupont HL (2006). A randomized, double-blind, multicenter study of rifaximin compared with placebo and with ciprofloxacin in the treatment of travelers' diarrhea. Am. J. Trop. Med. Hyg., 74: 1060-1066.
Thielman NM, Guerrant RL (2004). Clinical practice. Acute infectious diarrhea. N. Engl. J. Med., 350: 38-47.
Vargas M, Gascón J, Gallardo F, Jimenez De Anta MT, Vila J (1998). Prevalence of diarrheagenic *Escherichia coli* strains detected by PCR in patients with travelers' diarrhea. Clin. Microbiol. Infect., 4: 682-688.