CLASS 1 INTEGRONS AND MULTIDRUG RESISTANCE AMONG *ESCHERICHIA COLI* ISOLATES FROM HUMAN STOOLS

Souwalak Phongpaichit, Kornvika Wuttananupan and Wiwit Samasanti

Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Abstract. Three hundred and eighteen *Escherichia coli* isolates from stools of healthy volunteers and outpatients from a major university hospital in southern Thailand were tested for the presence of class 1 integrons using multiplex-PCR and for their susceptibility against 12 antimicrobial agents using standard disc diffusion method. Based on the presence of *intI1*, 162 isolates harbored class 1 integrons, which were more prevalent in isolates from outpatients compared with those from healthy volunteers. The majority (85%) of the isolates were resistant to at least one antimicrobial agent with the following percent resistance: streptomycin 66 %, tetracycline 60%, sulphamethoxazole 59%, ampicillin 52%, trimethoprim/sulphamethoxazole 47%, kanamycin 30%, nalidixic acid 27%, ciprofloxacin 23%, norfloxacin 22%, amoxicillin/clavulanic acid 16%, gentamicin 8%, and amikacin 2%. The most frequent pattern of multiresistant strains (11%) was sulphamethoxazole- trimethoprim/sulphamethoxazole -ampicillin-tetracycline-streptomycin. Multiple drug resistance was more frequent in integron-positive isolates (89%) than those in integron-negative *E. coli* (57%). These data indicate that human fecal *E. coli* is a reservoir of antibiotic-resistant genes that poses a significant risk of the spread of microbial resistance in the community.

INTRODUCTION

The emergence of antibiotic resistance among pathogenic and commensal bacteria has become a serious problem worldwide (Barlow *et al*, 2004). Normal intestinal microbiota are a reservoir for resistance genes, especially *Escherichia coli* because this species can occupy multiple niches, including human and animal hosts. The prevalence of resistance in commensal *E. coli* is a useful indicator of antibiotic resistance in bacteria in the community (Levy *et al*, 1988; Levin *et al*, 1997). The dissemination of antibiotic resistance genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among many bacteria (Ploy *et al*, 2000).

Commensal *E. coli* strains efficiently exchange genetic material with other pathogens such as *Salmonella*, *Shigella*, *Yersinia* and *Vibrio*, as well as pathogenic *E. coli* (Tauxe *et al,* 1989). Recently this exchange of many different and diverse genes responsible for antibiotic resistance has been linked to genetic structures called integrons, that integrate and mobilize individual gene cassettes encoding antibiotic resistance determinants (Recchia and Hall, 1995). Class 1 integrons are strongly associated with multiresistance seen in Enterobacteriaceae in the hospital environment (Martinez-Freijo *et al*, 1998). Integrons are also present in resistant intestinal *E. coli* isolated from subjects living in the community (Leverstein-van Hall *et al*, 2002; Skurnik *et al*, 2005).

Correspondence: Souwalak Phongpaichit, Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.
Tel/Fax: 66 (074) 446661
E-mail: souwalak.p@psu.ac.th

Resistance to antibiotics has a high prevalence in bacterial isolates from developing countries due to the overuse and improper use of antibiotics (Hart and Kariuki, 1998; Okeke *et al*, 2005). The aim of this study was to investigate the prevalence of class 1 integrons and antibiotic resistance in commensal *E. coli* isolates from stool samples of healthy subjects living in Songkhla Province and from outpatients at the major university hospital in southern Thailand.

MATERIALS AND METHODS

Bacterial isolations

Of the total of 318 *E. coli* isolates from human stool samples included in the study, 143 isolates were obtained from healthy volunteers, who had not taken antibiotics for at least one month and 175 isolates from stool samples of outpatients submitted for routine culture to the Microbiology Laboratory, Songklanagarind Hospital, which is the major teaching hospital in southern Thailand. All collections took place during the period of April 2004 to June 2005. All isolates were recovered from feces using standard microbiological procedures (Forbes *et al*, 2002). Biochemical confirmation of the strains was performed according to the bacteriological analytical manual completed test for *E. coli* (Feng *et al*, 2002). All isolates were stored in 10% glycerol at -70ºC until use.

PCR detection of class 1 integrons

Integrons were detected using multiplex PCR, targeting three conserved sequences of class 1 integrons (*intI1, qacEΔ1,* and *sul1*), as adapted from Ebner *et al* (2004). Primer pairs were purchased from a commercial source (QIAGEN Operon GmbH, Cologne, Germany). Primers, (reported from 5′ to 3′) included GGTTCGAATGTCGTAACCGC and ACG CCCTTGAGCGGAAGTATC for amplification of *intI1*, ATCAGACGTCGTGGATGTCG and CGAAGAACCGCACAATCTCG for amplification of *sul1,* and GAGGGCTTTACTAAGC TTGC and ATACCTACAAAGCCCCACGC for amplification of *qacEΔ1*. Template DNA was prepared by boiling overnight grown cultures. Boiled cultures were cooled on ice for 5 minutes and aliquots of 1 μl were used immediately for PCR using a PTC-100 Peltier thermocyler (MJ Research, Waltham, MA) with the following thermal cycling: (i) one cycle of 94ºC for 4 minutes; (ii) 10 "touchdown" cycles of 94ºC for 1 minute, 65ºC for 30 seconds (decreasing 1ºC/cycle), 70ºC for 2 minutes; (iii) 24 cycles of 94ºC for 1 minute, 55ºC for 30 seconds, 70ºC for 2 minutes; and (iv) one final cycle of 70ºC for 5 minutes. *Salmonella enterica* Typhimurium DT104, a known carrier of a class 1 integron, was used as positive control. The PCR products were visualized by ethidium bromide staining after agarose gel-electrophoresis. Presence of class 1 integrons was based on the detection of *intI1*.

Antimicrobial susceptibility testing

All isolates were tested for susceptibility to 12 antimicrobials or antimicrobial combinations: ampicillin (AMP), amoxicillin/clavulanic acid (AMC), tetracycline (TET), amikacin (AMK), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), sulphamethoxazole (SMX), trimethoprim/sulphamethoxazole (SXT), nalidixic acid (NAL), norfloxacin (NOR), and ciprofloxacin (CIP) using the Clinical and Laboratory Standards Institute (CLSI) standard disc diffusion method (CLSI, 2004). *E. coli* ATCC25922 was used as reference strain. Results were expressed as susceptible or resistant according to the criteria recommended by the CLSI. Intermediate isolates were counted as resistant to all the agents tested. A multiple drug resistance (MDR) phenotype was defined as resistant to ≥ 2 antimicrobial agents.

Statistical analysis

Comparisons of integron frequency and antimicrobial resistance were performed by a

chi-square test. Difference is considered significant at $p<0.05$.

RESULTS

PCR detection of class 1 integrons

Fig 1 shows a typical result of multiplex-PCR for detecting class 1 integron genes. The incidence of class 1 integrons in commensal *E. coli* from healthy subjects and outpatients were examined (Table 1). Class 1 integron is indicated by the presence of *intI1*, and was detected in 162 isolates (51%). Simultaneous presence of all three conserved genes (*qacEΔ1*, *intI1*, and *sul1*) was found in 74 (23%) of the 318 isolates. Integrons were more prevalent in isolates from outpatients (63%) than in isolates from healthy volunteers (36%).

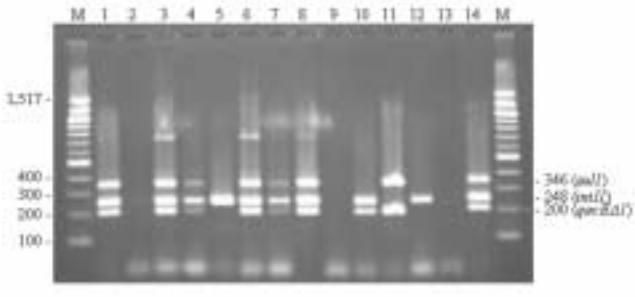

Fig 1–Multiplex-PCR detection of class 1 integron genes. Lane M, 100-bp DNA ladder; lanes 1 and 14, positive control *Salmonella enterica* Typhimurium DT104; lanes 2 and 13, negative control; and lanes 3-12, wild-type isolates. PCR amplification was carried out in 50 μl volumes containing 10 μl of 10X PCR buffer, 1 μl of each primer pair (50 pmole), 5 μl of 2.5 mM dNTPs mix, 1 μl of 0.5 U *Taq* polymerase, 1 μl of DNA template and 30 μl of PCR water.

Antimicrobial susceptibility

Antimicrobial susceptibility of all *E. coli* isolates is shown in Table 2. Overall, the highest percentage of resistance was found to streptomycin (66%), tetracyclines (60%), sulphamethoxazole (59%), ampicillin (52%), and trimethoprim/sulphamethoxazole (47%). Low resistance to quinolones (nalidixic acid 27%, norfloxacin 22%, ciprofloxacin 23%), aminoglycosides (amikacin 3%, gentamicin 9%, kanamycin 30%), and amoxicillin/clavulanic acid (16%) were observed. Resistance to all tested antimicrobial agents was higher in isolates from outpatients than in isolates from healthy subjects ($p<0.05$). However the difference for amikacin was not significant because of the low numbers involved.

Multiple-drug resistance phenotypes and class 1 integrons

Of the 318 *E. coli* isolates in this study, only 46 (14%) were susceptible to all the anti-

Table 1
Frequency of class 1 integron component genes in *E. coli* isolates from healthy volunteers and outpatients.

Gene	Healthy volunteers (*n*=143)	Outpatients (*n*=175)	Total (*n*=318)
		No. (%)	
qacEΔ1 + intI1 + sul1	24 (17)[a]	50 (29)[b]	74 (23)
IntI1	51 (36)[a]	111 (63)[b]	162 (51)
Sul1	24 (17)[a]	61 (35)[b]	85 (27)
None	92 (64)[a]	64 (37)[b]	156 (49)

[a,b] Values within the same row with different letters differ significantly ($p<0.05$)

Table 2
Frequency of resistance to 12 antimicrobial agents in *E. coli* isolates from healthy volunteers and outpatients and of class 1 integron-positive and integron-negative isolates.

Antimicrobial agents	Healthy volunteers (*n*=143)	Outpatients (*n*=175)	p-value	Integron-positive (*n*=162)	Integron-negative (*n*=156)	p-value	Total (*n*=318)
Penicillins							
Ampicillin	50 (35)[a]	114 (65)	<0.001	125 (77)	39 (25)	<0.001	164 (52)
Amoxicillin+clavulanic acid	8 (6)	44 (25)	<0.001	40 (25)	12 (8)	<0.001	52 (16)
Tetracyclines							
Tetracycline	70 (49)	121 (69)	<0.001	131 (81)	60 (38)	<0.001	191 (60)
Aminoglycosides							
Amikacin	1 (1)	7 (4)	≤0.1	7 (4)	1 (1)	<0.05	8 (2)
Gentamicin	4 (3)	23 (13)	<0.001	21 (13)	6 (4)	<0.01	27 (8)
Kanamycin	26 (18)	68 (39)	<0.001	68 (42)	26 (17)	<0.001	94 (30)
Streptomycin	77 (54)	132 (75)	<0.001	147 (91)	62 (40)	<0.001	209 (66)
Sulphonamides							
Sulphamethoxazole	67 (47)	121 (69)	<0.001	133 (82)	55 (35)	<0.001	188 (59)
Trimethoprim/sulphamethoxazole	41 (29)	108 (62)	<0.001	125 (77)	24 (15)	<0.001	149 (47)
Quinolones							
Nalidixic acid	17 (12)	70 (40)	<0.001	46 (28)	30 (19)	<0.01	87 (27)
Norfloxacin	11 (8)	60 (34)	<0.001	46 (8)	25 (16)	<0.01	71 (22)
Ciprofloxacin	13 (9)	59 (34)	<0.001	47 (29)	25 (16)	<0.01	72 (23)

[a]No. (%)

Table 3
Resistance patterns of class 1 integron-positive and class 1 integron-negative *E. coli.*

Resistance patterns (%)	
Integron-positive *E. coli* (*n*=162)	Integron-negative *E. coli* (*n*=156)
SMX-SXT-AMP-TET-STR (21) [a]	SMX-STR (7)
SMX-SXT-AMP-TET-KAN-STR (14) [b]	SMX (6)
SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-STR (7) [c]	TET (6) [k]
SMX-SXT-NAL-NOR-CIP-TET-GEN-KAN-STR (4)	SMX-TET-STR (6) [g]
SMX-SXT-AMP-AMC-TET-STR (3) [d]	STR (4) [f]
SMX-SXT-AMP-AMC-TET-AMK-KAN-STR (3)	SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-KAN-STR (4) [i]
SMX-SXT-NAL-AMP-TET-STR (3)	NAL-NOR-CIP-AMP-STR (3)
NAL-NOR-CIP-AMP-AMC-GEN-KAN-STR (3)	NAL-NOR-CIP-AMP-KAN-STR (3)
SMX-SXT-NAL-NOR-CIP-AMP-TET-GEN-KAN-STR (3) [e]	AMP-STR (2)
STR (2) [f]	AMP-TET (2)
SMX-TET-STR (2) [g]	KAN-STR (2)
SMX-SXT-NAL-NOR-CIP-AMP-KAN-STR (2)	SMX-TET (2)
SMX-SXT-NAL-NOR-CIP-AMP-TET-STR (2) [h]	SMX-TET-KAN-STR (2) [r]
SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-KAN-STR (2) [i]	SMX-SXT-NAL-NOR-CIP-AMP-TET-STR (2) [h]
AMP (1) [j]	KAN (1)
TET (1) [k]	SXT (1)
AMP-TET-STR (1) [l]	SXT-STR (1)
SMX-AMP-TET-STR (1) [m]	TET-STR (1)
SMX-SXT-TET-STR (1)	AMP-KAN-STR (1)
NAL-AMP-AMC-TET-KAN-STR (1)	SMX-AMP-TET (1)
SMX-SXT-NAL-AMP-AMC-TET-STR (1)	SMX-SXT-AMP-TET-STR (1) [a]
SMX-SXT-NAL-AMP-TET-KAN-STR (1) [n]	SMX-SXT-AMP-TET-KAN-STR (1) [b]
SMX-SXT-NAL-NOR-CIP-AMP-STR (1)	SMX-SXT-NAL-NOR-CIP-AMP-TET-GEN-KAN-STR (1) [e]
SMX-SXT-NAL-NOR-CIP-TET-KAN-STR (1)	AMP (16) [j]
SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-GEN-KAN-STR (1) [o]	AMP-AMC (1)
AMP-AMC-STR (1) [p]	NAL-STR (1)
CIP-TET-STR (1)	AMP-AMC-STR (1) [p]
SMX-KAN-STR (1)	AMP-GEN-AMK (1)
SMX-SXT-STR (1)	AMP-TET-STR (1) [l]
TET-KAN-STR (1) [q]	SMX-AMC-TET (1)
AMP-AMC-TET-STR (1)	SMX-SXT-NAL (1)
SMX-TET-KAN-STR (1) [r]	TET-GEN-STR (1)
SMX-NOR-AMP-TET-STR (1)	TET-KAN-STR (1) [q]
SMX-SXT-AMP-GEN-STR (1)	AMP-AMC-KAN-STR (1)
SMX-SXT-CIP-AMP-STR (1)	NAL-NOR-CIP-TET (1)
SMX-SXT-AMP-GEN-KAN-STR (1)	SMX-AMP-KAN-STR (1)
SMX-SXT-AMP-AMC-TET-KAN-STR (1)	SMX-AMP-TET-STR (1) [m]
SMX-SXT-NAL-NOR-CIP-TET-STR (1) [s]	NAL-NOR-CIP-TET-KAN (1)
SMX-SXT-NAL-NOR-CIP-TET-AMK-KAN (1)	SMX-SXT-NAL-TET-STR (1)
SMX-SXT-NAL-AMP-TET-GEN-AMK-KAN-STR (1)	SMX-SXT-AMP-AMC-TET-STR (1) [d]
SMX-SXT-NAL-NOR-AMP-AMC-TET-KAN-STR (1)	SXT-NAL-CIP-AMC-TET-STR (1)
SMX-SXT-NAL-NOR-CIP-AMP-AMC-KAN-STR (1)	SMX-SXT-NAL-AMP-TET-KAN-STR (1) [n]
SMX-SXT-NAL-NOR-CIP-AMC-TET-GEN.KAN-STR (1)	SMX-SXT-NAL-NOR-CIP-TET-STR (1) [s]
	SMX-SXT-NAL-AMP-AMC-TET-KAN-STR (1)
	SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-STR (1) [c]
	SMX-SXT-NAL-NOR-CIP-AMP-GEN-KAN-STR (1)
	SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-GEN-KAN-STR (1) [o]

AMC, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NOR, norfloxacin; SMX, sulphamethoxazole; STR, streptomycin; SXT, trimethoprim/sulphamethoxazole; TET, tetracycline.
[a,b,c,d,...] Resistance patterns found in both class 1 integron-positive and class 1 integron-negative isolates.

microbial agents tested, while 38 (12%), 29 (9%), 30 (9%), 13 (4%), 45 (14%), 44 (14%), and 73 (23%) were resistant to 1, 2, 3, 4, 5, 6, or >6 antimicrobial agents, respectively (Fig 2). Higher multiple resistance was observed in isolates from outpatients (140/175, 80%) compared with isolates from healthy subjects (94/143, 66%) ($p<0.05$). It is noteworthy that 31% of healthy subject isolates and 67% of outpatient isolates were resistant to more than 5 antimicrobials.

Of the 71 different combinations of resistance observed, 2 were most common: 1) resistance to 5 antibiotics (sulphamethoxazole, trimethoprim/sulphamethoxazole, ampicillin, tetracycline, and streptomycin) found in 36 (11%) isolates; and 2) resistance to 6 antibiotics (sulphamethoxazole, trimethoprim/sulphamethoxazole, ampicillin, tetracycline, kanamycin, and streptomycin) found in 25 (8%) isolates.

Resistance patterns among integron-positive and integron-negative *E. coli* are shown in Table 3. A total of 43 resistance patterns were observed among 162 integron-positive isolates. The most frequent pattern was SMX-SXT-AMP-TET-STR (21%), followed by SMX-SXT-AMP-TET-KAN-STR (14%) and SMX-SXT-NAL-NOR-CIP-AMP-AMC-TET-STR (7%). MDR phenotypes were common (89%). Only seven isolates (4%) were resistant to a single drug and ten isolates (6%) were susceptible to all antimicrobial agents tested. Seventy-eight percent of the integron-positive isolates were resistant to more than 5 antimicrobials.

Forty-seven different resistance patterns were observed among 156 integron-negative isolates. However, only 19 resistance patterns were shared with the integron-positive group. The most frequent pattern found in integron-negative isolates was SXT-STR (7%), followed by SMX (6%) and TET (6%). Thirty-one isolates (20%) were resistant to a single drug and 36 (23%) isolates were susceptible to all the tested drugs. An MDR phenotype was observed in 57% of the isolates, significantly lower than those from the integron-positive isolates ($p<0.05$). Only 21% of integron-negative isolates were resistant to more than 5 antimicrobials.

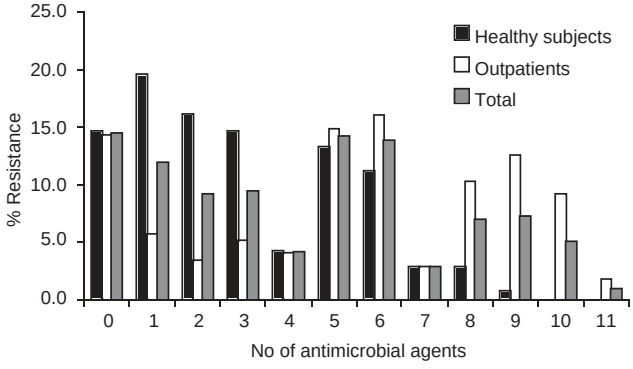

Fig 2–Distribution of multiple antimicrobial resistance of *E. coli* isolated from healthy subjects and outpatients.

DISCUSSION

In class 1 integrons, the 5′ conserved region encodes a site-specific recombinase (integrase, *intI1*) and a strong promoter or promoters that ensure expression of the integrated resistance gene cassettes. The 3′ conserved segment carries *qacEΔ1* that specifies resistance to antiseptics and quaternary ammonium disinfectant compounds, while *sul1* confers sulphonamide resistance, and there is an open reading frame of unknown function (Hall and Stokes, 1993). We found that only 23% of our *E. coli* isolates contained all three conserved genes (*qacEΔ1*, *intI1*, *sul1*). Sunde (2005) has reported that a high portion of class 1 integrons found in *E. coli* lack *sul1* and suggested that when screening for integrons, it is preferable to do an initial screening for the integrase gene. We detected *intI1* in 51% of the 318 isolates and 94%

integron-positive isolates were resistant to at least one antibiotic. Thus there were only ten isolates (6%) possessing *intI1* that failed to express antibiotic resistance. It is possible that these susceptible isolates may not have resistance gene cassette or the expression of their resistance gene cassette is weak. Solberg *et al* (2006) also found that 3 out of 68 *E. coli* isolates possessing *intI1* and resistant to SXT fail to yield gene cassettes. Other investigators have found that antibiotic susceptible *E. coli* and *Salmonella* can harbor specific gene cassettes within integrons but these gene cassettes have weak expression (Zhao *et al*, 2001; 2003; Sunde *et al*, 2005).

In agreement with other studies (Reyes *et al*, 2003; Mathai *et al*, 2004), we noted that class 1 integrons were common in *E. coli* isolates. This result is also in agreement with that of Skurnik *et al* (2005) who found that integrons persist in commensal *E. coli* isolates in subjects who have not taken antibiotics for at least one month. The frequency of class 1 integron in healthy subjects who have not taken antibiotics for at least one month in this study (36%) was higher than that reported by Skurnik *et al* (2005). It is accepted that antimicrobial use is the single most important factor responsible for increased antimicrobial resistance (McGarock, 2002; Rubin and Samore, 2002). However, the use of antibiotics may vary across countries. In addition to recent exposure to antibiotics, diet, deficient hygiene, poor living conditions, and living in developing countries can promote resistance in commensal intestinal *E. coli* (Skurnik *et al*, 2005). Furthermore, the frequency of integrons in *E. coli* from outpatients was significantly higher than those from healthy subjects. It is possible that outpatients may have recently taken antibiotics before visiting the hospital.

Our *E. coli* isolates demonstrated high resistance to antimicrobials commonly used for chemotherapy. Frequent resistance patterns of *E. coli* were resistance to ampicillin, tetracycline, streptomycin and sulphonamides, in agreement with previous reports (Skurnik *et al*, 2005; Hsu *et al*, 2006). These drugs have been extensively used in many developing countries (Hart and Kariuki, 1998; Okeke *et al*, 2000; Nys *et al*, 2004). In *E. coli*, acquired resistance to sulphonamides is frequently from the acquisition of 3 *sul*-type genes that encode dihydropteroate synthases with reduced affinity for sulphonamide (Sköld, 2000; Perreten and Boerlin, 2003). In this study, *sul1* was detected in 27% isolates, and 93% *sul1*-positive isolates were integron-positive (data not shown). However, 47% and 59% of *E. coli* isolates were resistant to SXT and SMX, respectively. It is possible that these sulphonamide-resistant *E. coli* may contain other *sul*-type genes. Infante *et al* (2005) reported that 35 % SXT-resistant *E. coli* isolates from healthy children in Bolivia and Peru possessed *sul1* and 75% had *sul2*.

Significantly higher resistance rates of all antimicrobial agents tested were observed in integron-positive isolates compared with integron-negative isolates. Humans carrying integron-positive commensal *E. coli* are a reservoir of integron-carrying isolates that can spread to other bacteria. The clinical importance of integrons is mainly that the use of one antibiotic may activate the expression and transfer of a whole gene cassette. As a result, a bacterial strain may become multidrug resistant due to exposure to only one antibiotic (Norrby, 2005). Therefore, restrictive use of all antibiotics is needed to prevent the spread of antibiotic resistance.

In summary, multidrug resistant *E. coli* were found to be common (88%) in commensal microbiota of healthy volunteers and outpatients from a major university hospital in southern Thailand. High incidence of class 1 integrons was found in resistant strains indicating widespread distribution of resistant determinants in this community. These results emphasize that intestinal *E. coli* is an important reservoir of antibiotic resistant genes.

ACKNOWLEDGEMENTS

This work was supported by a grant from Graduate School, Prince of Songkla University. We would like to thank the Clinical Microbiology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University for providing *E. coli* isolates from outpatients and Dr Brian Hodgson for critical review of this manuscript.

REFERENCES

Barlow RS, Pemberton JM, Desmarchelier PM, Gobius KS. Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrob Agents Chemother* 2004; 48: 838-42.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; fourteenth informational supplement (M100-S14). 2004; 24 (1).

Ebner P, Garner K, Mathew A. Class 1 integrons in various *Salmonella enterica* serovars isolated from animals and identification of genomic island SGI1 in *Salmonella enterica* var. Meleagridis. *J Antimicrob Chemother* 2004; 53: 1004-9.

Feng P, Weagant SD, Grand MA. Enumeration of *Escherichia coli* and the Coliform bacteria. In: Bacteriological analytical manual online. Chapter 4. 2002. [Cited 2006 Dec 12]. Available from: URL: http://www.cfsan.fda.gov/~ebam/bam-4.html

Forbes B, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 11th ed. St Louis, MO: CV Mosby, 2002.

Hall RM, Stokes HW. Integrons: novel DNA elements which capture genes by site-specific recombination. *Genetica* 1993; 90: 115-32.

Hart CA, Kariuki S. Antimicrobial resistance in developing countries. *BMJ* 1998; 317: 647-50.

Hsu SC, Chiu TH, Pang JC, Hsuan-Yuan CH, Chang GN, Tsen HY. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents* 2006; 27: 383-91.

Infante B, Grape M, Larsson M, *et al*. Acquired sulphonamide resistance genes in fecal *Escherichia coli* from healthy children in Bolivia and Peru. *Int J Antimicrob Agents* 2005; 25: 308-12.

Leverstein-van Hall MA, Paauw A, Box ATA, Blok HEM, Verhoef J, Fluit AC. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. *J Clin Microbiol* 2002; 40: 3038-40.

Levin BR, Lipstich M, Perrot V, *et al*. The population genetics of antibiotic resistance. *Clin Infect Dis* 1997; 24: S9-16.

Levy SB, Marshall B, Schluederberg S, Rowse D, Davis J. High frequency of antimicrobial resistance in human fecal flora. *Antimicrob Agents Chemother* 1988; 32: 1801-6.

Martinez-Freijo P, Fluit AC, Schmitz F-J, Grek VSC, Verhoef J, Jones ME. Class 1 integrons in gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *J Antimicrob Chemother* 1998; 42: 689-96.

Mathai E, Grape M, Kronvall G. Integrons and multidrug resistance among *Escherichia coli* causing community-acquired urinary tract infection in southern India. *APMIS* 2004; 112: 159-64.

McGarock H. Unjustified antibiotic prescribing in the community: a major determinant of bacterial antimicrobial resistance. *Pharmacoepidemiol Drug Saf* 2002; 11: 407-8.

Norrby SR. Integrons: adding another threat to the use of antibiotic therapy. *Clin Infect Dis* 2005; 41: 10-1.

Nys S, Okeke IN, Kariuki S, Dinant GJ, Driessen C, Stobberingh EE. Antibiotic resistance of fecal *Escherichia coli* from healthy volunteers from eight developing countries. *J Antimicrob Chemother* 2004; 54: 952-5.

Okeke IN, Fayinka ST, Lamikanra A. Antibiotic resistance in *Escherichia coli* from Nigerian

students, 1986-1998. *Emerg Infect Dis* 2000; 6: 393-6.

Okeke IN, Laxminarayan R, Bhutta ZA, *et al.* Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect Dis* 2005; 5: 481-93.

Perreten V, Boerlin P. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. *Antimicrob Agents Chemother* 2003; 47: 1169-72.

Ploy MC, Lambert T, Couty JP, Denis F. Integrons: an antibiotic resistance gene capture and express system. *Clin Chem Lab Med* 2000; 38: 483-7.

Recchia GD, Hall RM. Gene cassettes: a new class of mobile element. *Microbiol* 1995; 141: 3015-27.

Reyes A, Bello H, Dominguez M, Mella S, Zemelman R, Gonzalez G. Prevalence and types of class 1 integrons in aminoglycoside-resistant Enterobacteriaceae from several Chilean hospitals. *J Antimicrob Chemother* 2003; 51: 317-21.

Rubin MA, Samore MH. Antimicrobial use and resistance. *Curr Infect Dis Re*p 2002; 4: 491-7.

Sköld O. Sulfonamide resistance: mechanisms and trends. *Drug Resist Updates* 2000; 3: 155-60.

Skurnik D, Le Menach A, Zurakowski D, *et al*. Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. *Antimicrob Agents Chemother* 2005; 49: 3062-5.

Solberg OD, Ajiboye RM, Riley LW. Origin of class 1 and class 2 integrons and gene cassettes in a population-based sample of uropathogenic *Escherichia coli*. *J Clin Microbiol* 2006; 44: 1347-51.

Sunde M. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. *J Antimicrob Chemother* 2005; 56: 1019-24.

Tauxe RV, Cavanagh TR, Cohen ML. Interspecies gene transfer in vivo producing an outbreak of multiply resistant shigellosis. *J Infect Dis* 1989; 160: 1067-70.

Zhao S, Qaiyumi S, Friedman S, *et al*. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *J Clin Microbiol* 2003; 41: 5366-71.

Zhao S, White DG, Ge B, *et al*. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. *Appl Environ Microbiol* 2001; 67: 1558-64.