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Integron Expression in Multidrug-Resistant *Escherichia coli* Isolated from House Flies within the Hospital[†]

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Abstract

Escherichia coli is a serious cause of a variety of hospital-acquired infections and commonly contributes to the environment by house flies. Integrons, particularly class 1 integrons, are the genetic elements that play an important role in the horizontal transfer of antimicrobial resistance mechanism. This mechanism is commonly found in Enterobacteriaceae, especially *E. coli*. In this study, we aim to investigate the occurrence and antimicrobial resistance patterns of *E. coli* isolated from the house flies in Phayao hospital and to determine the gene expression of class 1 integrons in those isolates of *E. coli*. Totally, 70 isolates of *E. coli* were isolated from 60 house flies collected from the hospital. Fifty-seven of the isolates (81.43 %) were multidrug resistance (MDR) and highly resistant to β-lactams, tetracyclines, and sulfonamides. Of 57 isolates of MDR-*E. coli*, 20 isolates (35 %) were found to carry class 1 integron genes. Fifteen patterns of antimicrobial resistance occurred in the isolates of integron-positive *E. coli*. Most integron-positive *E. coli* isolates were resistant to 7 antimicrobials. Two isolates of these bacteria (10 %) were able to resist 13 out of 14 tested antimicrobials. Using PCR and sequencing analysis, an investigation showed that *dfrA17-aadA5*, *dfrA12-aadA2* gene cassette was the most prevalent cassette (n = 10; 50 %) among the integron-positive *E. coli* isolates. Our results indicated that the presences of multidrug resistance and class 1 integrons were common in *E. coli* isolated from the houseflies in hospital. Therefore, screening for integron-positive *E. coli* from the hospital environment might be necessary for prevention of nosocomial infections.

Keywords: Antimicrobial resistance, class 1 integron, *Escherichia coli*, house flies, nosocomial spread

Introduction

Antimicrobial resistance among pathogenic bacteria and commensal bacteria appears to spread rapidly in many regions and has become a serious problem worldwide [1]. The increase of antimicrobial resistances is caused by the inappropriate selection and abuse of antimicrobials for the infection therapy of humans and animals as well as for the prophylaxis and growth production of animals [2]. The prevalence of multidrug resistant (MDR) bacteria found in human and animal isolates all over the world is increasing. MDR bacteria is the bacteria that are resistant to at least one agent in 3 or more antimicrobial classes [3]; therefore, the control of MDR distribution among bacteria should be more concerned. It has been reported that the increased MDR is caused by the

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horizontal transfer mechanism of the mobile genetic elements among Gram-negative bacteria [2,4]. Infectious diseases caused by MDR-pathogenic *Escherichia coli* lead to the increased mortality and cost. Multidrug resistance in non-pathogenic *E. coli* is responsible for being the important reservoir of resistance genes. *E. coli* can transfer the resistance genes through the mobile genetic elements like integrons [5] among *E. coli* strains and other bacteria in Enterobacteriaceae. Mobile genetic elements, such as transposons and plasmids, are known to carry integrons responsible for the resistance against multiple antimicrobials. Recently, 4 classes of integrons (classes 1, 2, 3 and 4) were found to be associated with carrying the resistance gene cassettes. Class 1 integrons most frequently found in *E. coli* and are more than 100 gene cassettes associated with antimicrobial resistance [6].

House flies (*Musca domestica*) are commonly found in Thailand [7]. They play an important role to the dissemination of various pathogenic bacteria such as *Salmonella* sp., *Acinetobacter* sp., *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*, etc. [7,8] because of their ability to travel around up to 8 km within 24 h to find food and reproductive sites. Therefore, they can spread an antimicrobial resistance between animals and humans by carrying the relevant bacteria on the surface of their exoskeleton and in the alimentary canal [9]. Onwugamba *et al*. have revealed that there are many reported colonization of housed flies with enteropathogenic bacteria. It has been reported that house flies can transmit these bacteria due to it not only colonize but also cause disease in humans and animals [9]. Even though the information is still limited, the evidence of carriage, vector and transmission potential of antimicrobial resistance bacteria in Iran [10], Brazil [11], Czech Republic [12], and Germany [13] has been found. Conduction of the resistant pathogenic and non-pathogenic bacteria in the environment, particularly in a hospital, affects the public health. Hospital acts as an important reservoir of resistant bacteria causing nosocomial infections. Screening for integron-positive *E. coli* from the hospital environment might be necessary for the monitoring of nosocomial infections.

The Southeast Asia as well as Thailand has been reported on the increasing antimicrobial resistance in clinical samples [14] whereas the data of antimicrobial resistance in mechanical vectors was scarce. Thailand has been reported on the prevalence of antimicrobial resistance in many strains of Gram-positive and –negative bacteria from the clinical samples [15-17]. Accordingly, screening for resistant *E. coli* from house flies, the mechanical vector, is important to control and monitor the distribution of the resistant bacteria in the hospital. In addition, Thailand had a few data on antimicrobial resistance throughout country especially the resistance from transmission vectors of the resistant bacteria in hospital. Furthermore, data on antimicrobial resistance patterns and class 1 integron gene cassettes are also not enough. In this study, we aim to investigate the occurrence and antimicrobial resistance patterns of *E. coli* isolated from the house flies in Phayao hospital, and to characterize class 1 integrons in those isolates of *E. coli*.

Materials and methods

Bacterial isolation

Escherichia coli were isolated from 60 house flies. *E. coli* isolates were obtained from the house flies within Phayao hospital during June and August, 2015. Each fly was randomly collected and vigorously shaken in the 0.9 % saline. After removing the fly, saline was cross-streaked on Eosin-methyleneblue agar (Oxoid Limited, Basingstoke, United Kingdom) and incubated at 37 °C for 18 h. The typical colonies were picked to identify using the standard biochemical test [15]. *E. coli* ATCC 25922 was used as the reference-typed strain for all tests. All strains were stored at −80 °C until further study.

Antimicrobial susceptibility test

All *E. coli* isolates were investigated for the susceptibility to antimicrobials by disc diffusion method on Mueller-Hinton agar (Oxoid Limited, Basingstoke, United Kingdom) according to the Clinical and Laboratory Standards Institute (CLSI) guideline [18]. The susceptibility was investigated with 14 antimicrobial agents including penicillins (ampicillin, amoxicillin/clavulanic acid,

ampicillin/sulbactam), cephalosporins (cephalothin, cefotaxime), carbapenems (meropenem, imipenem), fluoroquinolones (ciprofloxacin, norfloxacin), aminoglycosides (amikacin, gentamicin), tetracyclines (tetracycline), phenicols (chloramphenical), and folate pathway inhibitor (trimethoprim-sulfamethoxazole). All antimicrobial agents were purchased from Oxoid Ltd. (Oxoid Limited, Basingstoke, United Kingdom). Intermediate isolates were determined to be resistant. *E. coli* ATCC25922 was used as the quality control strain. The isolate resistant to at least one agent in 3 or more antimicrobial classes was defined as a multidrug resistant *E. coli* (MDR-*E.coli*) [3].

Detection of class 1 integrons by PCR

Screening for integron was performed by directed colony PCR technique. The specific primers for integrase 1 gene (*intI*) were presented in **Table 1**. The amplification condition was performed by a cycle of pre-denaturation at 94 °C for 5 min, and 30 cycles of 94 °C for 30s (denaturing), 48 °C for 30 s (annealing), and 72 °C for 1 min (extension). The final extension was 72 °C for 7 min. The PCR products were determined by agarose gel electrophoresis. Amplicon size was determined by comparing with the DNA marker.

Characterization of variable region of class 1 integron

All *int1*-positive isolates were investigated for the presence of integrons using primers 3'CS and 5'CS for gene cassettes of class 1 integrons (**Table 1**). The PCR reaction was carried out by a cycle of pre-denaturation at 94 °C for 5 min, and 30 cycles of denaturing at 94 °C for 45 s, annealing at 48 °C for 45 s, and extension at 72 °C for 5 min. The final extension was 72 °C for 7 min. PCR products were determined by agarose gel electrophoresis.

Table 1 Primers used for PCR amplification to detect class 1 integrons.

Primer	Sequence (5' → 3')	Size of amplicon (bp)	Target genes	References
Int 1-F Int 1-R	GCATCCTCGGTTTTCTGG GGTGTGGCGGGCTTCGTG	457	Integrase1	[19]
In5'CS In3'CS	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	Variable	Class 1 integron	[20]

DNA sequencing and analysis of sequence data

Selected PCR products were purified by DNA extraction kit (Invitrogen) and analyzed by DNA sequencing. Purified DNA were sent to the commercial facility for sequencing (Krimogen). Sequences were BLAST and analyzed by the online BLAST of the National Center for Biotechnology Information (NCBI) website software.

Statistical analysis

Association between the resistance to antimicrobials and the integron expression was determined by Chi-square or Fisher's exact test (when $n \leq 20$ or number in cell < 5) with $p < 0.05$. Analyses were performed using SPSS software for Window version 22.0.

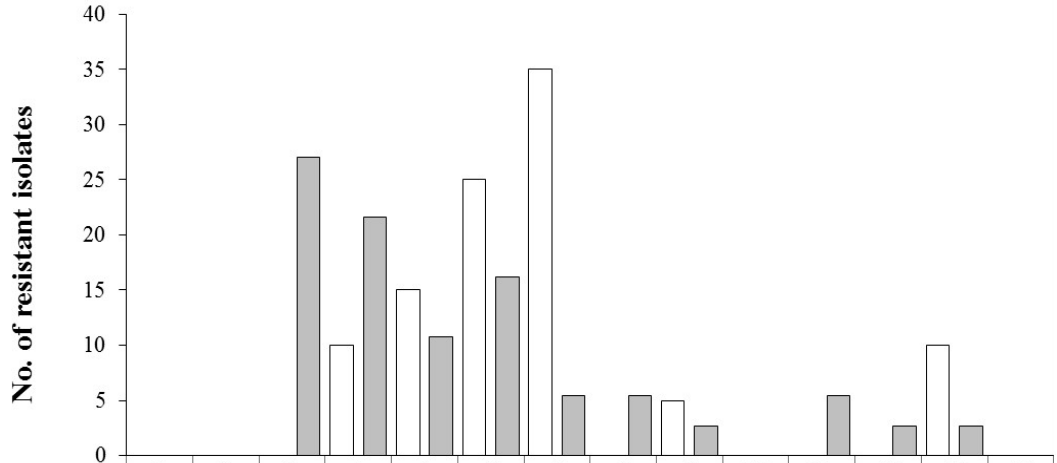
Results and discussion

Identification of antimicrobial resistant *E. coli* isolates

Totally, 70 isolates of *E. coli* were isolated from 60 house flies collected from the hospital. All 70 *E. coli* isolates were tested for the susceptibility to 14 antimicrobials. The result showed that 68 isolates (97.1 %) were resistant to at least one antimicrobial while 57 isolates (81.43 %) were

classified as MDR-*E. coli* isolates. The results indicated the high prevalence of antimicrobial resistance among *E. coli* isolates from the house flies in hospital. The multidrug resistant isolates were selected for integron detection. The percentages of the resistance to 14 antimicrobial agents for 57 isolates are shown in **Table 2**. Most isolates were resistant to cephalothin (94.7 %) and ampicillin (93.0 %), which indicated high resistant frequencies. High resistant frequency to ampicillin in our study was much higher than those of the resistance in *E. coli* from the healthy volunteer (35.0 %) and outpatient (65.0 %) stool from hospital in Thailand [21]. A low resistant rate was observed to meropenem, imipenem, and norfloxacin (lower than 15.0 %) and no one found 100 % susceptible.

The numbers of antimicrobial in co-resistance of MDR-*E. coli* strains are shown in **Figure 1**. Two isolates of the integron-positive isolates (10.0 %) and one of the integron-negative isolates (2.7 %) were able to resist 13 out of 14 tested antimicrobials. Most integron-positive isolates (35 %) were resistant to 7 antimicrobials while most isolates of the integron-negative isolates (27 %) were resistant to 3 out of 14 tested antimicrobials. Interestingly, the integron-positive isolates significantly exhibited high resistance to only trimethoprim/sulfamethoxazole while the resistance to other antimicrobials was not significantly different from the integron-negative isolates.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
□+int1 isolate	0	0	0	10	15	25	35	0	5	0	0	0	10	0
▨-int1 isolate	0.0	0.0	27.0	21.6	10.8	16.2	5.4	5.4	2.7	0.0	5.4	2.7	2.7	0.0

Figure 1 Frequency of multidrug resistance to 14 antimicrobial agents in the integron-positive (+int1) isolates and the integron-negative (-int1) isolates of *Escherichia coli*.

Table 2 Antimicrobial resistance and integrons association of *Escherichia coli* isolates.

Antimicrobial agents	No. (%) of resistant isolates[a]			*p
	Total isolates (n = 57)	Integron-positive isolates (n = 20)	Integron-negative isolates (n = 37)	
Penicillins				
Ampicillin	53 (93.0)	20 (100)	33 (89.2)	0.29
Ampicillin/sulbactam	32 (56.1)	11 (55.0)	21 (56.8)	0.90
Amoxicillin/clavulanic acid	11 (19.3)	3 (15.0)	8 (21.6)	0.73
Cephalosporins				
Cephalothin	54 (94.7)	19 (95.0)	35 (94.6)	1.00
Cefotaxime	33 (57.9)	12 (60.0)	21 (56.8)	0.81
Carbapenems				
Meropenem	5 (8.8)	2 (10.0)	3 (8.1)	1.00
Imipenem	6 (10.5)	2 (10.0)	4 (10.8)	1.00
Fluoroquinolones				
Norfloxacin	8 (14.0)	2 (10.0)	6 (16.2)	0.70
Ciprofloxacin	12 (21.1)	4 (20.0)	8 (21.6)	1.00
Aminoglycosides				
Amikacin	12 (21.1)	5 (25.0)	7 (18.9)	0.74
Gentamicin	20 (35.1)	10 (50.0)	10 (27.0)	0.08
Tetracyclines				
Tetracycline	44 (77.2)	17 (85.0)	27 (73.0)	0.35
Phenicols				
Chloramphenicol	22 (38.6)	11 (55.0)	11 (29.7)	0.06
Folate pathway inhibitor				
Trimethoprim-sulfamethoxazole	26 (45.6)	17 (85)	9 (24.3)	< 0.01

[a]Tested by disc diffusion method. Intermediate isolates were determined as resistant strains.

Table 3 Multidrug resistant profiles of the integron-positive *Escherichia coli* isolates.

Pattern type	Antimicrobial resistance	No. (%) of resistant strains (n = 20)
1	AMP, SAM, KF, STX	1 (5.0)
2	AMP, AKF, CTX, SXT	1 (5.0)
3	AMP, SAM, KF, CTX, SXT	1 (5.0)
4	AMP, SAM, KF, CN, TE	1 (5.0)
5	AMP, SAM, KF, SXT, TE	1 (5.0)
6	AMP, SAM, KF, SXT, AK, TE	1 (5.0)
7	AMP, SAM, KF, C, SXT, TE	1 (5.0)
8	AMP, KF, CTX, C, SXT, TE	1 (5.0)
9	AMP, KF, C, SXT, CIP, TE	1 (5.0)
10	AMP, KF, CTX, C, AK, TE	1 (5.0)
11	AMP, SAM, AMC, C, CIP, TE	1 (5.0)
12	AMP, KF, CTX, C, SXT, CN, TE	5 (25.0)
13	AMP, SAM, KF, SXT, AK, CN, TE	1 (5.0)
14	AMP, SAM, KF, CTX, C, SXT, CN, CIP, TE	1 (5.0)
15	AMP, AMC, SAM, KF, CTX, SXT, MEM, IPM, AK, CN, CIP, NOR, TE	2 (10.0)

Comparing with previous studies, the expression of class 1 integron in *E. coli* from the environment of Asia, including house flies (35 %; result from this study), Dongjiang river of China (82.3 %) [22] and aquaculture water in Iran (40.0 %) [23], was higher than that in Europe, such as wastewater in Poland (11.0 %) [24]. Considering the resistance-integron association, the rate of resistance to each tested antimicrobial was not different except trimethoprim-sulfamethoxazole (85.0 % for integron-positive and 24.3 % for integron-negative isolates).

The resistance pattern of each strain was also investigated. The resistance patterns of integron-positive isolates and the major resistance patterns of the integron-negative isolates were shown in **Tables 2** and **3**, respectively. The integron-positive isolates displayed 15 different patterns and only 2 patterns were shared by more than 2 isolates. While, the integron-negative isolates showed 28 different patterns and 6 patterns were shared by more than 2 isolates (**Table 2**). The resistance pattern AMP-KF-CTX-C-SXT-CN-TE was the most frequent pattern for the integron-positive isolates while pattern AMP-KF-TE was the most frequent pattern for the integron-negative isolates (**Tables 3** and **4**). Comparing with the previous report on clinical *E. coli* isolates, the number of the resistant patterns of the integron-positive isolates in this study (15 patterns) was less than that of the resistant patterns of the clinical *E. coli* isolates in other studies (43 patterns) [21].

Table 4 Multidrug resistant profiles of the integron-negative *Escherichia coli* isolates.

Pattern type	Antimicrobial resistance	No. (%) of resistant strains (n = 37)
1	AMP, KF, TE	4 (10.8)
2	AMP, SAM, KF	2 (5.4)
3	AMP, KF, CTX, AK	2 (5.4)
4	AMP, KF, CTX, C, CN, TE	2 (5.4)
5	AMP, SAM, KF, CTX, C, TE	3 (8.1)
6	AMP, AMC, SAM, KF, CTX, C, SXT, CN, CIP, NOR, TE	2 (5.4)

Integron and gene cassette characterization

All MDR-*E.coli* isolates were investigated for integrase genes and class 1 conserved region by PCR technique. Of the 57 isolates, 21 (36.8 %) were positive for a class 1 integrase gene by amplifying the *int 1*. The 21 *E. coli* isolates that carried *int 1* were further analyzed for class 1 integrons using 5'CS and 3'CS and the result showed that 20 (35 %) contained class 1 integron. The different integron patterns were shown in **Table 5**. Resistance gene cassettes carried on class 1 integron exhibited with the sizes ranging from 0.8 kb to 2.4 kb. Among 20 integron-positive isolates, there were 6 isolates containing one amplicon and 14 isolates containing 2 amplicons with different sizes. The co-existence of 2 different integrons was confirmed that these integrons carried different gene cassettes using sequence analysis. **Table 5** illustrated 7 different gene cassettes, including gene encoding resistance to trimethoprim (*dfrA7*, *dfrA12*, *drfA17*, *dhfr12*), and aminoglycosides (*aadA2*, *aadA5*). The most common type of cassette (*dfrA*) conferred resistance to trimethoprim, which represented 100 % among all cassettes. The frequently found trimethoprim-resistant (*dfrA*) gene cassette in the *E. coli* strains corresponded to the previous reports [2,25]. The array *dfrA17-aadA5*, *dfrA12-aadA2* encoding resistance to trimethoprim and aminoglycosides was frequently found in class 1 integrons containing *E. coli* isolates in this study. The prevalence of *dfrA* linked with *aadA* array in *E. co*li agreed with those reported earlier in other countries [2,25]. In Thailand, there was no report on these arrays in the integron containing *E. coli* isolates.

Table 5 Gene cassette arrays of class 1 integrons of *Escherichia coli* isolates.

Integron group	Approximate size of amplicon (kb)	Gene cassettes[a]	No. of strains carrying gene cassettes
1	0.8	*dfrA7*	2
2	1.6	*dfrA17-aadA5*	3
3	2.2	*dfrA12-aadA2*	1
4	0.8, 2.2	*dfrA7*, *dhfr12-aadA2*	1
5	1.5, 2.1	*dfrA12*, *dfrA12-aadA2*	2
6	1.6, 2.1	*dfrA17-aadA5*, *dfrA12-aadA2*	10
7	1.6, 2.4	*dfrA17-aadA5*, *dfrA12-orfF-aadA2*	1

[a]Resistant genes: *dfrA7, dfrA12, drfA17* and *dhfr12*, encoding dihydrofalate reductase; *aadA2* and *aadA5,* encoding aminoglycoside adenyltransferase; *orfF*, unknown open reading frame.

House flies carrying integron-positive *E. coli* are the important reservoir of integron containing isolates that can be distributed to other bacteria in environment and also in humans. The importance of integron expression implies that using one antimicrobial could contribute to the expression and transfer of a whole gene cassette. Bacteria receiving that gene might become multidrug resistance although they were exposed to only one antimicrobial agent [26].

Conclusions

The resistance to multiple antimicrobial agents of *E. coli* from house flies in the Northern Thailand hospital was commonly found (81.43 %). House flies may harbor MDR-*E. coli* for long time, and transfer in the environment causing the reservoir and infection risk to patients. The expression of class 1 integrons in this study revealed the potential for house flies to contribute to the environmental existence and probable distribution of MDR-*E. coli* in hospital. Our data could be the useful information for monitoring the antimicrobial resistance in environment through the insect vector.

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