Antibiotic Resistance Patterns of *Enterococcus* spp. Isolated from *Musca domestica* and *Chrysomya megacephala* in Ubon Ratchathani

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Background: *The housefly Musca domestica and the blowfly Chrysomya megacephala are found worldwide and are medically significant as mechanical vectors of various pathogens from unsanitary locations to food, resulting in diseases in humans.*
Objectives: *This study aimed to test the antimicrobial activity against Enterococcus spp. isolated from M. domestica and C. megacephala by standard disk diffusion and minimal inhibitory concentration (MIC), and to study the potential of M. domestica and C. megacephala to transfer multi-drug resistant enterococcus to humans.*
Material and Method: *Seven hundred adult flies were collected from fresh-food markets, garbage piles, restaurants, school cafeterias, and rice paddy fields in Muang Ubon Ratchathani and Warinchamrap in Ubon Ratchathani Province. Antimicrobial susceptibility for Enterococcus spp. isolated from adult flies was performed by disk diffusion test and minimum inhibitory concentration (MIC) determination.*
Results: *One hundred and twenty isolates of Enterococcus spp. were taken from 67 M. domestica and 53 C. megacephala. Standard disk diffusion showed the Enterococcus spp. isolates exhibited susceptibility to ampilcillin (99.2%), chloramphenicol (74.2%), tetracycline (75.0%), vancomycin (50.8%), and erythromycin (42.5%). The MICs of antimicrobial agents for all isolates were ≤0.25-8 μg/mL for vancomycin, 1->16 μg/mL for tetracycline, 4->16 μg/mL for chloramphenicol, and 0.5-8 μg/mL for ciprofloxacin.*
Conclusion: *The study demonstrated the potential of M. domestica and C. megacephala to carry Enterococcus spp. Nine antimicrobial susceptibility patterns were obtained among the 120 enterococci isolates.*

Keywords: *Enterococcus spp., Musca domestica, Chrysomya megacephala*

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Musca domestica and *Chrysomya megacephala* are medically significant flies as they have been reported as mechanical carriers of multiple pathogens that can cause diseases in humans[1]. These pathogens include numerous bacterial species, viruses, and helminth eggs. In Thailand, an investigation in Chiang Mai indicated that *C. megacephala* and *M. domestica* were mechanical carriers of pathogenic enteric bacteria[2]. Previous surveys indicated that both are the most prevalent fly species in the central and northern parts of Thailand.

Research indicated that *M. domestica* and *C. megacephala* make up 70% of flies collected in Ubon Ratchathani Province[3]. *C. megacephala* specifically presented the potential as a vector of pathogenic enteric bacteria to humans, including *Escherichia coli* O157:H7 (EHEC) and *Salmonella* Typhi. Moreover, other human pathogens such as *Enterococcus* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were also found[4]. *Enterococcus* spp. is a gram positive bacteria and causes infection in humans, especially in immuno-compromised hosts. Multi-drug resistant enterococci including vancomycin resistant *Enterococcus* (VRE) were reported. The resistant strains encode genes that confer vancomycin resistance, namely *van* gene, and are resistant to vancomycin with low to high levels depending on the *van* types. VRE can be carried by healthy people who have come into contact with the bacteria. However, flies are commonly mechanical vectors of pathogens, but there are no data about the patterns of susceptibility to bacterial isolates from these flies. The

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high distribution of *M. domestica* and *C. megacephala* acts as vectors and transfers bacterial contaminates in the environment and to humans. If these flies contain bacteria with resistance to antimicrobials, the drug resistance is transferred to bacteria in the same family and induces pathogenic bacteria to resist antibiotics. The source of the infection limits the choice of therapy[1,2,4].

The objective of the present study was to isolate and identify *Enterococcus* spp. associated with populations of *M. domestica* and *C. megacephala* collected from Ubon Ratchathani Province and test the antimicrobial activity against the *Enterococcus* spp. by standard disk diffusion and minimal inhibitory concentration (MIC). It is anticipated that the data will describe the potential of adult flies as vectors to drug-resistant *Enterococcus* and assist in management plans for the control of flies.

Material and Method

Fly collection and bacterial identification

Flies were collected from fresh-food markets, garbage piles, restaurants, school cafeterias, and paddy fields from December 2012 to June 2013 using sterile insect sweep nets. These sites were locations at which flies had the opportunity to feed on waste or unsanitary materials and human food items, and all were in Muang Ubon Ratchathani and Warinchamrap districts in Ubon Ratchathani Province of North-East Thailand. *M. domestica* and *C. megacephala* were individually identified under a dissecting microscope with the taxonomic keys of Kurahashi and Chowanadisai[5]. Each fly was individually transferred into a separate 20 ml sterile glass vial using sterilized forceps. Two milliliters of sterile buffer peptone water was added to each vial and then shaken vigorously for two minutes to create a wash from each fly. Subsequently, 0.1 ml of the wash solution vial was inoculated onto blood agar plates. The isolated *Enterococcus* spp. were then identified by morphological and biochemical tests using Bergey's Manual of Determinative Bacteriology[6].

Antimicrobial susceptibility testing

Phenotypic antibiotic resistance was defined by the testing by MIC and standard disk diffusion of 120 isolates. Ampicillin, tetracycline, chloramphenicol, erythromycin, and vancomycin were used in the standard disk diffusion test. MICs were performed in chloramphenicol, tetracycline, vancomycin, and ciprofloxacin.

The disk diffusion test was performed on Mueller-Hinton agar (MHA) (Hardy Diagnostics, Santa Maria, USA) using Kirby-Bauer method. Antimicrobial disks (Oxoid Ltd, Hampshire, England) included ampicillin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), erythromycin (15 μg) and vancomycin (30 μg). *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 were used as antimicrobial-sensitive controls. The results were interpreted according to the guidelines of Clinical and Laboratory Standards Institute (CLSI)[7]. MICs of various antimicrobials were tested by a Sensititre gram-positive plate (Trek diagnostic systems, Biosciences Inc., Magellan, USA) and performed according to the manufacturer's instructions. The concentrations of antimicrobials were 0.06->16 μg/mL.

Results

Seven hundred flies were collected from fresh-food markets, garbage piles, restaurants, school cafeterias, and paddy fields in Muang Ubon Ratchathani and Warinchamrap districts in Ubon Ratchathani Province of Northeastern Thailand. Of these, 120 isolates (17.14%) of *Enterococcus* spp. were obtained from 67 *M. domestica* and 53 *C. megacephala* (Table 1).

Antimicrobial susceptibility

Enterococcus spp. isolated from *M. domestica* had a susceptibility to ampicillin of 98.5%, 76.1% to tetracycline, 70.2% to chloramphenicol, 32.8% to erythromycin, and 44.8% to vancomycin (Fig. 1). The 53 isolates of *Enterococcus* spp. isolated from *C. megacephala* exhibited a susceptibility to ampicillin of 100%, 73.6% to tetracycline, 79.3% to chloramphenicol, 54.7% to erythromycin, and 58.5% to vancomycin. MIC revealed that all *Enterococcus* spp. isolates from both *M. domestica* and *C. megacephala* were susceptible to vancomycin, whereas 89.6% and 81.1% of isolates from *M. domestica* and *C. megacephala*, respectively had reduced susceptibility to ciprofloxacin (Table 2). Nine antimicrobial susceptibility patterns were obtained from the 120 enterococci isolates (Table 3).

Discussion

Flies are considered to carry many pathogenic bacteria in hospitals, households, and residential areas. *M. domestica* and *C. megacephala* were found to be the dominant species of synanthropic flies in Thailand[3,8-10]. These flies may be capable of transferring common pathogens of human disease[2,3].

Table 1. Number of *C. megacephala* and *M. domestica* carrying Enterococcus collected at various human habitations in Ubon Ratchathani province from December 2012 to June 2013

Sources	No. of *M. domestica*		No. o*f C. megacephala*	
	Tested	Carrying enterococcus	Tested	Carrying enterococcus
Fresh-food markets	70	16	70	15
Garbage piles	70	14	70	11
Paddy fields	70	8	70	12
Restaurants	70	21	70	16
School cafeterias	70	8	70	3
Total	350	67	350	57

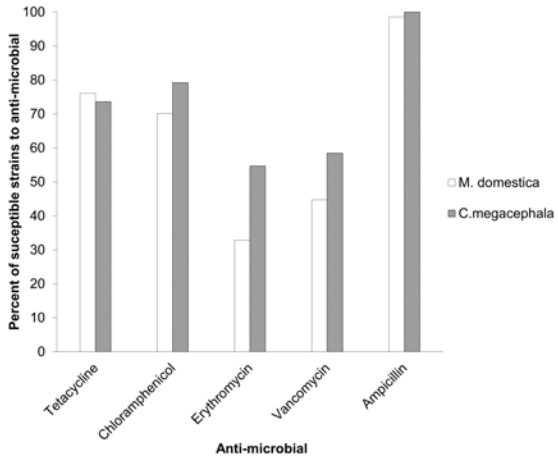

Fig. 1 Percentages of susceptibility to various antimicrobials for *Enterococcus* spp. isolated from *M. domestica* and *C. megacephala* by standard disk diffusion.

However, little is known regarding the drug resistance of bacteria associated with flies.

The present study revealed that the potential for *Enterococcus* spp. from *M. domestica* and *C. megacephala* was 17.1%. *Enterococcus* spp. is gram-positive cocci that can cause disease in humans, especially in immuno-compromised hosts. These bacteria find common commensal organisms in the intestines of humans and are important in the causes of urinary tract infection, bacteremia, endocarditis, and meningitis. In the setting of the present study, enterococci were tested using phenotypic antimicrobial activity. It was shown that by standard disk diffusion, ampicillin had the highest susceptibility to enterococci. Erythromycin exhibited susceptibility of 32.8% in contrast with ampicillin. In the present study, almost all isolates were susceptible to vancomycin by MIC.

The present study suggested that flies in Muang Ubon Ratchathani and Warinchamrap Districts in Ubon Ratchathani Province of North-East Thailand can carry enterococci on their external surfaces. Flies from the collection sites mentioned above could contribute to the dispersal of bacteria to humans. Graham et al studied the efficiency of houseflies and the dispersal of the antibiotic resistance of enterococci[11]. Recent studies involving *M. domestica* suggested *E. faecalis* was digested in the mid-gut but proliferated in the external environment. Then, environmental samples at the end of the assay were contaminated by fluorescing *E. faecalis*, demonstrating that the flies disseminated *E. faecalis.* This experiment concluded that *M. domestica* had the vector potential for *E. faecalis* as seen the bio-enhanced vector of *E. faecalis*[12]. Consequently, enterococci with reduced susceptibility to antimicrobial isolated from *M. domestica* and *C. megacephala* may proliferate in the environment and transfer the resistance to other bacteria in those areas, particularly in hospitals.

In the present study, it was found that enterococci reduced susceptibility to ciprofloxacin and in this study almost all isolates were susceptible to vancomycin based on MIC determination except one isolate from *C. megacephala.* Therefore, the potential transfer of isolates with reduced susceptibility to vancomycin could rarely occur. Flies may also transfer reduced susceptible antimicrobial enterococci from the study sites. Enterococcal infections are usually treated with penicillin, the drug of choice such as ampicillin, aminoglycosides, and tetracyclines. The present study should test susceptibility to penicillin and high gentamycin (120 µg) in the future.

Enterococci were isolated from flies from fresh-food markets, garbage piles, restaurants, school cafeterias, and paddy fields close to human habitation and food-preparation areas. However, enterococci isolated from flies in Ubon Ratchathani showed higher

Table 2. MICs of antimicrobials for the Enterococcus isolated from *M. domestica* and *C. megacephala*

Antimicrobials (susceptible ranges)	No. of isolates giving MICs (μg/mL)							
	M. domestica (n = 67 isolates)							
	≤0.25	0.5	1	2	4	8	16	>16
Chloramphenicol	0	0	0	0	0	29	34	4
Tetracycline	0	0	50	0	1	0	0	16
Vancomycin	0	16	19	30	2	0	0	0
Ciprofloxacin	0	7	0	33	20	7	0	0
	C. megacephala (n = 53 isolates)							
	≤0.25	0.5	1	2	4	8	16	>16
Chloramphenicol	0	0	0	0	1	33	16	3
Tetracycline	0	0	43	1	1	0	0	8
Vancomycin	1	8	11	28	4	1	0	0
Ciprofloxacin	0	0	10	42	1	0	0	0

Table 3. Antimicrobial susceptibility patterns were obtained from 120 *Enterococcus* spp. isolates

Antimicrobial susceptibility patterns	AM	TE	C	E	V	No. of isolates
I	S	S	S	S	S	74
II	S	S	R	S	S	3
III	S	R	S	S	S	9
IV	R	S	S	S	S	1
V	S	S	S	R	S	11
VI	R	R	S	R	S	1
VII	S	R	R	R	S	8
VII	S	R	S	R	S	10
IX	S	S	R	R	S	3

AM = ampicillin, TE = tetracycline, C = chloramphenicol, E = Erythromycin, V = vancomycin

susceptibility to ampicillin and decreased susceptibility to erythromycin by standard disk diffusion testing. Enterococci isolated from food displayed a higher resistance to erythromycin, ciprofloxacin, and levofloxacin but had susceptibility to penicillin and gentamycin[13]. However, regarding the differences found in their patterns of susceptibility to organisms, it is remarkable that the highest concentration of resistant bacteria was found in the Ubon Ratchathani Province. It is suggested that there is a need to review management strategies for houseflies and blowflies.

Conclusion

The flies collected from fresh-food markets, garbage piles, restaurants, school cafeterias, and paddy fields in Ubon Ratchathani Province may be vectors of human pathogenic enterococci. The potential for these flies to act as *Enterococcus* species vectors was 17.1%. These bacteria had reduced susceptibility to antibiotics such as erythromycin, ciprofloxacin, tetracycline, and cholramphenicol; this may lead to failure in the treatment of enterococcal infection in humans. The control of fly populations may be a practical method to reduce the transference of enterococci in this region.

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Potential conflicts of interest

None.

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รูปแบบของเชื้อ Enterococcus spp. ต่อการดื้อยาปฏิชีวนะ ที่แยกได้จากแมลงวันบ้าน (Musca domestica) และแมลงวันหัวเขียว (Chrysomya megacephala) ในอุบลราชธานี

ธารินี ไชยวงศ์, ธันยากาญจ์ ศรีวรมาศ, มารุตพงศ์ ปัญญา, สุรศักดิ์ แว่นรัมย์, ภาวนา พนมเขต

ภูมิหลัง: *แมลงวันบ้าน (Musca domestica) และแมลงวันหัวเขียว (Chrysomya megacephala) เป็นแมลงวันที่มีความสำคัญทางการแพทย์ และสามารถแพร่กระจายได้ไปทั่วโลกรวมทั้งในประเทศไทย แมลงตัวเต็มวัยสามารถเป็นพาหะนำเชื้อโรคได้หลายชนิดจากสถานที่ที่ไม่ถูกสุขลักษณะสู่อาหารของคนและเป็นสาเหตุทำให้เกิดการก่อโรคในคน*

วัตถุประสงค์: *การศึกษานี้มุ่งหวังเพื่อทดสอบความไวของยาปฏิชีวนะต่อเชื้อ Enterococcus spp. ที่แยกได้จากแมลงวันบ้านและแมลงวันหัวเขียวด้วยวิธี standard disk diffusion และการทดสอบ minimum inhibitory concentration (MIC) และศึกษาความสามารถในการนำเชื้อ Enterococcus spp. ที่ดื้อยาในคน*

วัสดุและวิธีการ: *เก็บแมลงตัวเต็มวัยจากสถานที่ต่างๆ จากอำเภอเมืองและอำเภอวารินชำราบในสถานที่ดังนี้ ตลาดสด ถังขยะ ร้านอาหาร โรงอาหารภายในโรงเรียน และทุ่งนา เชื้อ Enterococcus spp. ที่แยกได้จากแมลงวันทั้งสองชนิด ในการศึกษาครั้งนี้รวมทั้งสิ้น 120 ตัวอย่าง*

ผลการศึกษา: *แมลงวันบ้านและแมลงวันหัวเขียวที่ได้จากอำเภอเมืองและอำเภอวารินชำราบมีศักยภาพ ในการเป็นพาหะนำเชื้อ Enterococcus spp. พบว่าเชื้อ Enterococcus spp. ที่แยกได้จากแมลงวันทั้งสองชนิด ไวต่อยา ampicillin ร้อยละ 99.2 chloramphenicol ร้อยละ 74.2 tetracycline ร้อยละ 75.0 vancomycin ร้อยละ 50.8 และ Erythromycin ร้อยละ 42.5 ด้วยวิธี standard disk diffusion ผลการทดสอบ MIC ต่อยาต่างๆ ดังนี้ vancomycin 0.5-4 μg/mL tetracycline 1-4 μg/mL chloramphenicol 8-≥16 μg/mL และ ciprofloxacin 0.5-8 μg/mL ตามลำดับ*

สรุป: *การศึกษาครั้งนี้แสดงให้เห็นศักยภาพของแมลงวันบ้านและแมลงวันหัวเขียวต่อการเป็นพาหะนำเชื้อ Enterococcus spp. มีความไวของยาปฏิชีวนะต่อเชื้อ enterococci 120 สายพันธุ์ทั้งหมด 9 รูปแบบ*