Original Article

Determination of Antimicrobial Susceptibility, Antimicrobial Resistance Genes and *in vivo* Testing of Antimicrobial Susceptibility of *Avibacterium paragallinarum*

Tippayaporn Noonkhokhetkong [1] Kridda Chukiatsiri [2] Jiroj Sasipreeyajan [1]

Niwat Chansiripornchai [1*]

Abstract

Avibacterium paragallinarum is the causative agent of infectious coryza, an acute upper respiratory disease in chickens. The aim of this study was to characterize the antimicrobial susceptibility, resistance genes and the *in vivo* testing of antimicrobial susceptibility in chickens. Eighteen *A. paragallinarum* isolated in Thailand were tested for antimicrobial susceptibility by broth microdilution method. A high prevalence of resistance MIC pattern to oxytetracycline, doxycycline, streptomycin, ciprofloxacin, erythromycin and sulfamethoxazole-trimethoprim was found. Thirteen isolates showed multiple antimicrobial drug resistance (66.7%). Resistance genes of *tet(A), tet(B), tet(M), erm(A), erm(B),* bla_{ROB-1} and *sul2* were found and the resistance genes of *tet(A), tet(B)* and *tet(M)* were revealed to be the highest percentage of resistance genes discovered (66.7%).The chickens were challenged with *A. paragallinarum* strains, which are sensitive and do not harbor any antimicrobial resistance genes against amoxicillin and doxycycline. Three days later, the challenged chickens were treated with amoxicillin and doxycycline. The treatment by sensitive antimicrobial drugs reduced the clinical signs in the challenged groups, but *A. paragallinarum* could be isolated from the infraorbital sinus 7 days after treatment. In conclusion, the sensitive antimicrobial agent could reduce the clinical signs of infectious coryza challenged by the sensitive antimicrobial and no harbor of antimicrobial resistance genes of *A. paragallinarum*.

Keywords: antimicrobial agents, *Avibacterium paragallinarum*, chickens, resistance genes

[1]*Avian Health Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Faculty of Animal Science and Technology, Maejo University, Chiangmai 50290, Thailand*
***Corresponding author: E-mail**: cniwat@chula.ac.th*

บทคัดย่อ

การตรวจวิเคราะห์ความไวต่อยาต้านจุลชีพ ยีนดื้อยาต้านจุลชีพ และการทดสอบความไวต่อยาต้านจุลชีพในสัตว์ทดลองของเชื้อ *เอวิแบคทีเรียม พารากัลลินารุม*

ทิพยาพร โนนคู่เขตโขง [1] ***กฤดา ชูเกียรติศิริ*** [2] ***จิโรจ ศศิปรียจันทร์*** [1] ***นิวัตร จันทร์ศิริพรชัย*** [1]*

เอวิแบคทีเรียม พารากัลลินารุม เป็นสาเหตุของโรคหวัดหน้าบวม ซึ่งเป็นโรคระบบทางเดินหายใจแบบเฉียบพลันในไก่ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาความไวต่อยาต้านจุลชีพ ยีนดื้อยาต้านจุลชีพ และการทดสอบความไวต่อยาต้านจุลชีพในสัตว์ทดลองของเชื้อ *เอวิแบคทีเรียม พารากัลลินารุม* นำเชื้อ *เอวิแบคทีเรียม พารากัลลินารุม* จำนวน 18 สายพันธุ์ที่แยกได้ในประเทศไทย มาทดสอบความไวต่อยาต้านจุลชีพด้วยวิธี broth microdilution พบความชุกอย่างสูงของการดื้อยาต่อยาออกซีเตตราซัยคลิน ดอกซีซัยคลิน สเตรปโตมัยซิน ซิโปรฟลอกซาซิน อิริโทรมัยซิน และซัลฟาเมทอกซาโซล-ไตรเมทโทรปริม จำนวน 13 เชื้อพบการดื้อยาตั้งแต่ 3 ชนิด (ร้อยละ 66.7) พบยีนดื้อยา *tet(A) tet(B) tet(M) erm(A) erm(B)* bla_{ROB-1} และ *sul2* โดยยีนดื้อยา *tet(A) tet(B)* และ *tet(M)* พบเป็นร้อยละที่สูง (ร้อยละ 66.7) ทำการให้เชื้อ *เอวิแบคทีเรียม พารากัลลินารุม* ซึ่งเป็นเชื้อที่ไวและไม่มียีนดื้อยาต่อยาแอมพิซิลลินและดอกซีซัยคลินในไก่ สามวันต่อมาทำการรักษาด้วยยาอะมอกซีซิลลินและดอกซีซัยคลิน การรักษาด้วยยาต้านจุลชีพที่มีความไวต่อเชื้อ สามารถลดอาการทางคลินิกในกลุ่มที่ได้รับเชื้อแต่ยังคงสามารถแยกเชื้อ *เอวิแบคทีเรียม พารากัลลินารุม* จากไซนัสใต้ตาได้ที่ 7 วันหลังจากทำการรักษา สรุปยาต้านจุลชีพสามารถลดอาการทางคลินิกของโรคหวัดหน้าบวมในไก่ที่ได้รับเชื้อ *เอวิแบคทีเรียม พารากัลลินารุม*ที่มีความไวต่อยาต้านจุลชีพและไม่มียีนดื้อยา

คำสำคัญ: *เอวิแบคทีเรียม พารากัลลินารุม* ยาปฏิชีวนะ ยีนดื้อยาไก่

[1] *หน่วยปฏิบัติการวิจัยสุขภาพสัตว์ปีก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ10330 ประเทศไทย*

[2] *คณะสัตวศาสตร์และเทคโนโลยี มหาวิทยาลัยแม่โจ้ เชียงใหม่ 50290 ประเทศไทย*

***ผู้รับผิดชอบบทความ** E-mail: cniwat@chula.ac.th*

Introduction

Avibacterium paragallinarum is a gram negative and non-motile bacterium whichis a causative agent of avian infectious coryza, an acute upper respiratory disease in chickens (Blackall et al., 2005). The disease results in poor growth performance in broilers and a marked reduction (10-40%) in egg production in layers (Blackall and Soriano, 2008). Clinical signs of this disease involve nasal passage and sinuses with serous to mucoid nasal discharge, facial edema and conjunctivitis (Blackall, 1999). Chickens of all ages are susceptible to *A. paragallinarum*, but the disease is usually less severe in young chickens. The incubation period is shortened and the course of the disease tends to be longer in mature chickens (Blackall and Soriano, 2008). *A. paragallinarum* are classified into three serovars named A, B and C using a slide agglutination test (Page, 1962). In Thailand, *A. paragallinarum* infection has been found in both industrial and native chickens (Neramitmansuk and Neramitmansuk, 1985). Currently, outbreaks of the three serovars have been reported (Chukiatsiri and Chansiripornchai, 2007; Chukiatsiri et al., 2010). Vaccination is a common method for prevention of infectious coryza. Unfortunately, there is no cross protection between serovars, so commercial vaccine productions are used in combination with many serovars including bivalent (serovar A and C) vaccine, trivalent (serovar A, B and C) vaccine and tetravalent (serovar A, B, C and B variant) vaccine. However, difference between field and vaccine strains cause outbreaks although farms are vaccinated (Chukiatsiri and Chansiripornchai, 2007).

Various antimicrobial drugs are useful for alleviating the severity and course of infectious coryza, but drug resistance in *A. paragallinarum* does occur especially if treatment is discontinued and when there is no elimination of carriers (Blackall and Soriano, 2008). Thus, emergence of the antimicrobial resistance to *A. paragallinarum* has been reported in many countries. In 2007, more than 75% of *A. paragallinarum* were resistant to neomycin, streptomycin and erythromycin and 88.9% of the isolates were resistant to two or more antibiotics. Around 72% of the isolates contain plasmids pYMH5 which are encoded in functional streptomycin, sulphonamides, kanamycin and neomycin resistance genes (Hsu et al., 2007). In Thailand, antimicrobial resistance to *A. paragallinarum* outbreak isolated from vaccinated chickens has revealed resistance to amoxicillin, erythromycin, sulfamethoxazole-trimethoprim and doxycycline (Chukiatsiri and Chansiripornchai, 2007). Many antimicrobial resistant genes are linked to these antimicrobial resistance characters. β-lactamase gene (bla_{ROB-1}) has been detected in *Haemophilus parasuis* (San Millan et al., 2007). *tet*(B) and *tet*(M) are frequently found in tetracycline resistance genes (*tet* genes) of Genus *Haemophilus* (Kehrenberg, 2006). Moreover, *sul*2is frequently found as sulphonamides resistance gene of *Haemophilus influenza* (Enne et al., 2002). Macrolides resistance genes including *erm*(A), *erm*(B) and *tet*(C) are frequently found in Genus *Aggregatibacter,* which is in the same Family of *Pasteurellaceae* (Kehrenberg,

2006). Selection of an appropriate antimicrobial drug to alleviate suffering of chickens from infectious coryza needs information on antimicrobial susceptibility and resistance genes. The aim of this study was to characterize the antimicrobial susceptibility, resistance genes and *in vivo* testing of antimicrobial susceptibility of *A. paragallinarum* in chickens.

Materials and Methods

Bacteria: Eighteen isolates of *A. paragallinarum* were obtained from chickens showing the typical clinical signs of infectious coryza studied by Chukiatsiri et al. (2012). Chocolate agar plate and supplemented test medium broth (TMB) (Chukiatsiri et al., 2012) were used for the culture and maintenance of *A. paragallinarum* cultures under aerobic conditions at 37^0C overnight for TMB and at 37^0C with 5% CO_2 overnight for the chocolate agar plate.

Antimicrobial susceptibility test: Antimicrobial susceptibility testing by broth microdilution method was modified from a prior study (Blackall, 1988). Briefly, eighteen isolates were grown in supplemented TMB broth at 37^0C for 2-6 hours and then diluted to 5 x 10^5 CFU/ml. The tested antimicrobial drugs included amoxicillin**,** ampicillin**,** ceftiofur, cephalexin, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, gentamicin, nalidixic acid, oxytetracycline, spectinomycin, streptomycin andsulfamethoxazole–trimethoprim. A concentration of antimicrobial drugs was diluted two-fold from 256 to 0.25 µg/ml in a 96 well plate, followed by addition of the bacterial suspension. An interpretation of the minimum inhibitory concentration (MIC) results was analyzed according to the standards used by Blackall (1988) and Clinical and Laboratory Standards institute (CLSI, 2011).

Detection of resistance genes: *A. paragallinarum* was cultured on chocolate agar overnight and harvested, then the bacteria was extracted for DNA by being added to 200 µl sterile PBS, vortexed and heated in an AccuBlock™ Digital Dry Bath (Labnet international, Inc., USA) at 98^0C for 5 min, and then, centrifuged at 5,000 x g for 5 min. Supernatant was collected and used as DNA template for PCR amplification of the resistance genes by specific oligonucleotide primers and annealing temperature (Table1). A polymerase chain reaction (PCR) was conducted by heating at 98^0C for 20 min 30 sec, 25 cycles of denaturation at 94^0C for 1 min, annealing for 1 min, an extension at 72^0C for 2 min and then, a final extension step at 72^0C for 10 min. The PCR product was separated onto a 0.7% agarose gel by electrophoresis and visualized under UV light.

In vivo testing of antimicrobial susceptibility

Chickens and experimental design: One hundred thirty-five-days female layer chickens, Babcock 308, were obtained from a commercial hatchery. The chickens were randomly divided into 10 groups of 10 chickens each. The chickens in groups 1-3, 4-6 and 7-9 were challenged with 98, 111492 and B1E1, respectively. Group 10 served as the negative control group. Three days after challenge, the chickens in groups 2, 5 and 8 were administered with amoxicillin and the chickens in groups 3, 6 and 9 were administered with doxycycline (Table 4). The chickens were euthanized at 8 days after challenge and the left and right infraorbital sinuses of the euthanized chickens were swabbed and cultured for *A. paragallinarum* on a blood agar plate which was cross-streaked with *Staphylococcus aureus*. Each group of chickens was maintained in a separate unit and raised in metallic cages at environmental temperature. The chickens were fed *ad libitum* before and during the

Table 1 Oligonucleotide primers used for detection of antimicrobial resistance genes in *A. paragallinarum*

Gene	Primers	5′ -----> 3′ sequence	Fragment size (bp)	Annealing Temp. (^{0}C)	References
Tetracycline					
tet(A)	tetA-L	GGCGGTCTTCTTCATCATGC	502	65	Lanz et al. (2003)
	tetA-R	CGGCAGGCAGAGCAAGTAGA			
tet(B)	tetB-L	CATTAATAGGCGCATCGCTG	930	65	Lanz et al. (2003)
	tetB-R	TGAAGGTCATCGATAGCAGG			
tet(M)	tet1	GCTCA(T/C)GTTGA(T/C)GCAGGAA	500-600	60	Barbosa et al. (1999)
	tet 2	AGGATTTGGCGG(C/G)ACTTC(G/T)A			
Sulphonamides					
sul2	sulII-a	CGGCATCGTCAACATAACCT	721	65	Lanz et al. (2003)
	sulII-b	TGTGCGGATGAAGTCAGCTC			
Erythromycin					
erm(A)	erm(A)-F	ATGAACCAGAAAAACCCTAAAG	732	54	Matter et al. (2007)
	erm(A)-R	TTAGTGAAACAATTTGTAACTATTG			
erm(B)	erm(B)-F	GAAAAGGTACTCAACCAAATA	639	54	Chung et al. (1999)
	erm(B)-R	AGTAACGGTACTTAAATTGTTTAC			
Penicillin					
bla_{ROB-1}	bla_{ROB}-1-F	CATTAACGGCTTGTTCGC	852	65	Matter et al. (2007)
	bla_{ROB}-1-R	CTTGCTTTGCTGCATCTTC			

experiment. The guidelines and legislative regulations on the use of animal scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as is certified in permission no. 1031042.

Bacterial strains and Antimicrobial drugs: Three isolates of the *A. paragallinarum* strains 98, 111492 and B1E1 were selected by the broth microdilution method and by examining resistance genes which were sensitive and not resistant to penicillin and tetracycline (Table 3). The strains 98, 111492 and B1E1 were cultured in supplemented TMB broth and incubated at 37^0C overnight with shaking. The bacteria were adjusted to a concentration of 10^8 CFU/ml. The chickens were challenged by intranasal inoculation of 0.2 ml bacteria. At seventy two hours after challenge, amoxicillin (Virbac Animal Health, Thailand) and doxycycline (Better Pharma Co Ltd, Thailand) were oral administered once a day for a 5 consecutive day period at a concentration of 20 mg/kg body weight. The amoxicillin and doxycycline were selected for *in vivo* testing because these drugs are frequently used to treat poultry respiratory tract infection.

Scoring for clinical signs: Presence of the clinical signs of infectious coryza of all chickens was examined daily after challenge. Scoring of clinical signs was recorded as described previously (Soriano et al., 2004): 0 : no sign, 1 : nasal discharge or slight facial swelling, 2 : nasal discharge and moderate facial swelling, 3 : abundant nasal discharge and several facial swelling, 4 : as in 3 but including swelling of wattles. The disease score for each group was calculated for mean daily clinical sign score which equals the total clinical sign scores divided by the number of chicken of every group.

Statistical analysis: The clinical sign scores were analyzed using the Kruskal Wallis test with SPSS for Windows (SPSS Inc, Chicago). Statistical difference was considered significant at $p < 0.05$.

Results

Antimicrobial susceptibility test: The antimicrobial susceptibility patterns of all the isolates are presented in Tables 2 and 3. The resistance was most often observed in oxytetracycline (13 isolates, 72.2%) followed by doxycycline, streptomycin, ciprofloxacin, erythromycin, sulfamethoxazole-trimethoprim (12 isolates, 66.7%), gentamicin (10 isolates, 55.6%), enrofloxacin, spectinomycin (9 isolates, 50%), ampicillin and ceftiofur (1 isolates, 5.6%). All isolates were sensitive to amoxicillin. Thirteen isolates (72.2%) were resistant to two or more antimicrobials. Strains 98 and 111492 were sensitive to all the antimicrobials in this study.

Detection of antimicrobial resistance genes: *tet(A), tet(B), tet(M)* and *erm(A)* were detected in the highest number of isolates (12 isolates, 66.7%) followed by*erm(B)*(11 isolates, 61.1%), *sul2* (3 isolates, 16.7%) and bla_{ROB-1}(1 isolates, 5.6%). Thirteen isolates (72.2%) had at least one resistance gene and 12 isolates (66.7%) had three or more resistance genes. The relationships between antimicrobial susceptibility and resistance genes are listed in Table 3. Twelve isolates (66.7%) were resistant to tetracycline (doxycycline and oxytetracycline) as determined by broth microdilution method and also had the resistance genes of *tet(A)*, *tet(B)*and *tet(M)*. One strain was resistant to oxytetracycline, but did not have these tetracycline resistance genes. Twelve isolates (66.7%) were resistant to erythromycin and 11 isolates had the resistance genes of *erm(A)*and *erm(B)*except 1 isolate that had only *erm(A)* genes. Twelve isolates (66.7%) were resistant to sulfamethoxazole-trimethoprim, but only 3 isolates (16.7%) had resistance genes (*sul2*). One isolate (5.6%) was resistant to ampicillin and had resistance genes (bla_{ROB-1}).

Table 2 MIC of fourteen antimicrobial drugs for *A. paragallinarum* isolates

Antibiotics	Breakpoints	Number of strains with MIC of (µg/ml)											Resistance
		≤0.25	0.5	1	2	4	8	16	32	64	128	≥256	(%)
Ampicillin[a]	≥8	8	6	1	1	1	1	-	-	-	-	-	5.6
Amoxicillin[b]	≥8	14	2	2	-	-	-	-	-	-	-	-	0
Ceftiofur[b]	≥4	11	4	2	-	1	-	-	-	-	-	-	5.6
Cephalexin	ND[c]	3	-	2	5	3	5	-	-	-	-	-	ND[c]
Doxycycline[b]	≥8	3	3	-	-	-	-	2	-	5	5	-	66.7
Oxytetracycline[b]	≥8	1	-	-	1	3	-	-	1	-	-	12	72.2
Ciprofloxacin[b]	≥2	2	3	1	1	1	-	1	-	2	3	4	66.7
Enrofloxacin[b]	≥1	9	-	-	-	2	0	5	-	1	-	1	50
Sulfamethoxazole–trimethoprim[a]	≥2	4	-	2	-	-	-	-	-	-	2	10	66.7
Erythromycin[a]	≥4	2	1	2	1	-	1	1	1	3	4	1	66.7
Gentamicin[b]	≥8	3	2	1	-	2	2	5	2	1	-	-	55.6
Spectinomycin[b]	≥64	2	-	-	-	-	1	3	3	4	3	2	50
Streptomycin[a]	≥8	3	-	2	1	-	-	1	-	-	-	11	66.7
Nalidixic acid	ND[c]	2	2	2	1	1	1	-	1	3	2	3	ND[c]

[a] The breakpoints of MIC values reported by Blackall (1988)
[b] The breakpoints of MIC values reported by CLSI (2011)
[c] Not defined.

In vivo testing of antimicrobial susceptibility: Three isolates of *A. paragallinarum* (98, 111492 and B1E1) which were sensitive to amoxicillin and doxycycline and did not harbor the resistance genes of penicillin

(bla_{ROB-1}) and tetracycline ((*tet(A)*, *tet(B)* and*tet(M)*) were studied for the *in vivo* testing of antimicrobial susceptibility. The mean clinical sign scores for treated and untreated chickens challenged with the different strains of *A. paragallinarum* are shown in Table 4. The mean clinical sign scores of treated chickens in all groups were lower than those of the positive control starting from the fourth day post inoculation (DPI).The mean clinical sign scores of treated chickens which were challenged with strain 98 and received amoxicillin and doxycycline were significantly lower than those of the positive control chickens on the 4 DPI ($p < 0.05$).The mean clinical sign scores of treated chickens challenged with strain 111492 and receiving amoxicillin and doxycycline were significantly lower than those of the positive control chickens on the 5 and 6 DPI ($p < 0.05$). Moreover, the mean clinical sign scores of the chickens challenged with strain B1E1 and receiving amoxicillin were significantly lower than those of the positive control chickens on the 4 and 5 DPI ($p < 0.05$), but the mean clinical signs of chickens receiving doxycyclinehad no significant difference from the positive control chickens.

The highest number of positive bacterial re-isolations from the mucous of the infraorbital sinuses was found in the positive control group of all challenge strains, followed by all the groups treated with amoxicillin and doxycycline. However, no significant differences were found between the treatment groups and the positive control group in each challenged strain. No clinical signs and bacterial cultures were found in the negative control group.

Discussion

In this study, eighteen *A. paragallinarum* isolates from Thailand were tested for antimicrobial susceptibility by the broth microdilution method and most of the isolates were resistant to at least one antimicrobial agent. Only two isolates (11.1%), strains 98 and 111492, were not resistant to all antimicrobial drugs. The prevalence of broth microdilution resistance could be arranged from high to low resistance as follows oxytetracycline, doxycycline, streptomycin, ciprofloxacin, erythromycin and sulfamethoxazole-trimethoprim. The results were in accord with many previous studies (Blackall, 1988; Poernomo et al., 2000; Hsu et al., 2007; Chukiatsiriet al., 2012). Compared to Chukiatsiri et al. (2012), who performed the disk diffusion method against the same eighteen *A. paragallinarum* strains, the antimicrobial susceptibility patterns were similar to the current

Table 3 Antimicrobial susceptibility and resistance genes of *A. paragallinarum* isolates

Strains	serovar	Antimicrobial resistance profile	Antimicrobial resistance gene(s)
98	A		
423	A-2	ERT	*erm(A)*
746	A	OXT	
102090	A	DOX/OXT/SXT/STR	*tet(A) /tet(B)/tet(M)*
111492	A		
112179	A	DOX/OXT/CIP/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
B1E1	A-2	STR	
IR1	A-2	CIP/ENR	
CHS0809	A	DOX/OXT/CIP/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
CMU1009	A	AMP/CEF/DOX/OXT/CIP/SXT/ERT/ENR/STR	*tet(A)/tet(B)/tet(M)/erm(A)/erm(B)/*bla_{ROB-1}
1687	B	DOX/OXT/CIP/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
102984	B	DOX/OXT/CIP/ENR/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
211108	B	DOX/OXT/CIP/ENR/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
CMA0509	B	DOX/OXT/CIP/ENR/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
F1CM0809	B	DOX/OXT/CIP/ENR/SXT/ERT/GEN/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)*
102943	C	DOX/OXT/CIP/ENR/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)/sul2*
102947	C-2	DOX/OXT/CIP/ENR/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)/sul2*
115757	C	DOX/OXT/CIP/ENR/SXT/ERT/GEN/SPC/STR	*tet(A) /tet(B)/tet(M)/erm(A)/erm(B)/sul2*

AMP: ampicillin; DOX: doxycycline; OXT: oxytetracycline; CEF: ceftiofur; CIP: ciprofloxacin; ENR: enrofloxacin; SXT: Sulfamethoxazole–trimethoprim;ERT: erythromycin; GEN: gentamicin: SPC: spectinomycin; STR: streptomycin

Table 4 Mean clinical sign scores and infraorbital sinuses isolation of experimental layers

Group	Challenge strain	drugs	Mean clinical sign scores (Day post inoculation) 1	2	3	4	5	6	7	Infraorbital sinuses
1	98	-	0.8^a	0.8^a	1.2^a	1.6^a	1^a	0.9^a	0.3^a	$9/10^a$
2	98	amoxicillin	0.8^a	1.1^a	1.1^a	0.7^b	0.5^a	0.5^a	0^a	$6/10^a$
3	98	doxycycline	0.8^a	1.1^a	1.4^a	0.9^b	0.8^a	0.3^a	0.2^a	$8/10^a$
4	111492	-	0.7^a	0.8^a	1^a	1.1^a	1.2^a	0.9^a	0.6^a	$8/10^a$
5	111492	amoxicillin	0.8^a	1.4^a	1.2^a	0.6^a	0.2^b	0.2^b	0.2^a	$4/10^a$
6	111492	doxycycline	0.8^a	1.4^a	1.2^a	0.6^a	0.2^b	0.2^b	0.2^a	$7/10^a$
7	B1E1	-	0.9^a	1.2^a	1.6^a	1.6^a	1.2^a	0.9^a	0.7^a	$9/10^a$
8	B1E1	amoxicillin	1.1^a	1.2^a	1.6^a	0.8^b	0.7^b	0.4^a	0.3^a	$4/10^a$
9	B1E1	doxycycline	0.8^a	1.2^a	1.8^a	1.2^a	0.8^a	0.5^a	0.4^a	$6/10^a$
10	-	-	0^b	0^b	0^b	0^b	0^b	0^b	0^b	$0/10^b$

research but the number of resistance strains for each antimicrobial drug was not the same; this may have been caused by the different antimicrobial susceptibility methods. The antimicrobial susceptibility results showed that 66.7% of the isolates were resistant to three or more antimicrobial drugs. Thus, most of the *A. paragallinarum* isolates from Thailand were multiple antimicrobial drugs resistance.

For the resistance genes, *tet(A)*, *tet(B)* and *tet(M)* genes which were resistant to oxytetracycline and doxycycline, was found at the highest number (66.7%), according with the high resistance rate to oxytetracycline and doxycycline (72.2% and 67.7%) and was the same as the high number of *erm(A)* and *erm(B)* genes (61.1%) which showed resistance to erythromycin (66.7%).One strain was resistant to oxytetracycline but did not have these tetracycline resistance genes; this strain may carry other tetracycline resistance genes that were not included in our study. Three isolates (16.7%) were found in the*sul2* gene, similar to the work of Hsu et al. (2007), which found multidrug resistance plasmid (pYMH5) in *A. paragallinarum* that encoded functional sulfonamide, kanamycin, streptomycin and neomycin resistance genes.

In the chicken experiment, after challenged with *A. paragallinarum* isolates which were sensitive to selected antimicrobial drugs and had no resistance genes, they were then treated with sensitive antimicrobial drugs. The sensitive antimicrobial drugs could reduce the clinical signs in all treated groups and at 7 DPI most of chickens showed no clinical signs, according with the study of Zhao et al. (2009). Infraorbital sinuses isolation showed a low rate of elimination of *A. paragallinarum* in infraorbital sinuses of chickens. The results are similar to previous reports which showed that after 5-7 days of treatment, if chickens were continuously treated with appropriate antimicrobial drugs, clinical signs would disappear completely (Blackall and Hinz, 2008) and the case report of Chukiatsiri and Chansiripornchai (2007), which showed that if layers were treated with susceptible drugs for 7 days, clinical signs of infectious coryza (nasal discharge and facial swelling) would decreased.

In conclusion, antimicrobial agent which was sensitive and did not have the resistance genes could reduce the clinical signs of infectious coryza although the antimicrobial drugs did not completely eliminate the bacteria from chickens in the period of observation.

Acknowledgements

This work was financially supported by the 90thAnniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) and the Graduate School of Faculty of Veterinary Science, Chulalongkorn University. We would like to thank the staff of Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University for their support.

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