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Antimicrobial Sensitivity of Avian Pathogenic Escherichia coli (APEC) Isolated from Chickens During 2007-2010

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Short Communication

Antimicrobial Sensitivity of Avian Pathogenic *Escherichia coli* (APEC) Isolated from Chickens During 2007-2010

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Abstract

Fifty isolates of Avian pathogenic *Escherichia coli* (APEC) isolated from chickens during 2007-2010 were *in vitro* tested to 21 antimicrobial discs. The highest sensitivity of APEC was to fosfomycin (84%). The second sensitivity against enrofloxacin and norfloxacin was 70%. The antimicrobial sensitivity to colistin, gentamicin, kanamycin, sulfametroxazole+trimethoprim, amoxicillin+clavulanic acid and doxycycline were 66, 64, 62, 60, 58 and 52%, respectively. The antimicrobial resistance of APEC to tylosin, tilmicosin, tiamulin, lincomycin, amoxicillin, ampicillin, erythromycin, cephalexin and neomycin were 100, 100, 100, 94, 86, 82, 80, 72 and 62%, respectively.

Keywords: APEC, antimicrobial drugs, chickens, sensitivity test

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บทคัดย่อ

ความไวต่อยาต้านจุลชีพของ อี. โคไล ก่อโรคสัตว์ปีกที่แยกได้จากไก่ระหว่างปี 2007-2010

นิวัตร จันทร์ศิริพรชัย [1*] *สราวุธ มูลจันที* [1] *พงษ์ไทย บุญคำ* [2]

เชื้อแบคทีเรีย อี. โคไล สายพันธุ์ก่อโรคในสัตว์ปีก จำนวน 50 เชื้อ ที่แยกได้ในไก่ระหว่างปี 2007-2010 นำมาทดสอบนอกตัวสัตว์ด้านความไวต่อแผ่นยาต้านจุลชีพจำนวน 21 ชนิด เชื้อ อี. โคไล ดังกล่าว มีความไวสูงสุดต่อฟอสโฟมัยซิน ร้อยละ 84 และมีความไวรองลงมาต่อเอนโรฟลอกซาซิน และนอร์ฟลอกซาซิน ร้อยละ 70 ความไวต่อโคลิสติน เจนตามัยซิน กานามัยซิน ซัลฟาเมททอกซาโซล+ไตรเมทโทรปริม อะมอกซิซิลลิน+กรดคลาวูลานิค และดอกซีซัยคลิน ร้อยละ 66, 64, 62, 60, 58 และ 52 ตามลำดับ พบการดื้อยาของเชื้อ อี. โคไล ก่อโรคสัตว์ปีกต่อไทโลซิน ทิลมิโคซิน ไทอะมูลิน ลินโคมัยซิน อะมอกซีซิลลิน แอมพิซิลลิน อิริโทรมัยซิน เซฟฟาเลคซิน และนีโอมัยซิน ร้อยละ 100, 100, 100, 94, 86, 82, 80, 72 และ 62 ตามลำดับ

คำสำคัญ: เชื้อ อี. โคไล ก่อโรคสัตว์ปีก ยาต้านจุลชีพ ไก่ การทดสอบความไวของเชื้อ

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Introduction

A major problem of the poultry industry around the world is respiratory tract infection, which can have a major economic impact due to increased losses through the death of birds, higher condemnation rates and additional treatment costs. Avian pathogenic *Escherichia coli* (APEC) has frequently been found as a cause also known as colibacillosis. APEC is a Gram-negative rod and non-spore forming bacterium. It can be found at all ages of poultry, most frequently between 4-5 weeks old. The outbreaks of the disease have been found in all types of poultry, especially, broilers raised in high density flocks. Predisposing causes of APEC infection include poor quality of chicks, bad ventilation and poor management. Clinical signs in naturally infected poultry range from subacute to chronic while sudden death usually occurred in younger chicks. The major pathological lesions of APEC infection in poultry are air sac disease, pericarditis, perihepatitis, peritonitis, salpingitis, synovitis, panophthalmitis, coligranuloma, omphalitis and yolk sac infection (Chansiripornchai, 2009). APEC can cause the primary infection, however the secondary infection triggered by other respiratory pathogens especially mycoplasma has frequently been found to be the cause of outbreaks. Control of APEC infection is mainly by management and vaccination. The result of vaccination against APEC infection with bacteria inactivated by formaldehyde and sonication is still variable and generally unsatisfactory. Vaccination of broiler breeding hens provides passive immunity to protect their offsprings during the first 2 weeks of age (Melamed et al., 1991). Recently, Rawiwet and Chansiripornchai (2009) reported that genetically modified live vaccine tended to prevent APEC infection in broilers.

APEC is a bacterial infection so the effective antimicrobials can be used to treat the infected birds especially at early infective stage. Application of appropriate antibiotics is always important to reduce the emergence of the antimicrobial resistant strain of APEC and to reduce the suffering and mortality of poultry from the APEC infection. Moreover, the suitable selection of antimicrobials to treat bacterial infection in poultry will reduce the duration of disease and increase the efficacy of disease treatment resulting in a higher profit of poultry production and a reduction in the resistance to existing antimicrobials. A decade ago, an *in vitro* antibiotic pattern of Thai APEC was reported (Chansiripornchai et al., 1995). The report provided a list of appropriate antimicrobials for poultry veterinary practitioners to use against APEC in the Thai poultry industry. The aim of this study was to reveal *in vitro* antimicrobial patterns of Thai APEC isolated between 2007 and 2010 A.D.

Materials and Methods

APEC identification: Fifty swabs taken from APEC suspected cases were sent to the Avian Health Research Unit or the Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University between 2007 and 2010 A.D. The swabs were cultured on 5% sheep blood and MacConkey agar (Oxoid, Hampshire, UK) and

Table 1 Sensitivity patterns of 50 APEC isolates isolated during 2007-2010 against 21 antimicrobial discs

Antimicrobial discs (μg)	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxycillin (10)	0	7(14)	43(86)
Ampicillin (10)	0	9(18)	41(82)
Amoxycillin + Clavulanic acid (20+10)	29(58)	7(14)	14(28)
Cephalexin (30)	14(28)	0	36(72)
Colistin (10)	33(66)	5(10)	12(24)
Doxycycline (30)	26(52)	9(18)	15(30)
Enrofloxacin (5)	35(70)	3(6)	12(24)
Erythromycin (15)	0	10(20)	40(80)
Fosfomycin (50)	42(84)	4(8)	4(8)
Gentamicin (10)	32(64)	6(12)	12(24)
Kanamycin (30)	31(62)	5(10)	14(28)
Lincomycin (2)	0	3(6)	47(94)
Lincospectin (100)	14(28)	21(42)	15(30)
Neomycin (30)	5(10)	14(28)	31(62)
Norfloxacin (10)	35(70)	5(10)	10(20)
Oxytetracycline (30)	21(42)	4(8)	25(50)
Sulfametroxazole+Trimethoprim (23.75+1.25)	30(60)	3(6)	17(34)
Tetracycline (30)	21(42)	13(26)	16(32)
Tiamulin (30)	0	0	50(100)
Tilmicosin (15)	0	0	50(100)
Tylosin (150)	0	0	50(100)

incubated overnight at 37^0C. The typical color and appearance colony of *E. coli* were picked and streaked again on blood agar plates, then re-streaked on EMB agar (Oxoid, Hampshire, UK). The green metallic sheen isolates were considered to be *E. coli* and the presumptive colonies were confirmed by specific biochemical tests (Bauer et al., 1974). The APEC isolates were stored at -20^0C in tryptic soy broth (Oxoid, Hampshire, UK) containing 15% glycerol until use.

Antimicrobial susceptibility tests: All 50 APEC isolates were tested against 21 antimicrobial discs. The antimicrobial sensitivity test was performed according to Bauer Kirby disc diffusion method (Bauer et al., 1966). Briefly, McFarland 0.5 standardized suspension of bacteria (10^8 cfu/ml) was swabbed over the surface of a blood agar plate (Oxoid, Hampshire, UK) and paper discs containing single concentrations of each antimicrobial agent were placed onto the inoculated surface. Following 37^0C incubation overnight, the diameters of inhibition zone were measured. The inhibition zone for each isolate was interpreted as sensitive, intermediate or resistant to a particular drug as described elsewhere (NCCLS, 2002).

Results and Discussion

The disc diffusion method revealed the sensitivity of antimicrobial discs against Thai APEC isolates. The result could provide a useful list of antimicrobials for poultry veterinary practitioners to treat colibacillosis. The antimicrobial sensitivity patterns of APEC isolates to 21 antimicrobial discs are revealed in Table 1. The tested APEC revealed the highest sensitivity to fosfomycin, which showed 84% sensitive and 8% resistant. This drug is relatively new and has not frequently been used in the poultry industry. Fosfomycin attacks bacteria by inhibiting cell wall synthesis. It shows broad spectrum activities *in vitro* against Gram positive bacteria and Enterobacteriaceae, especially *E. coli* isolated from human urinary tract infection (García-Rodriguez et al., 1997). *In vivo* study also revealed good efficacy of fosfomycin against APEC infection in poultry (Fernández et al, 2002). Enrofloxacin and norfloxacin, belonging to fluoroquinolone group, showed efficacy in *in vivo* treatment of APEC in Thai broilers (Chansiripornchai and Sasipreeyajan, 2004; Chansiripornchai, 2009). In this study, the APEC performed better and similar sensitivity to enrofloxacin (70%) and norfloxacin (70%) compared to the previous study of enrofloxacin (64.62%) and norfloxacin (70.65%) (Chansiripornchai et al., 1995). Currently, enrofloxacin and norfloxacin are prohibited from use in the Thai poultry industry (Chansiripornchai, 2011) due to their cross-resistance fluoroquinolones used for treatment of human enteric infection (García-Rodriguez et al., 1995). This may result in the better sensitivity of APEC isolates to both drugs. Moreover, the 4th generation of fluoroquinolone group tends to slowly develop the antimicrobial resistance because the drugs act on both DNA gyrase (type II and IV topoisomerase), the essential enzyme for DNA replication (Everett et al., 1996; Kim et al., 2011). The APEC revealed a higher sensitivity (66%) to colistin than the previous report of 48.19% (Chansiripornchai et al., 1995). Colistin is less frequently applied in cases of APEC infection due to its gastrointestinal tract activity, but APEC mainly causes infection of the respiratory system. Gentamicin revealed lower sensitivity (64%) to the tested APEC than the previous study of 83.52% (Chansiripornchai et al., 1995). Since it is available as injection preparation only, thus it is not the first choice for treatment of poultry disease. However, it is frequently selected if treatment by other antimicrobials fails. The APEC revealed higher resistance to amoxicillin (86%) and ampicillin (82%) than the previous report of amoxicillin (37.50%) and ampicillin (41.94%) (Chansiripornchai et al., 1995). Amoxicillin and ampicillin are frequently used for respiratory tract infection, therefore, they tend to show a higher rate of antimicrobial resistance for APEC infection. In

contrast to amoxicillin+clavulanic acid showed a higher sensitivity compared to amoxicillin alone. Amoxicillin+clavulanic acid is a relatively new antimicrobial used in the poultry industry and still provides a higher rate of sensitivity for APEC. The APEC showed higher sensitivity to sulfomethoxazole+trimethoprim (60%) than the previous study of sulfomethoxazole+trimethoprim (12.09%) (Chansiripornchai et al., 1995). The APEC revealed a higher sensitivity to doxycycline, oxytetracycline and tetracycline of 52, 42 and 42%, respectively than the previous report of 2.94, 2.0 and 4.0%, respectively (Chansiripornchai et al., 1995). Three drugs including tilmicosin and tylosin, belonging to the macrolide group and tiamulin, belonging to the pleuromutilin group are mainly active against Gram+ bacteria and mycoplasma, but these drugs are generally not effective against APEC infection (Chansiripornchai, 2011). In this study, the tested APEC revealed 100% resistance to these 3 drugs. In conclusion, the tested APEC revealed the various antimicrobial patterns to the tested antimicrobial discs. These data could preliminarily support the poultry veterinary practitioners to select appropriate antimicrobials for poultry application. However, the antimicrobial disc sensitivity test should be routinely performed to suggest or confirm the application of these antimicrobials in poultry industry.

References

Bauer, W.H., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Path. 45(4): 493-496.

Bauer, W.H., Davis, B.R. and Martin, W.J. 1974. Biochemical characterization of *Escherichia coli*. US Department of Health, Education and Welfare. Public Health Service, CDC Atlanta, GA: 15.

Chansiripornchai, N. 2009. Comparative efficacy of enrofloxacin and oxytetracycline for different administration times in broilers after experimental infection with avian pathogenic *Escherichia coli*. Thai J Vet Med. 39(3): 231-236.

Chansiripornchai, N. 2011. Antimicrobial drugs. In: Applications of Drugs and Vaccines in Poultry. Tiranasar Co Ltd, Bangkok: 14-57.

Chansiripornchai, N., Pakpinyo, S. and Sasipreeyajan, J. 1995. The *in vitro* antimicrobial sensitivity testing of *Escherichai coli* isolated from commercially reared chickens. Thai J Vet Med. 25(4): 275-283.

Chansiripornchai, N. and Sasipreeyajan, J. 2004. The efficacy of norfloxacin given in broiler chickens after experimental infection with *E. coli*. Proceeding of the 30th Thai Veterinary Medicine Association, 11-12 Nov 2004, p. 357-362.

Everett, M.J., Jin, Y.F., Ricci, V. and Piddock, L.J. 1996 Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. Antimicrob. Agents Chemother. 40(10): 2380-2386.

Fernández, A., Lara, C., Loste, A. and Marca, M.C. 2002. Efficacy of calcium fosfomycin for the treatment of experimental infections of broiler chickens with *Escherichia coli* O78:K80. Vet Res Commun. 26(6): 427-436.

García-Rodríguez, J.A., Fresnadillo, M.J., García, M.I., García-Sánchez, E., García Sánchez, J.E. and Trujillano, I. 1995. Multicenter Spanish study of ciprofloxacin susceptibility in gram-negative bacteria. Eur J Clin Microbiol Infect Dis. 14(4): 456-459.

García-Rodríguez, J.A., Trujillano Martín, I., Baquero, F., Cisterna, R., Gobernado, M., Liñares, F., Martín-Luengo, F. and Piédrola G. 1997. *In vitro* activity of fosfomycin trometamol against pathogens from urinary tract infections: a Spanish multicenter study. J Chemother. 9(6): 394-402.

Kim, I.S., Lee, N.Y., Kim, S., Ki, C.S. and Kim, S.H. 2011 Reduced levofloxacin susceptibility in clinical respiratory isolates of *Haemophilus influenzae* is not yet associated with mutations in the DNA gyrase and topoisomerase II genes in Korea. Yonsei Med J. 52(1): 188-191.

Melamed, D., Leitner, G. and Heller, E.D. 1991. A vaccine against avian colibacillosis based on ultrasonic inactivation of *Escherichia coli*. Avian Dis. 35(1): 17-22.

NCCLS. 2002. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. 2nd ed M31-A2 Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards.

Rawiwet, V. and Chansiripornchai, N. 2009. The efficacy of *E. coli* aroA-live vaccine in broilers against avian *E. coli* serotype O78 infection. Thai J Vet Med. 39(4): 337-342.