World J Microbiol Biotechnol (2008) 24:1741–1746
DOI 10.1007/s11274-008-9667-9

ORIGINAL PAPER

Concordance of antibiotic resistance and ERIC-PCR DNA fingerprint pattern in *Escherichia coli* isolated from farmer and broiler in the same farm

Kannika Na Lampang · Virasakdi Chongsuvivatwong · Rawee Teanpaisan · Viroj Kitikoon

Received: 28 April 2007 / Accepted: 9 January 2008 / Published online: 30 January 2008

Abstract The study was conducted to quantify the concordance of antibiotic resistance and ERIC-PCR DNA fingerprint pattern in *Escherichia coli* (*E. coli*) isolated from farmers and their broilers of 95 broiler farms in Songkhla province, Thailand. Four hundred and fifty-seven and 460 *E. coli* isolates from both groups produced 35 patterns of antibiotics resistance. Mono-resistance to doxycycline (23.2%) in isolates from farmers and multiple resistance to doxycycline, nalidixic acid, norfloxacin and ciprofloxacin (17.8%) were the most common finding in broilers. Twenty-seven farms had 44 within-farm concordant patterns of resistance. From simulation, the frequency of concordance was significantly higher than concordance by chance alone ($P < 0.05$). Out of these 44 matched sets, only four had the same DNA fingerprint pattern. Concordance by DNA pattern was also not associated with phenotypic resistance. Clonal spread is therefore not a good explanation of the concordance in this population. Other mechanisms need further analysis.

Keywords Antibiotics · Broiler · *Escherichia coli* · Farmer · Resistance

Introduction

As a result of evolutionary pressure for bacteria to survive in the environment with increased use of antimicrobials for humans and animals, antimicrobial resistance has become a global public problem leading to treatment failure and severity of infection (WHO 1997). Many studies have shown the spread of antimicrobial resistance from animal to human (Miles et al. 2006; Ojeniyi 1989; Oppegaard et al. 2001; Saida et al. 1981; van den Bogaard et al. 2001, 2002). Yet the extent at the population level is not known. Studies are often based on animals alone without close contact with human. Same phenotypical resistance patterns can be a result of chance alone and are often not supported by genotyping matching. Thus the evidence of cross infection is not strong. A population-based study on antibiotic resistance in humans in close contact with animals with phenotypic and genotypic matching is needed to assist control programs in the planning and for the evaluation of this problem.

Thailand has a large amount of poultry production. In 2005, there were over 30,000 households producing approximately 150 million broilers (Department of Livestock Development 2006c). Antibiotic use is known to be heavy. The amount of antibiotics used in 1998 was approximately USD 23 million (Thai Drug Control Division 2003) but details on animal species and the level of antibiotic resistance among the bacteria in the farm has rarely been reported. From our previous study, antibiotic use in broiler farms of southern Thailand is very common. Since broiler farmers are in close contact with the animals, they are good candidates to test the existence of animal to human transmission of antibiotic resistance microbials. The objectives of this study were (1) to document the prevalence and pattern of antibiotic resistance, (2) to test whether the concordance could be explained by chance and (3) to examine the extent of clonal spread of antibiotics resistance to *E. coli* within the same farm sampled from farmers and broilers in farms in rural southern Thailand.

K. Na Lampang (✉) · V. Chongsuvivatwong · V. Kitikoon
Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
e-mail: kna_lampang@hotmail.com

R. Teanpaisan
Department of Stomatology, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Materials and methods

Study design

The study is a cross-sectional survey conducted from November 2004 to April 2005.

Study site

Songkhla is a province with a population of 1.5 million and is located in the southern part of Thailand bordering Malaysia to the west. It plays an important role in the food industry as the number of households involved in animal food production was 34,976 (Department of Livestock Development 2006b). Out of these, 402 broiler farms produced 1,223,264 broilers in 2005 (Department of Livestock Development 2006a, c). The products are both locally consumed and exported to nearby provinces. Antibiotic use has been reported to be heavy. Major types include enrofloxacin, amoxicillin, doxycycline and colistine (Na Lampang et al. 2007).

Sampling technique

From the list of the farms registered at the Songkhla Provincial Livestock Office, 101 broiler farms were selected by simple random sampling. Selection criteria included: at least 500 broilers in the farm, safety from political unrest and accessibility by paved road. After giving consent at each selected farm, a randomly selected farmer who had been in close contact with the broilers and had never taken antibiotics in the previous 2 months was asked permission for a fecal swab. Three broilers were randomly selected from the same farm and cloacal swabs were carried out. All swabs were kept in transport media and sent to the laboratory on the same day.

E. coli isolation

On the day of arrival at the laboratory, the cloacal swabs from the broilers were mixed with 0.9% sterile normal saline solution and cultured on MacConkey agar (Merck) at 37°C overnight. Suspected *E. coli* colonies were inoculated onto Urea agar (Merck), Triple iron agar, Simmons' citrate agar and Motility-indole-lysine media (Difco). Identification of *E. coli* was done based on biochemical reactions standard (Quinn et al. 1994). Three to five colonies were randomly selected and subcultured for antibiotics resistance pattern and genotype analysis. Only isolates from farms with *E. coli* from the farmers and broilers were used in the analysis.

Antibiotic resistance testing

Antibiotic resistance tests were performed by the standard disc diffusion technique recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The selection criteria of antibiotics testing discs depended on the regularly use of antibiotics in the broiler farm, potential public health importance and recommended from the guideline of antibiotic susceptibility testing from NCCLS. Resistance testing discs contained amoxicillin/clavulanic (20:10 μg), nalidixic acid (30 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), doxycycline (30 μg), ceftriaxone (30 μg), cefuroxime (30 μg) and cephalothin (30 μg) (Oxoid). The isolates were considered resistant if the diameter of inhibition zone was less than or equal to the resistance breakpoint recommended by NCCLS guidelines. Quality control of diameters of inhibition zone against the level of resistance was based on standard *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

Genotyping *E. coli* isolates

Isolates from farmers and broilers from the same farm with the same antibiotics resistance pattern were used for genotype matching test by Enterobacterial Repetitive Intergenic Concensus (ERIC) Polymerase Chain Reaction (PCR) described by Meacham et al. (2003). This method does not require the large quantities of DNA, is not time consuming and the equipment is not expensive. *E. coli* DNA was extracted from overnight growth of a pure culture. The cells were lysed by boiling for 15 min. The lysate was then centrifuged at 15,000*g* for 1 min. PCR amplifications were performed in 25 μl volumes containing 5 mM $MgCl_2$, 2 U of Platinum *Taq* polymerase, 0.4 mM (each) deoxynucleoside triphosphates, 10 ng of crude template DNA and 25 pmol of the ERIC2 primer (5′-AAGTAAGTGACTGGGGTGA GCG-3′). The PCR amplification started with hot start at 94°C for 2 min, denature at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 4.5 min after 35 cycles a final extension for 1 min at 72°C. The amplification products were resolved by gel electrophoresis in 2% agarose gels stained with 10 mg/ml ethidium bromide and normalized using a 100 base pair ladder (Invitrogen). Gels were run at 80 V for 1.5 h. Strips of ERIC-PCR DNA patterns of *E. coli* from the same farms were read under direct inspection and of the bands belonging to the broilers and the farmers were compared.

Data management and statistical analysis

After resistance testing was complete, the data were entered into a computer using Epidata version 2.1 (The Epidata Association, Odense, Denmark) using a double-entry validation process followed by data cleaning. The data set was subsequently analysed using Stata Version 7.0 (Stata Corporation, College Station, Tx, USA) and R software version 2.4.1 (R development Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Tables were created to display the prevalence of individual resistance, frequency of various multiple resistance patterns and the numbers of concordant resistance and DNA fingerprints.

To test whether concordance of resistance pattern took place beyond chance alone, a "Monte Carlo" simulation approach was used. Patterns of resistance of individual *E. coli* isolate were sorted by species of hosts and farm. Within the same farm, on same species of host, any pattern duplicating with an existing one was removed to obtain unique records by pattern, host species and farm. The total number of concordant patterns was calculated and taken as the "observed pairs of concordance". In the simulation process, records of patterns from the same host species were reordered at random and consecutively allocated into the farm. The total number of concordant patterns was calculated to obtain "the pairs of concordance from simulation". The sampling process was repeated 10,000 times giving 10,000 pairs of concordance farm simulations. A *P*-value or the probability of getting an extreme concordant pair as much as or beyond the observed value was calculated. We would reject the null hypothesis that the observed concordance would have occurred purely by chance, if the *P*-value (2 sided) is less than 0.05. Finally, contingency tables between concordance of resistance pattern and of DNA fingerprint were created. The amount of agreement was determined using an odds ratio and 95% CI.

Results

Number of *E. coli* isolates

Of 101 pairs of specimens sampled from the farm, 95 provided *E. coli* isolates from both the farmers (457 isolates) and the broilers (460 isolates)

Prevalence of antibiotic resistant and multiple drug resistant *E. coli*

From Table 1, human *E. coli* isolates were most often resistant to doxycycline (35.5%). In contrast nalidixic acid resistance was most frequently observed among the isolates from broilers (87.8%). The prevalence of resistance for amoxicillin/clavulanic acid, ceftriaxone and cefuroxime was low in both groups. Isolates from broilers were generally more resistant than those from the farmers. Table 2 shows that half of *E. coli* isolates from farmers, in contrast to only 6% of those from broilers, were sensitive to all agents tested. The highest level of multi-drug resistant of six combination agents was found in *E. coli* from broilers.

Table 1 Prevalence of antibiotic resistance *E. coli* isolated from farmers and broilers

Agents	Farmer (457 isolates)		Broiler (460 isolates)	
	Resistance	%	Resistance	%
Doxycycline (do)	162	35.5	239	52.0
Nalidixic acid (na)	108	23.6	404	87.8
Ciprofloxacin (cip)	60	13.1	258	56.1
Norfloxacin (nor)	42	9.2	191	41.5
Cephalothin (kf)	25	5.5	55	12.0
Cefuroxime (cxm)	3	0.7	18	3.9
Amoxicillin/clavulanic (amc)	3	0.7	5	1.1
Ceftriaxone (cro)	0	0	3	0.7

Pattern of antibiotics resistance from *E. coli*

From Table 3, there were 35 patterns of resistance. Eighteen patterns were found in isolates from the farmers and 31 patterns in those from the broilers. The first five most common combinations comprised 51.8% of the resistance patterns of all isolates.

The concordance of antibiotics resistance

Of 457 and 460 isolates of *E. coli* from the farmers and broilers, there were 200 unique records of pattern by farm

Table 2 Prevalence of antibiotic multiresistant from *E. coli*

Number of resistant antibiotics	Farmer (n = 457)		Broiler (n = 460)	
	Colonies	%	Colonies	%
0	230	50.3	28	6.1
1	128	28.0	100	21.8
2	53	11.6	92	20.0
3	23	5.0	113	24.6
4	15	3.3	91	19.8
5	8	1.8	31	6.8
6	0	0	5	1.1

Table 3 *E. coli* resistance to antibiotics drug patterns

Drug combination pattern*	Farmer		Broiler		Total	
	No. of colonies	%	No. of colonies	%	No. of colonies	%
do	106	23.2	21	4.6	127	13.9
do-na-nor-cip	15	3.3	82	17.8	97	10.6
Na	12	2.6	77	16.7	89	9.7
na-nor-cip	18	3.9	66	14.4	84	9.2
do-na	29	6.4	49	10.7	78	8.5
na-cip	15	3.3	36	7.8	51	5.6
do-na-cip	3	0.7	32	7.0	35	3.8
do-cro-kf-na-nor-cip	5	1.1	28	6.1	33	3.6
do-kf-na	1	0.2	9	2.0	10	1.1
Others 26 patterns	23	5.0	32	7.0	55	6.0
Sensitive	230	50.3	28	6.1	258	28.1
Total	457	100	460	100	917	100

* Abbreviation for antibiotic agents (see Table 1)

among the farmers *E. coli* and 310 among those from the broilers. Thirty-three farms had at least one concordance pair. The observed number of pairs of concordance was 44.

Results of simulation

The 10,000 simulated samples gave a mean and standard deviation of pairs of concordance of 34.6 and 4.4, respectively, with a distribution resembling a normal distribution (Fig. 1). For a one-sided analysis, 134 simulation records gave 44 pairs or higher giving a *P*-value of 0.016. On the low end, the number of records within the concordance of 25 pairs (equidistant from the mean as those as the high extreme) was 178. Combining these and the aforementioned high end gave a two-sided *P*-value of 0.032, thus rejecting the hypothesis that the observed concordance occurred by chance alone.

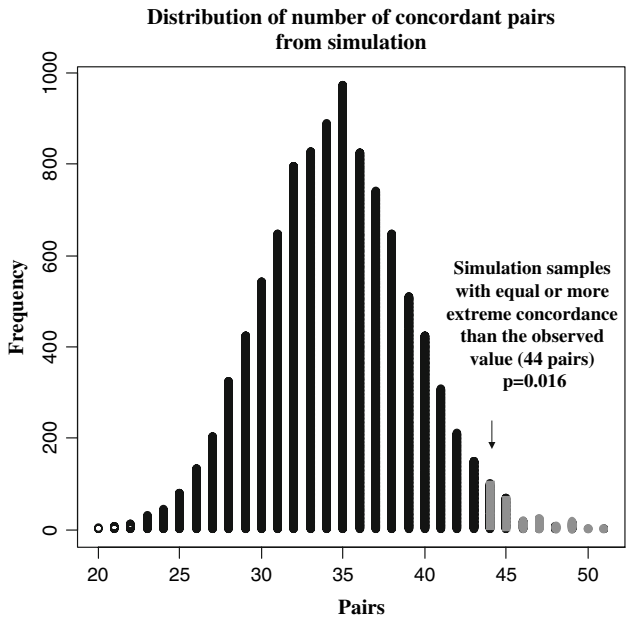

Fig. 1 Distribution of number of concordant pairs from simulation

The concordance of DNA fingerprint in *E. coli*

Nine of the 44 observed concordant pairs were actually fully sensitive to all antibiotics tested. Of the remaining 35 concordant pairs with at least one antibiotic resistance, only four had a concordant DNA fingerprint. Two of these four were mono resistant to doxycycline. The other two were resistant to the combination of doxycycline and nalidixic acid.

On the other hand, there were 20 farms with concordance DNA fingerprint but different resistance pattern with same fingerprint matched set. Table 4 summarizes the relationship between concordance of DNA fingerprint and that of resistance pattern. There was no evidence of association between the two concordance assessment techniques.

Table 4 Concordance of antibiotic resistance and fingerprint pattern of *E. coli*

		Concordance by DNA fingerprint		
		Yes	No	Total
Concordance by antibiotic resistance pattern	Yes	4	23	27
	No	20	48	70
	Total	24	71	95

OR = 0.42; 95% CI = 0.09–1.46

Chi-squared (1df) = 2.18; *P*-value = 0.14

Discussion

Antibiotic resistance is common among study *E. coli* from both species of hosts. The prevalence and the level of multiple drug resistance are more serious among the isolates from the broilers than those from the farmers. With eight common antibiotics tested, identical antibiotic resistance pattern was found in a quarter of isolates from the 44 matched sets. Observed concordance was significantly higher than by chance alone. Sharing both phenotype and genotype pattern was rare (4.2%). Concordance of pattern by both methods has no association with each other.

E. coli isolates from broilers in our study clearly exhibited a higher resistance rate than those from farmer. While antibiotic use was heavy among broilers, only farmer who had not taken antibiotic within the previous 2 months were selected for our study. The difference in prevalence of resistance might be explained by this difference in selection criteria of antibiotic exposure. Lower levels of exposure to antibiotics in the farmers increase the likelihood that the transmission of an antibiotic resistance organism would be in a downward direction, i.e. from broiler to farmer rather than in the opposite direction.

Resistance to antibiotic agents classified as critically important for human medicine (WHO 2005), such as ceftriaxone and amoxicillin/clavulanic, were rare in our study sample. However, the high prevalence of resistance to quinolones and doxycycline, which are important agents for public health control of infectious diseases such as cholera, ricketsia infection and leptospirosis, are worrisome.

Multi-drug resistance was found in *E. coli* from farmers and broiler, but was more frequent in broiler isolates. The evidence of *E. coli* resistance to multiple drugs in this study is not different from those results from other countries (Al-Ghamdi et al. 1999; Miles et al. 2006; Saenz et al. 2001).

The rate of the identical resistance pattern of *E. coli* isolates from humans and animals in the same farm was more common in our study (26.3%) than previously reported (4%) by Nijsten et al. (1996). Most previous studies failed to consider the explanation of concordance by chance. Such probability would be high if the number of antibiotics tested is small or the prevalence of full sensitivity is high. With eight agents of antibiotic tested and the prevalence of full sensitivity being 50.3 and 6.1% in *E. coli* from farmers and broilers, our simulation shows that there was an average of 34.6 concordant pairs out of 95 farms being tested. Thus concordance by chance should never be ignored. However, in our study population, the concordance of the resistance pattern was not caused by chance alone.

Our identical fingerprint pattern across *E. coli* from the two types of hosts of 25.3% were more common than that in previous studies (Stobberingh et al. 1999; van den Bogaard et al. 2001, 2002), with all had small sample sizes. With internal inconsistency between results from our two methods, clonal spread, although was possible in four incidence, was not the main mechanism for multiple-drug resistance transmission in our study sample.

While we could reject the hypothesis of concordance by chance and could prove that clonal spread plays very little role, if any, yet the mechanisms of plasmid conjugation and transposon transfer need to be further examined for these isolates. Additionally, concordance in the same farm may be the result of co-evolution, i.e. exposure to the same antibiotics in the same farm environment. This would need further investigation.

Ethical consideration

This study was approved by the Ethics committee of the Faculty of Medicine, Prince of Songkla University.

Acknowledgments This study was a part of the thesis of the first author to fulfill the requirement of the Ph.D. degree in Epidemiology Unit, Prince of Songkla University, Thailand. Funding for this work was supported by the Royal Golden Jubilee Ph.D. Program of the Thailand Research Fund. We would like to give our thanks to all broiler farmers and the veterinarian from Songkhla Provincial Livestock Office, Songkhla, Thailand.

References

Al-Ghamdi MS, El-Morsy F, Al-Mustafa ZH et al (1999) Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. Trop Med Int Health 4:278–283

Department of Livestock Development, Ministry of Agriculture and Cooperative (2006a) Statistics of livestock in Thailand (1996–2005). http://www.dld.go.th/ict/yearly/yearly48/book/stat/annex01.xls. Cited 18 May 2006

Department of Livestock Development, Ministry of Agriculture and Cooperatives (2006b) Number of animal in Thailand 2005. http://www.dld.go.th/ict/yearly/yearly48/book/stock/report07.xls. Cited 5 May 2006

Department of Livestock Development, Ministry of Agriculture and Cooperatives (2006c) Number of poultry in Thailand 2005. http://www.dld.go.th/ict/yearly/yearly48/book/stock/report08.xls. Cited 5 May 2006

Meacham KJ, Zhang L, Foxman B et al (2003) Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J Clin Microbiol 41:5224–5226

Miles TD, McLaughlin W, Brown PD (2006) Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. BMC Vet Res (in press). DOI 10.1186/1746-6 148-2-7

Na Lampang K, Chongsuvivatwong V, Kitikoon V (2007) Pattern and determinant of antibiotics used on broiler farms in Songkhla province, sounthern Thailand. Trop Anim Health Prod 39:355–361

Nijsten R, London N, van den Bogaard A et al (1996) Antibiotic resistance among Escherichia coli isolated from faecal samples of pig farmers and pigs. J Antimicrob Chemother 37:1131–1140

Ojeniyi AA (1989) Direct transmission of *Escherichia coli* from poultry to humans. Epidemiol Infect 103:513–522

Oppegaard H, Steinum TM, Wasteson Y (2001) Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. Appl Environ Microbiol 67:3732–3734

Quinn PJ, Carter ME, Markey B et al (1994) Enterobacteriaceae. In: Clinical veterinary microbiology. Wolfe Publishing, London

Saenz Y, Zarazaga M, Brinas L et al (2001) Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. Int J Antimicrob Agents 18:353–358

Saida K, Ike Y, Mitsuhashi S (1981) Drug resistance and R plasmids of *Escherichia coli* strains isolated from pigs, slaughterers, and breeders of pigs in Japan. Antimicrob Agents Chemother 19: 1032–1036

Stobberingh E, van den Bogaard A, London N et al (1999) Enterococci with glycopeptide resistance in turkeys, turkey farmers, turkey slaughterers, and (sub)urban residents in the south of The Netherlands: evidence for transmission of vancomycin resistance from animals to humans? Antimicrob Agents Chemother 43:2215–2221

Thai Drug Control Division (2003) The value of antibiotics in human and animal. http://www.app1.fda.moph.go.th/drug/zone_search/files/sea001_c10.asp. Cited 7 Nov 2006

van den Bogaard AE, London N, Driessen C et al (2001) Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 47:763–771

van den Bogaard AE, Willems R, London N et al (2002) Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 49:497–505

World Health Organization (1997) The medical impact of antimicrobial use in food animals. Report of WHO Meeting. Berlin, Germany, 13–17 October 1997. http://whqlibdoc.who.int/hq/1997/WHO_EMC_ZOO_97.4.pdf. Cited 11 Nov 2006

World Health Organization. (2005) Critically important antibacterial agents for human medicine for risk management strategies of non-human medicine. http://www.who.int/foodborne_disease/resistance/FBD_CanberraAntibacterial_FEB2005.pdf. Cited 27 Nov 2006