aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 3 (6): 409-416, 2008
ISSN 1683-9919

Antimicrobial Susceptibilities of *Escherichia coli* Isolates from Food Animals and Wildlife Animals in Sarawak, East Malaysia

[1]K. Apun, [1]Y.L. Chong, [2]M.T. Abdullah and [1]V. Micky
[1]Department of Molecular Biology,
[2]Department of Zoology, Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

Abstract: A total of 133 *E. coli* strains isolated from different food animals and wildlife sources in Sarawak, Malaysia were screened to determine their antibiotic-resistance pattern using the disk diffusion methods. The animal sources were broiler and village chickens, cattle, bats and rodents. All *E. coli* isolates were tested for their resistance patterns towards 12 commonly used antibiotics: ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, nalidixic acid, neomycin, nitrofurantoin, ofloxacin, streptomycin, snlfamethoxazole-trimethoprim and tetracycline. In general, the most frequently encountered form of resistance in all samples was resistance to tetracycline (41.35%) and snlphamethoxazole-trimethoprim (19.55%). Low levels of resistance were for gentamicin, nitrofurantoin and ofloxacin, which demonstrated less than 7% resistance of the total samples being assessed. The Multiple Antibiotic Resistance (MAR) indices were highest for broiler chicken isolates (0.479) and low for bat isolates (0.013). All isolates from both broiler chicken samples were multidrug-resistant *E. coli*. A high percentage of the isolates from bat (84.62%) and rodent (68.57%) samples were not resistant (totally susceptible) to all the antibiotics tested. The resnlts in this study thus suggest that wildlife do not present a high risk of spreading antibiotic-resistant *E. coli* to the environment. The higher value of MAR indices as well as prevalence of multiple-resistance patterns of *E. coli* isolates from food animals demonstrated that indiscriminate use of antibiotics should be discouraged in food animals to overcome future resistance problem.

Key words: Antimicrobial agents, MAR indices, *Escherichia coli*, wildlife, Sarawak

INTRODUCTION

Normal intestinal microflora of hnmans and animals comprise enormous reservoir of resistance genes for potentially pathogenic bacteria. Thus, these bacteria may serve as major indicators of selection pressure exerted by antibiotics use in veterinary and human medicines (Okoli *et al*., 2005). Indiscriminate usage of antibiotic is probably the most important factor that leads to the emergence selection and dissemination of antibiotics resistant microorganisms especially *E. coli* strains to antimicrobial agents used in animal husbandry and for human prescription (Witte, 1998; Van de Bogaard and Stobberingh, 2000). The problem of such resistant organisms evolving and being transmitted, like zoonotic bacteria, through the food chain from food-producing animals to man is becoming increasingly important throughout the world.

The fate and safety of food-animal products in Malaysia, especially those supplied by backyard industries and when antimicrobial agents are indiscriminately used are of serious concern due to high multidrugs resistant strains being reported by Son *et al*. (1999). Resistance to antimicrobial drug can

Corresponding Author: K. Apun, Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

arise either from new mutations in the bacterial genome or through the acquisition of genes coding for resistance via mobile elements, such as plasmids, transposons, bacteriophages, integrons and transformation (Levy, 2002). Once acquired, resistance genes can be transferred between bacteria (Singer *et al*., 2003), which consequently can give rise to emergence of a new bacterium with resistance factor. Thus there is a need to monitor how widespread is the occurrence of antibiotics resistance among different type of animals especially among food-producing animals.

Antimicrobial susceptibility profiling and Multiple-Antibiotic-Resistance (MAR) test are low-cost screening tools to detect the ecology of antimicrobial resistance of bacteria in selected populations and animal hosts (Guan *et al*., 2002). MAR indexing can be used to complement microdilution broth test and molecular techniques with additional information about the origin of contamination and as a useful tool for better risk assessment by identifying contamination from high-risk envirouments (Guan *et al*., 2002). Therefore, this study was designed with two objectives: to isolate *E. coli* strains in food and wild animals and to compare their antimicrobial susceptibility and multiple drug resistance patterns to commouly used antimicrobial agents using the disk diffusion method. Bats and rodents were used to represent wildlife that has no direct contact with human. This might help to better understand the distribution of multiple antibiotic-resistant *E. coli* strains in the enviroument in relation to their origins.

MATERIALS AND METHODS

Bacterial Strains

A total of 133 *E. coli* isolates recovered from food animals (n = 85), bats (n = 13) and rodents (n = 35) were used in this study. Sampling was done between April 2004 and September 2005, which covered the Northern and Southern regions of Sarawak, Malaysia. Samples from broiler and village chickens were obtained from five different farms at Kota Samarahan and Kuching, by collecting the cloacal swabs. Anal swabs and faeces from bats and rodents samples were obtained from three different forests, namely Niah National Park, Miri; Bako National Park, Kuching and a secondary forest near the Universiti Malaysia Sarawak campus in Kota Samarahan District. The samples were cultured on EMB (Eosin Methylene Blue) agar (Oxoid, UK) as well as MacConkey agar, non-selective medium with respect to members of the enteric bacteria (Gordan and FitzGibbon, 1999), to isolate presumptive *E. coli*. Presumptive *E. coli* were tested to confirm that they matched the biochemical characteristics of *E. coli* based on the standard methods of Methyl red positive, Voges-Proskauer negative, H_2S negative, indole positive and citrate negative (Krieg and Holt, 1984). Approximately 20% of the isolates used in this study were further tested by using a commercial identification kit, API 20E system (BioMerieux, France) to further confirm the *E. coli* isolates.

Antimicrobial Susceptibility Test

Disk diffusion method was carried out to determine the antimicrobial agent sensitivity profiles of the *E. coli* isolates towards 12 selected antimicrobial agents as listed: ampicillin (AMP), 10 μg U^{-1}; carbenicillin (Car), 100 μg U^{-1}; cephalothin (Cep), 30 μg U^{-1}; chloramphenicol (Chl), 10 μg U^{-1}; gentamicin (Gen), 10 μg U^{-1}; nalidixic acid (Nal), 30 μg U^{-1}; neomycin (Neo), 30 μg U^{-1}; nitrofurantoin (Nit), 300 μg U^{-1}; ofloxacin (Ofx), 5 μg U^{-1}; streptomycin (Str), 10 μg U^{-1}; sulfamethoxazole-trimethoprim (Stx), 25 μg U^{-1} and tetracycline (Tet), 30 μg U^{-1}. This method is in accordance with National Committee for Clinical Laboratory Standards (NCCLS) as previously reported by Saenz *et al*. (2001) and Sayah *et al*. (2005). The agents were chosen on the basis of their importance in treating human or animal *E. coli* infections, their use as feed additives to promote growth in animals and on the basis of their ability to provide diversity for representation of different antimicrobial agent classes. The breakpoints used to categorize isolates as resistant or not resistant to

each antimicrobial agent were those recommended by the NCCLS System. To verify that susceptibility test results are accurate, reference strain *E. coli* ATCC 25922 was included periodically as an internal control in this study.

Data Analysis

Differences in the mean disk diffusion zones diameter between species groups were assessed by using univariate analysis and the significant differences between species groups as well as between sample sources were generated using two-way ANOVA (SPSS 13.0; Norusis and SPSS Inc., 2005). The MAR (multiple antibiotic resistance) index was a measure of the extent of antimicrobial agent resistance for isolates in the group studied. It was calculated as described by Krumperman (1983) and Guan *et al*. (2002). It is defined as a/b, where a represents the number of antibiotics to which the isolate were resistant and b represents the total number of antibiotics to which the isolate was exposed. According to Krumperman (1983), MAR index values of more than 0.2 indicate that the isolates recovered from samples originating from high-risk sources.

RESULTS

Antimicrobial Agent Resistance in *E. coli* Isolates from Different Animal Sources

The most frequently encountered form of resistance in all samples was resistance to tetracycline (41.35%) and sulphamethoxazole-trimethoprim (19.55%). Low level of resistance was to gentamicin, nitrofurantoin and ofloxacin, which demonstrated less than 7% resistance of the total samples being assessed (Table 1). *E. coli* isolates from food animal samples exhibited higher percentage of resistance towards all antimicrobial agents tested as compared to wildlife (bats and rodents), with the exception of cephalothin. None of the isolates from wildlife were resistant to chloramphenicol and gentamicin.

The patterns of antimicrobial agent resistance for different type of food animal (broiler chickens, village chickens and cattle) isolates were also examined to determine whether there were any common patterns of resistance between the different animals (Table 1). *E. coli* isolates from broiler chicken samples showed highest levels of resistance towards all antimicrobial agents tested. Surprisingly, resistance to chloramphenicol, gentamicin, nalidixic acid and ofloxacin were only shown by isolates from broiler chicken samples. *Escherichia coli* isolates from broiler chicken further showed exceptional

Table 1: *E. coli* isolates exhibiting resistance to antimicrobial agent and their sources

	Percentage of isolate resistance (%)							
	Food animals				Wildlife			
Antimicrobial agents	Broiler chicken (n = 28)	Village chicken (n = 45)	Cattle (n = 12)	All food animals (n = 85)	Bat (n = 13)	Rodent (n = 35)	All wildlife (n = 48)	Overall (n = 133)
Amp	50.00	8.89	8.33	22.35	0.00	8.57	6.25	16.54
Chl	46.43	0.00	0.00	15.29	0.00	0.00	0.00	9.77
Car	50.00	8.89	16.67	23.53	7.69	0.00	2.08	15.79
Gen	10.71	0.00	0.00	3.53	0.00	0.00	0.00	2.26
Nal	64.29	0.00	0.00	21.18	0.00	2.86	2.08	14.29
Str	71.43	15.56	0.00	31.76	7.69	2.86	4.16	21.80
Tet	95.86	55.56	25.00	63.53	0.00	2.86	2.08	41.35
Nit	17.86	0.00	0.00	7.06	0.00	5.71	4.16	6.02
Cep	14.29	8.89	8.33	10.59	0.00	20.00	14.58	12.03
Neo	50.00	2.22	25.00	21.18	0.00	5.71	4.16	15.04
Ofx	25.00	0.00	0.00	8.24	0.00	5.71	4.16	6.77
Sxt	82.14	4.44	0.00	29.41	0.00	2.86	2.08	19.55

Amp: Ampicillin; Chl: Choramphenicol; Car: Carbenicillin; Gen: Gentamicin; Nal: Nalidixic acid; Str: Streptomycin; Tet: Tetracycline; Nit: Nitrofurantoin; Cep: Cephalothin; Neo: Neomycin; Ofx: Ofloxacin and Sxt: Sulfamethoxazole-trimethoprim

high resistance towards tetracycline (95.86%) and sulphamethoxazole-trimethoprim (82.14%). All isolates from village chicken and cattle samples demonstrated less than 50% resistance towards all the antibiotics tested except for tetracycline (55.56%) resistance in village chicken isolates.

Antibiotic Resistance Patterns and Multiple Antibiotic Resistance (MAR) Profiles for *E. coli* Isolates from Environmental Sample Sources

The MAR indices were compared between sample sources (Table 2). The MAR indices were highest for broiler chicken isolates (0.479) and lowest for bat isolates (0.013). Multidrug resistance was also evaluated with *E. coli* isolates from different sample sources (Table 2). It was observed that all the isolates from broiler chicken samples were multidrug-resistant *E. coli*. High percentage of the isolates from bat (84.62%) and rodent (68.57%) samples showed no resistance (totally susceptible) towards all the antibiotics tested. Among all the samples tested, only isolates from bat samples do not demonstrate multidrug resistance patterns.

Differences in the Disk Diffusion Zone for *E. coli* Isolates from Domestic and Wildlife Animals

Mean disk diffusion zone diameters were examined for differences between types of samples collected (Table 3). Overall, the smallest diffusion zones, indicating greatest resistance, were found with isolates from broiler chicken samples. Isolates from bats however exhibited the largest diffusion zones, indicating greater susceptibility, for almost all agents tested, followed by isolates from rodents.

Table 2: Multidrug-resistant *E. coli* isolates according to type of sample sources

Environmental sample source (n = 133)	MAR index	Percentage of isolates resistant to				
		0 agent	1 agent	2 agents	3 agents	>3 agents
Broiler chicken (n =28)	0.479	0.00	0.00	10.71	3.57	85.72
Village chicken (n = 45)	0.087	37.78	33.33	17.78	8.89	2.22
Cattle (n =12)	0.069	41.67	33.33	25.00	0.00	0.00
Rodent (n =35)	0.040	68.57	22.85	2.86	2.86	2.86
Bat (n = 13)	0.013	84.62	15.38	0.00	0.00	0.00

Table 3: Mean disk diffusion zone diameters, p-value and resistance breakpoints for *E. coli* isolates recovered from different sources

Antimicrobial agents	Breakpoints (mm)	Mean disk diffusion zone diam (mm)						
		Food animals				Wildlife		
		Broiler chicken (n = 28)	Village chicken (n = 45)	Cattle (n = 12)	All food animals (n = 85) (p-value[a])	Bat (n = 13)	Rodent (n = 35)	All wildlife (n = 48) (p-value[a])
Amp	≤13	12.1	17.1	15.4	15.2(0.000)	17.8	17.1	17.3(0.246)
Chl	≤12	13.2	21.0	22.2	18.6(0.000)	22.6	21.2	21.6(0.001)
Car	≤19	14.4	20.8	20.9	18.7(0.000)	22.8	22.4	22.5(0.457)
Gen	≤12	18.9	20.5	20.8	20.0(0.023)	20.8	20.1	20.3(0.212)
Nal	≤13	12.4	20.3	20.6	17.7(0.000)	21.0	20.7	20.8(0.555)
Str	≤11	8.7	14.8	15.7	12.9(0.000)	14.6	14.2	14.3(0.608)
Tet	≤14	7.3	12.1	15.1	11.0(0.000)	19.0	19.1	19.1(0.909)
Nit	≤14	15.9	18.3	18.4	17.5(0.000)	19.2	18.6	18.7(0.397)
Cep	≤14	17.1	18.2	16.1	17.6(0.042)	17.8	16.2	16.7(0.044)
Neo	≤12	13.5	17.4	17.0	16.1(0.000)	17.9	16.3	16.8(0.048)
Ofx	≤12	19.2	26.9	25.9	24.2(0.000)	28.8	27.9	28.1(0.510)
Sxt	≤10	8.3	24.4	24.8	19.1(0.000)	27.5	26.7	26.9(0.322)

[a]Test for significant differences in disk diffusion zones. The mean difference is significant at the 0.05 level; Amp: Ampicillin; Chl: Choramphenicol; Car: Carbenicillin; Gen: Gentamicin; Nal: Nalidixic acid; Str: Streptomycin; Tet: Tetracycline; Nit: Nitrofurantoin; Cep: Cephalothin; Neo: Neomycin; Ofx: Ofloxacin and Sxt: Sulfamethoxazole-trimethoprim

Among *E. coli* isolates from cattle, broiler and village chicken samples, it was observed that their mean disk diffusion zone diameters from the entire antibiotic assayed showed significantly differences (p-value<0.05) between these food animals. When mean disk diffusion zone diameters were compared between wildlife isolates, no significant difference (p-value>0.05) was observed for almost all of the antimicrobial agents tested.

DISCUSSION

Results in this study revealed that among the 12 antibiotics tested, tetracycline is the most common antimicrobial agents that *E. coli* isolates were resistant to, regardless of different food animal sample sources (Table 1). It is also worth mentioning that *E. coli* isolates particularly from broiler chickens showed more than 90% resistant to tetracycline, as compared to other sample sources. These data are in accordance with previous study by Kang *et al*. (2005) suggesting that resistance to tetracycline among commensal *E. coli* isolates was the most common in poultry samples. In the study by Schroeder *et al*. (2002), more than 50% of 534 *E. coli* isolates from food animals (Turkey, cattle, chicken and swine) were resistant to tetracycline. The high prevalence of tetracycline resistance is not surprising as this antibiotic is under the lists of FDA approved animal drug (US Food and Drug Administration, Center for Veterinary Medicine, Ouline Green Book, 2006). Tetracycline are commonly used to increase weight gain, improve feed efficiency and also for control of bacterial enteritis caused by *E. coli* (Mathew *et al*., 1998). In the USA, tetracyclines are frequently used as growth promoters for food animals and as pesticides in agriculture (Levy, 2002). The high occurrence of tetracycline resistance in this study probably reflects tetracycline's long history of use in the animal farm industry. The high level of resistance among *E. coli* or other enteric bacteria is of concern due to potential of cross-resistance with antibiotic used in human medicine. This is because tetracyclines are commouly used as the first-line defense to certain human infections. The important medical uses of tetracylines could be affected if the human pathogens concerned acquired *tet* genes from the animal envirourment. Therefore the use of this antibiotic must be carefully monitored as Rahman *et al*. (2008) cautioned that if the current practice of the use of this antibiotic is sustained, emergence of resistant *E. coli* will follow and soon a grave situation will arise when this drug and others will no longer be available to treat life-threatening infections caused by resistant *E. coli* strains

Overall, *E. coli* isolates obtained from food animals (village chickens, broiler chickens and cattle) demonstrated higher levels of resistance to all antibiotics tested as compared to wildlife (bats and rodents) isolates except for cephalothin (Table 1). Present results are in agreements with Sayah *et al*. (2005) whereby highest levels of resistant *E. coli* isolates were observed in food animals (such as dairy cattle, beef cattle, swine, sheep, goats and horses), followed by companion animals (such as dogs and cats) and wildlife. This pattern of resistance was further supported by MAR index and mean diffusion zone diameter. Food animal samples' isolates displayed higher average MAR indices than did wildlife isolates (Table 2). It was demonstrated that *E. coli* isolates from broiler chicken samples possess highest levels of multidrug resistance patterns with MAR indices greater than 0.4 and were among all the isolates that demonstrated greatest resistance (smaller diffusion zones) towards all the antibiotics tested (Table 3). The MAR indices in the present study were relatively higher than those reported by Guan *et al*. (2002) for chicken isolates (0.1286). These results are consistent with other reports that the MAR indices of fecal *E. coli* from wild animals were generally low, while human and livestock isolates had higher indices (Guan *et al*., 2002). Earlier studies by Dombek *et al*. (2000) and Krumperman (1983) also reported that *E. coli* isolates from humans, chickens and dairy cows have higher resistance indices than strains obtained from wild animals.

Results from the present study on the differences among the resistance patterns of *E. coli* isolates between food animal and wildlife samples can be attributed to the different level of exposure towards

antibiotics. The diets of the animal hosts might be the key factor contributing to this phenomenon. As broiler chickens are reared commercially, the feeds are usually supplemented with antibiotics for the purpose of growth and feed efficiency improvements. Apart from that, antibiotics are sometimes used therapeutically to treat infections. According to Glisson *et al.* (2004), antibiotics, such as enrofloxacin, tetracycline and sulfadimethoxine, are usually used to reduce morbidity and mortality caused by colibacillosis in broiler chickens. In typical livestock farming, the long-term administration of antimicrobial agents for improving growth might result in subsequent selection and acquisition of these drugs in the microflora community of the gastrointestinal tracts of these animals, which include the most prevalent enteric bacteria, *E. coli*. It was observed that the percentage of resistance and the MAR index for *E. coli* isolates from village chicken samples were lower as compared to broiler chicken samples. Village chickens are normally reared for self-consumption and not kept in a proper coop. The food sources of these animals are basically either from the food scraps from household, worms, dried-corns or from the vicinity. Antibiotics are not added in the diets of this animal. The results of this study thus confirm that prior exposure to antibiotic contributes to the resistance of the bacteria (Witte, 1998). Therefore from a public health viewpoint, the indiscriminate use of antibiotics in food animals should be discouraged to overcome future resistance patterns.

Escherichia coli isolates from wildlife exhibited a significantly lower prevalence of antimicrobial agent resistance compared to food animals. In the present study, *E. coli* isolates recovered from bat and rodent samples show no resistance to chloramphenicol and gentamicin and only 6.25 and 2.08% resistance towards ampicillin and tetracycline, respectively (Table 1). The *E. coli* isolates from wildlife had an average MAR index of only 0.172 (Table 2). The low resistance frequency in *E. coli* isolates from wildlife sources could be attributed to several factors, such as the diet and the ecological niches of the animal hosts. In this study, bats and rodents were sampled from primary forests as well as from secondary forests. The food sources available for these animals are almost completely and naturally found in the forests and their vicinity. There is also less contact of these animal hosts with human as the hosts are living away from human habitation. Consequently, the likelihoods that these animals being exposed to antibiotics are minimal thus rendering the majority of the *E. coli* isolates being recovered from them susceptible to most of the antibiotics tested. The results in this study thus suggest that wildlife do not present a high risk of spreading antibiotic-resistant *E. coli* to the enviroument.

When comparison was made between different types of food animal sources, it was found that regardless of antibiotics used, significant differences were observed ($p<0.05$) for their mean disk diffusion zone diameter (Table 3). Thus, it is agreed that for food animal samples, different sources of animal hosts resulted in different patterns of resistant-bacteria profiles. On the contrary, apart from chloramphenicol, cephalothin and nitrofurantoin, all other antimicrobial agents generated no significant differences ($p>0.05$) in their mean disk diffusion zone diameter for wildlife *E. coli* isolates. Based on the resistance pattern of bats and rodents, it can be concluded that wild animals may show the same resistance profiles towards many antibiotics. The significant difference among food animals indicates that differences in the mean disk diffusion zone diameters of the *E. coli* isolates are able to discriminate among food animals and between wildlife from enviroumental sources. This finding suggests that mean disk diffusion zone diameters of the bacteria can be used as a possible alternative method in source-tracking contamination studies from enviroumental sources.

CONCLUSION

This study provides an overview on the distribution and prevalence of antimicrobial agent resistance in *E. coli* isolates from a variety of animal sources in Sarawak, Malaysia. High MAR indices as well as high prevalence of multiple-resistance patterns of isolates were observed in broiler chicken

samples. Wildlife, however, does not present a high risk of spreading antibiotic-resistant *E. coli* to the envirounent. As concerns about resistant organisms being transported through the food chain continue to increase, it is important to educate the public and farmers on the risks and consequences associated with indiscriminate use of antibiotics in animal for food sources such as in broiler chicken. To be effective, strict policy and legislation is thus required to deter offenders from using these drugs in animal of food sources.

ACKNOWLEDGMENTS

This research was supported by a fundamental grant from Universiti Malaysia Sarawak [Vot No. 01(111)/485/2004(222)].

REFERENCES

Dombek, P.E., L.K. Johnson, S.T. Zimmerley and M.J. Sadowsky, 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Applied Environ. Microbiol., 66: 2572-2577.

Glisson, J.R., C.L. Hofacre and G.F. Mathis, 2004. Comparative efficacy of enrofloxacin, oxytetracylcine and sulfadimethoxine for the control of morbidity and mortality caused by *Escherichia coli* in broiler chickens. Avian Dis., 48: 658-662.

Gordan, D. M. and F. FitzGibbon, 1999. The distribution of enteric bacteria from Australian mammals: Host and geographical effects. Microbiology, 145: 2663-2671.

Guan, S., R. Xu, S. Chen, J. Odumeru and C. Gyles, 2002. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. Applied Environ. Microbial., 68: 2690-2698.

Kang, H.Y., Y.S. Jeong, J.Y. Oh, S.H. Tae, C.H. Choi, D.C. Moon, W.K. Lee, Y.C. Lee, S.Y. Seol, D.T. Cho, and J.C. Lee, 2005. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. J. Antimicrob. Chem., 55: 639-644.

Krieg, N.R. and J.G. Holt, 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1, Williams and Wilkins, Baltimore.

Krumperman, P.H., 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol., 46: 165-170.

Levy, S.B., 2002. Factors impacting on the problem of antibiotic resistance. J. Antimicrob. Chem., 49: 25-30.

Mathew, A.G., W.G. Upchurch and S.E. Chattin, 1998. Incidence of antibiotic resistance in fecal *Escherichia coli* isolated from commercial swine farms. J. Anim. Sci., 76: 429-434.

Norusis, M.J. and SPSS Inc., 2005. SPSS 13.0 Guide to Data Analysis. Upper Saddle River. 1st Edn., Prentice Hall, N.J.

Okoli, C.I., K.F. Chah, P.T.E. Ozoh and A.B.I. Udedibie, 2005. Antimicrobial resistance profile of *Escherichia coli* isolates from tropical free range chickens. Online J. Health Allied Sci., 4: 1-12.

Rahman, M., B.M. Rahman and B. Rahman, 2008. Antibiogram and plasmid profile analysis of isolated *Escherichia coli* from broiler and layer. Res. J. Microbiol., 3: 82-90.

Saenz, Y., M. Zarazaga, L. Brinas, M. Lantero, F. Ruiz-Larrea and C. Torres, 2001. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. Int. J. Antimicrob. Agents., 18: 353-358.

Sayah, R.S., J.B. Kaneene, Y. Johnson and R. Miller, 2005. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. Appl. Environ. Microbiol., 71: 1394-1404.

Schroeder, C.M., J. Meng, S. Zhao, C. DebRoy, J. Torcolini, C. Zhao, P.F. McDermott, D.D. Wagner, R.D. Walker and D.G. White, 2002. Antimicrobial resistance of *Escherichia coli* O26, O103, O111, O128, and O145 from animals and humans. Emerg. Infect. Dis., 8: 1409-1414.

Singer, R.S., R. Finch, H.C. Wegener, R. Bywater, J. Walter and M. Lipsitch, 2003. Antibiotic resistance the interplay between antibiotic use in animals and human beings. Lancet Infect. Dis., 3: 47-51.

Son, R., L. Samuel, G. Rusul and M.I. Zuraini, 1999. RAPD-PCR analysis, antibiotic resistance and plasmid profiles of *Escherichia coli* isolated from village and broiler chicken. Malays. Applied Biol., 28: 51-60.

Van de Bogaard, A.E. and E.E. Stobberingh, 2000. Epidemiology of resistance to antibiotics links between animals and humans. Int. J. Antimicrob. Agents, 14: 327-335.

Witte, W., 1998. Biomedicine: Medical consequences of antibiotic use in agriculture. Science, 279: 996-997.