Polish Journal of Microbiology
2012, Vol. 61, No 4, 323–326

SHORT COMMUNICATION

Identification and Antibacterial Resistance of Bacteria Isolated from Poultry

FADI. E. EL-RAMI[1], FAWWAK. T. SLEIMAN[2] AND ALEXANDER. M. ABDELNOOR[1*]

[1] Department of Microbiology and Immunology, Faculty of Medicine, American University of Beirut
Beirut Lebanon
[2] Department of Animal and Veterinary Sciences, Faculty of Agricultural and Food Sciences,
American University of Beirut, Beirut Lebanon

Received 11 october 2011, revised 5 July 2012, accepted 4 September 2012

Abstract

Food-borne infections are among the prominent health hazards. Antibacterial agents (ABA) are usually administered to poultry in Lebanon as antibiotic growth promoters (AGP), which might lead to the dissemination of resistant bacterial strains. The aims of this study were to isolate potential food borne pathogens from poultry and investigate an association between AGP usage and antibacterial resistance (ABR). Isolates were obtained from the culture of cloacae swabs and identified. *Escherichia coli* was the predominant isolate. There was a significant association between the use of tetracycline and gentamicin as AGP and the number of *E. coli* isolates resistant to these ABA.

Key words: antibacterial agents, antibacterial resistance, association; confidence interval, growth promoting factor

The abuse or misuse of antibacterial agents (ABA) as growth promoters (AGP) for livestock is of major concern in Lebanon and other developing countries. Antibacterial resistance (ABR) of enteric bacteria isolated from poultry attracts attention due to its direct influence on public health, through elevating the morbidity, mortality, and treatment costs of infectious diseases caused by cross-resistance to drugs used in human medicine (Warren *et al.*, 2008). Increased exposure of intestinal bacterial flora to ABA which is administered for disease prophylaxis, treatment, and growth promotion is one of the causes for the emergence of resistant strains (Gyles, 2008).

ABA have been used intensively in poultry farming since 1950's, with blurry figures representing the annual administered amounts for "non-therapeutic use", namely disease prevention and growth promotion, ranging from 27.3 million to 16 million pounds of ABA in USA at 2001, according to the Union of Concerned Scientists and the Institute of Medicine respectively (Shea, 2003).

Taking into consideration the recommendations of the Swann report (Swann Report, 1969) and WHO report (WHO, 1997) to phase out the use of ABA as AGP many European countries cut down the non-therapeutic use of ABA. On January 1, 2006, the European Union (EU) abandoned the use of AGP in feed for livestock (Regulation EC No. 1831/2003). Fruitful results were reflected in decreased ABR in zoonotic bacteria without loss of food productivity (Aarestrup *et al.*, 2001; Phillips *et al.*, 2004).

On the other hand, many countries, including Lebanon, continue to use AGP in food production (Bywater and Casewell, 2000; Phillips *et al.*, 2004). It is noteworthy to mention that recently in the United States, during the 111th Congress, a legislation entitled: the Preservation of Antibiotics for Medical Treatment Act of 2009 (H.R. 1549; S. 619) was introduced to restrict the non-therapeutic use in livestock and poultry of "critical antimicrobial animal drugs" such as penicillin, tetracycline, macrolide, aminoglycosides, and sulfonamides. This controversial legislation was supported by some federal agency officials and was opposed by most U.S. livestock and poultry producers, driven by concerns about animal welfare, food safety, and possible increases in production costs (Johnson, 2010).

The aims of this study were to identify potentially pathogenic Gram-negative isolates obtained from poultry farms in Lebanon, identify ABAs used as AGP by farms and determine their ABR patterns, and relate the

* Corresponding author: A.M. Abdelnoor, Department of Microbiology and Immunology, Faculty of Medicine, American University of Beirut, P.O. Box 11-0236, Riad el-Solh, 11072020, Beirut Lebanon; **e-mail: aanoor@aub.edu.lb**; F. Sleiman, Department of Animal and Veterinary Sciences, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236; **e-mail: sleimanf@aub.edu.lb**

Table I
Antibacterial resistance patterns among *E. coli* isolates in layers' farms

Farm		B	E	C	D	F	A
Location		Terbol	AUB farm	Rayak	Rayak	AUB farm	Zahle
Bird Strain		Hyline W-98	Hi-line	Hyline W-98	Hyline W-98	Hi-line	Hyline W-98
AGP		TE, ERY, STREP	–	TE, ERY, STREP	SXT	–	TE
Age of birds		6 days	2 months	4 months	11 months	18 months	20 months
N		12	9	21	14	9	17
Number of isolates Resistant to	AMC	3	0	0	0	0	0
	CRO	0	0	0	0	0	0
	GN	7	2	13	0	0	0
	TE	12	7	21	3	0	16
	CIP	9	4	18	0	0	1
	SXT	12	2	15	0	0	9

Abbreviations: AGP = Antibiotic Growth Promoter; AMC: amoxicillin/clavulanic acid; GN: gentamicin; TE: tetracycline; CIP: ciprofloxacin; SXT: sulphamethaxazole/trimethoprim; CRO: ceftriaxone; ND: not done; *N*: number of *E. coli* isolates; – : no AMA was administered as AGP. AUB; American University of Beirut.
Total number of layers (Farms A, B, C, D, E and F) = 103,000.
The average age of layers was 9.2 ± 7.7 months.

AGP used to the resistance patterns. The results might alert the public health authorities to take the necessary measures to control the use of AGP in animal feeds.

During a 2-month period, 111 cloacae swabs were collected from six layer farms. Four of six layer farms used AGP (farms A, B, C and D). The average age of layers was 9.2 ± 7.7 months. A questionnaire was used to record name of farm (A to F), location, breed/strain, age of birds and AGP administered (Table I).

Sterile cotton swabs were used to take specimens from live chickens. The cloacae swabs were placed in tryptic soy broth at room temperature. Prior approval by the Animal Care Committee was obtained.

Only isolation of Gram-negative rods, excluding *Salmonella* species, was attempted. All broth cultures were sub-cultured on MacConkey agar plates (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) and the most dominant characteristic colony was selected and sub-cultured on MacConkey agar plates. The final pure culture was identified using the API 20E kit (BioMerieux, Paris, France). A number of species were identified from 111 isolates (Table II), and some of them such as *E. coli, K. pneumoniae, P. aeruginosa* and *Enterobacter cloacae,* are causes of various human infections, such as pneumonia, meningitis and urinary tract infections (Moniri and Dastehgoli, 2005, Warren *et al.*, 2008; Harajly *et al.*, 2010). Two of the isolates, *Hafnia alvei* and *Pseudomonas putida* are not human pathogens but it is worth noting that the latter bacterium is the only patented living organism. Its discoverer reported that it degrades oils (Connors *et al.*, 1997; Van Beilen and Funhoff, 2007).

Antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute, CLSI (CLSI ,2006) guidelines was performed.

Bacterial inoculums were streaked on a Mueller-Hinton II agar plate (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) to obtain confluent growth. All isolates were tested for six antimicrobial agents (Oxoid, Basingstoke, UK): amoxicillin/clavulanic acid (30 μg ml^{-1}), ceftriaxone (30 μg ml^{-1}), gentamicin (10 μg ml^{-1}), tetracycline (30 μg ml^{-1}), ciprofloxacin (5 μg ml^{-1}), and sulfamethoxazole/ trimethoprim (SXT) (25 μg ml^{-1}). Selection of ABAs tested was based primarily on antibacterial agents that were used as a food additive.

The majority of isolates were resistant to tetracycline (76 isolates; 68.5%) followed by SXT (49 isolates; 44.1%), ciprofloxacin/enrofloxacin (34 isolates; 30.6%), gentamicin (26 isolates; 23.4%) and amoxicillin/clavulanic acid (11 isolates; 9.9%). Only 1 isolate was resistant to ceftriaxone (0.9%) (Table II).

E. coli isolates were used to investigate a link between ABA administration and ABR patterns because they were the predominant isolates obtained (82 of 111 isolates) and *E. coli* is used as an indicator of food and water contamination (Angulo *et al.*, 2004).

Relative Risk (RR) and 95% Confidence Interval (CI) were determined to see if an ABA administered as a AGP was associated with the number of *E. coli* isolates resistant to the ABA in a statistically significant manner. RR and CI were determined by the method described by Hutchon, DJR (Hutchon, 1999). An RR more than one and a CI that does not include the number "1" indicated significance.

Tetracycline was used as an AGP in 3 of 6 and gentamicin in 2 of 6 layer farms. There was a significant

Table II
Number of isolates resistant to each antibacterial agent

Isolate	Number isolated	Number of isolates resistant to					
		AMC	CRO	GN	TE	CIP	SXT
E. coli	82	3	0	22	59	32	38
K. pneumonia	1	0	0	0	1	0	1
E. cloacae	5	1	0	0	0	0	0
P. vulgaris	5	1	0	0	5	0	0
P. mirabilis	7	0	0	3	6	1	4
Ps. aeroginosa	1	1	0	0	1	0	1
Ps. Putida	6	3	1	0	2	1	1
H.alvei	1	1	0	0	1	0	0
E. sakozaki	1	1	0	1	1	0	1
C. freundi	2	0	0	0	0	0	0
Total	111	11	1	26	76	34	49

Abbreviations: AMC: amoxicillin/clavulanic acid; GN: gentamicin; TE: tetracycline; CIP: ciprofloxacin; SXT: sulphamethaxazole/trimethoprim; CRO: ceftriaxone; ND: not done.

association between tetracycline or gentamicin used as an AGP and the number of resistant *E. coli* isolates to these ABA (RR for Tetracycline = 19.1, 95% CI = 2.8 to 130.3. RR for Gentamicin = 4.2, 95% CI = 2.5 to 6.9) (Table III).

On the other end, there were no or few isolates that were resistant to an ABA that was not used as a AGP. None of the layer farms administered ceftriaxone or amoxacillin/clavulonic acid as an AGP and none of the isolates were resistant to ceftriaxone while only 3 of 82 *E. coli* isolates were resistant to amoxacillin/clavulonic acid.

Interestingly, 32 of 82 (39%) *E. coli* isolates from farms that did not use ciprofloxacin (a quinolone) as an AGP and 38 of 68 (56%) isolates from farms that did not use SXT as an AGP were resistant to these ABAs. Cross resistance with other ABAs that block bacterial porins and/or modify bacterial influx/efflux pumps, thus preventing the assimilation of SXT and ciprofloxacin to their intracellular targets, might be involved (Boerlin and Reid-Smith, 2008; Warren *et al.*, 2008).

Deciphering the intricacies of bacterial resistance against SXT has been a controversial issue. In our study, no significant association existed between the use of SXT as an AGP and the number of resistant isolates to this ABA (RR = 0). Our findings indicate that in farms not using SXT as an AGP, 38 of 68 (56%) isolates were resistant. Moreover, in farm D where SXT was used as an AGP, none of the *E. coli* isolates from these birds were resistant to SXT (Table I). It therefore appears that

Table III
Antibacterial agent(s) used as an AGP and number of *E. coli* isolates resistant and susceptible to the AGP(s)

	Number of isolates resistant to AGP	Number of isolates susceptible to AGP
Tetracycline* as AGP	49	1
Tetracycline* not an AGP	10	22
Gentamicin† as AGP	20	13
Gentamicin† not an AGP	2	47
Sulfamethoxazole/Trimethoprim‡ as AGP	0	14
Sulfamethoxazole/Trimethoprim‡ not as AGP	38	30
Ciprofloxacin§ not as as AGP	32	50
Ceftriaxone§ not as an AGP	0	82
Amoxicillin/Clavulinic§ acid not as an AGP	3	79

AGP = Antibiotic Growth Promoter.
* Relative Risk for Tetracycline = 19.1, 95% Confidence Interval = 2.8 to 130.3
† Relative Risk for Gentamicin = 4.2, 95% Confidence Interval = 2.5 to 6.9
‡ Relative Risk for Sulfamethoxazole/Trimethoprim = 0
§ Ciprofloxacin, Ceftriaxone and Amoxicillin/Clavulonic acid were not used as an AGP in any of the farms.

abuse or misuse of SXT is not the only factor involved in the development of resistance, warm climates and poor hygiene may play a role (Lester *et al.,* 1990). Moreover, Huovinen *et al.* (1995) reported that *E. coli* isolates in developing countries were more resistant to SXT than those from developed countries.

It may be argued that *E. coli* isolated from older birds are longer exposed to ABAs and are more likely to develop resistance to ABAs than isolates from younger birds. Referring to Table I, the number of resistant isolates from birds did not appear to correlate with the age of the birds.

The dissemination of resistant strains is of concern since the Lebanese ministry of agriculture permits the use AGP in poultry farming and estimates that Lebanon produces 144,023 tons of poultry food products, exports 894 tons of its local production of poultry food products, and the consumption per person of poultry meat in 2007 was 24.3 kg (Ministry of Agriculture, 2007).

In conclusion, ABR epidemiology is sparked by the concern of transmitting drug resistant food borne pathogens to humans, either through food supply or direct contact with animals, contributing to human illnesses with elevated rates of treatment failures. Our findings suggest a relationship between ABA administration and emergence of ABR of *E. coli* isolates obtained from the normal flora of layers, arising fears of consequent dissemination of resistant bacteria to humans and urging the need to reevaluation and change in practice. Abolishing the use of AGP alone is not enough (Van Den Bogaard *et al.*, 2000) if uncoupled with prudent ABA therapeutic usage, eradication programs for specific pathogens, and ABR surveillance programs coordinating their efforts on the national, continental, and international level to maintain an achievable high quality of food and health.

Literature

Aarestrup F.M., A.M. Seyfarth, H.D. Emborg, K. Pedersen, R.S. Hendriksen and F. Bager. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob. Agents Chemother.* 45: 2054–2059.

Angulo F.J., N.L. Baker, S.J. Olsen, A. Anderson and T.J. Barrett. 2004. Antimicrobial use in agriculture: controlling the transfer of antimicrobial resistance to humans. *Sem. in Ped. Inf.* Dis. 15: 78–85.

Boerlin P. and R.J. Reid-Smith. 2008. Antimicrobial resistance: its emergence and transmission. *Anim. Health Res*. Rev. 9: 115–126.

Bywater R.J. and M. Casewell. 2000. An assessment of the impact of antibiotic resistance in different bacterial species and of the contribution of animal sources to resistance in human infections. *J. Antimicrob. Chemother*. 46: 643–645. (Erratum: 46: 1052).

Clinical and Laboratory Standards Institute. 2006. *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S16*. CLSI, Wayne, PA, USA. http://www.sld.cu/galerias/pdf/servicios/medicamentos/nccls.jan2006parte01.pdf.

Connors N., R. Prevoznak, M. Chartrain, J. Reddy, R. Singhvi, Z. Patel, R. Olewinski, P. Salmon, J. Wilson and R. Greasham. 1997. Conversion of indene to cis -(1S),(2R)-indandiol by mutants of Pseudomonas putida F1. *J. Ind. Micr. and Biot.* 18: 353–359.

FAO/WHO Regional Meeting on Food Safety for the Near East. 2005. The impact of current food safety systems in the near east/eastern Mediterranean region on the human health, Amman, Jordan.

Gyles C.L. 2008. Antimicrobial resistance in selected bacteria from poultry. *Anim. Health Res. Rev*. 9: 149–58.

Harajly M., M. Khairallah, J. Corkill, G.F. Araj and G. Matar. 2010. Frequency of conjugative transfer of plasmid-encoded ISEcp1 – $bla_{CTX-M-15}$ and aac(6')-lb-cr genes in Enterobacteriaceae at a tertiary care center in Lebanon – role of transferases. *Ann. Clin. Microbiol. Antimicrob*. 9: 19.

Huovinen P., L. Sundstrom, G. Swedberg and O. Skold. 1995. Trimethoprim and sulfonamide resistance. *Antimicrob. Agents. Chemother*. 39: 279–89.

Hutchon D.J.R. 1999. Calculator for confidence intervals of relative risk. http://www.hutchon.net/ConfidRR.htm.

Johnson J.R, M.R. Sannes, C. Croy, B. Johnston, C. Clabots, M.A. Kuskowski, J. Bender, K.E. Smith, P.L. Winokur and E.A. Belongia. 2007. Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerging. Inf. Dis* 13: 838–846.

Johnson R. 2010. Potential Trade Implications of Restrictions on Antimicrobial Use in Animal Production. Congressional Research Service, 7–5700, R41047, USA. http://www.fas.org/sgp/crs/misc/R41047.pdf.

Lester, S.C., P.M. del Pilar, F. Wang, I.P. Schael, H. Jiang and T.F. O'Brien. 1990. The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. *N. Engl. J. Med.* 323: 285–289.

Ministry of Agriculture, Report: Agriculture in Lebanon 2006 and 2007: http://www.agriculture.gov.lb/arabic/prod_final_06_07.pdf.

Moniri, R. and M. Dastehgoli. 2005. Fluoroquinolone-resistant *Escherichia coli* isolated from healthy broilers with previous exposure to fluoroquinolones: Is there a link? *Microb. Ecol. Heal. Dis.* 17: 69–74.

NARMS. 2008. Retail Meat Complete Annual Report, US Department of Health and Human Services, CDC, Atlanta, GA http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm237109.htm.

Phillips I., M. Casewell, T. Cox, B. de Groot, C. Friis, R. Jones, C. Nightingale, R. Preston and J. Waddell. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J. Antimicrob. Chemother*. 53: 28–52.

Shea K.M. 2003. Antibiotic Resistance: What Is the Impact of Agricultural Uses of Antibiotics on Children's Health? *Pediatrics* 112: 253–258.

Swann Report. 1969. Joint Committee on the use of antibiotics in animal husbandry. HMSO UK.

Van Beilen J.B. and E.G. Funhoff. 2007. Alkane hydroxylases involved in microbial alkane degradation *Appl Microbiol Biotechnol.* 74: 13–21.

Van Den Bogaard A.E., N. Bruinsma and E. Stobberingh. 2000. The effect of banning avoparcin on VRE carriage in the Netherlands. *J. Antimicrob. Chemother.* 46: 146–147.

Warren R.E., V.M. Ensor and P. O'Neill. 2008. Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β-lactamases in the UK. *J. Antimicrob. Chemother* 61: 504–508.

WHO. 1997. The medical impact of the use of antimicrobials in food animals. Publication WHO/EMZ/ZOO/97.4. World Health Organisation, Geneva, Switzerland.