Iranian Journal of Veterinary Research, Shiraz University, Vol. 13, No. 3, Ser. No. 40, 2012

Short Paper

Determination of antibiotic resistance pattern in *Escherichia coli* strains isolated from animal faeces in a farm house

Amin, A.[1*]; Irfanullah, M.[2]; Hameed, A.[3]; Andaleeb, S.[4] and Ayaz Khan, M.[1]

[1]Gomal Center of Biochemistry and Biotechnology (GCBB), Gomal University, D. I. Khan (KPK), Pakistan; [2]M. Phil. Biotechnology, Gomal Center of Biochemistry and Biotechnology (GCBB), Gomal University, D. I. Khan (KPK), Pakistan; [3]Department of Microbiology, Faculty of Biological Sciences, Quaid I Azam University, Islamabad, Pakistan; [4]Centre of Virology and Immunology, National University of Science and Technology (NUST), Islamabad, Pakistan

[*]**Correspondence:** A. Amin, Gomal Centre of Biochemistry and Biotechnology (GCBB), Gomal University, D. I. Khan (KPK), Pakistan. E-mail: dani_amin79@yahoo.com

(Received 10 May 2011; revised version 21 Feb 2012; accepted 5 Mar 2012)

Summary

The present study aimed to evaluate the antibiotic resistance pattern of *Escherichia coli* strains isolated from animals to ascertain the levels of antibiotic resistance pervasiveness. A total of 28 *E. coli* strains were isolated from faecal samples and the antibiotic resistance pattern of *E. coli* strains was determined by means of disc diffusion assay. The resistance pattern determined for all strains was amoxicillin, streptomycin, cefepime, azteronam, amoxicillin/clavulanic acid, ciprofloxacin and ceftriaxone. About 50-75% of the strains were reported as resistant to more than five antibiotics (multidrug-resistant). This might result in broadening of the antibiotic resistance canvas among animals and from animals to human taking the animal food products or living in close contact with them.

Key words: *Escherichia coli*, Resistance pattern, R plasmid, Spread of resistance

Introduction

The clinical hazards of antibiotics resistance has a wide history that prevails over decades and even today over and unnecessary use of antibiotics has brought new challenges to public health (Levy and Marshall, 2004). In addition to other animals, wild animals and pets contribute a major part (greater than 70%) in the stretch of infections. This reality is supported by the outcomes of many studies which reported that the majority of outbreaks in humans over a decade (1990-2000) involved transmission through animals including the spread of multidrug-resistant (MDR) zoonotic pathogens (through zoo animals to humans) (Kasravi *et al*., 2010). Most of such microorganisms are gram negative bacterial pathogens (Bender and Shulman, 2004).

The approximation of antimicrobials usage in feed of farm animals and poultry leads to modification of intestinal flora by creating a selective pressure in favor of resistant bacterial pathogens that get transferred into humans through the environment and food chain (Diarra *et al*., 2007). Likewise, the genetic modifications, receptor insensitivity and decreased drug uptake and R factors contribute significantly in the spread of resistance to antimicrobials (O'Brien, 2002).

Escherichia coli is an inhabitant of normal flora of the gastrointestinal tract of humans and animals, and is believed to facilitate the food digestion through enzyme synthesis, however, few are potentially pathogenic (Levine, 1987). Such strains may induce colibacillosis, omphalitis, cellulitis, swollen head syndrome, coligranuloma, in

chicken (Amara *et al*., 1995), urinary tract infections, septicemia, and neonatal meningitis in human (Ewers *et al*., 2004; Johnson *et al*., 2008).

This study was conducted to determine the resistance pattern among *E. coli* strains isolated from different farm animals.

Materials and Methods

Sample collection

The fresh faecal samples of 7 different animals were obtained from an animal house in Malana village district D. I. Khan (KPK) Pakistan during April 2010. Overall, 28 *E. coli* strains were isolated from all representative faecal samples. The faecal samples were stored at -20°C till the complete examination.

Sample processing and identification

The microbial colonies were obtained by serial dilution method. Colonies were cultured on sterile nutrient agar plates and the isolated *E. coli* strains were identified by biochemical tests (Krier and Holt, 1984).

Antimicrobial agents

Nine different antimicrobial discs (Oxoid) were tested using ofloxacin 5 µg, amoxicillin 25 µg, cefepime 30 µg, amoxicilin-calvulanic acid 30 µg, ciprofloxacin 5 µg, ceftriaxone 10 µg, aztreonam 30 µg, meropenem 25 µg and streptomycin 10 µg. The standard antimicrobial powders were purchased commercially for MIC viz: Meropenem (Astra Zeneca, UK), amoxicillin-clavulanic acid and amoxicillin (GlaxoSmithKline and Beecham Pakistan), ceftriaxone (Macter (pvt) Ltd., Pakistan) streptomycin (Tabrose Pharma Pakistan) and ciprofloxacin (Ampson Pharmaceuticals (pvt) Ltd., Pakistan). The antibiotics used during the study were selected on the basis of information regarding their use in feed and frequency of usage against infections.

Antimicrobial resistance screening

Disc diffusion assay

Susceptibility tests were performed by Bauer-Kirby (Bauer *et al*., 1966) disc diffusion method on Muller Hinton agar (Oxoid). The results were expressed as susceptible/resistant according to diameter of zone of inhibition around each antibiotic disc (National Committee for Clinical Laboratory Standards, 2002).

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the antimicrobial agents were determined by agar dilution method (National Committee for Clinical Laboratory Standards, 2002). The sterilized Muller Hinton agar (oxoid) media was cooled to 50°C and about 19 ml of this was added to sterilized test tubes that contained 1 ml of different concentration of antibiotic. This mixture was thoroughly mixed and poured into pre-labeled sterile petri plates. Petri plates having only growth media were prepared with a similar procedure to serve for comparison with the petri plate containing antibiotic. The concentrations of the antibiotics used in this test ranged from 30 mg to 0.117 mg/ml. The suspensions of the microorganisms having density adjusted to 0.5 McFarland turbidity standard were inoculated onto the series of agar plates using micropipette (0.05 µl approximately). The plates were then incubated at 37°C for 24 h.

Results

The fresh faeces and cloacal swabs were collected from various animals in a farm house (Table 1 and 2). A sum of 28 *E. coli* strains was isolated from animal faeces. Almost all strains showed resistance towards cephalosporin antibiotics (100%). It was also observed that about 50 to 75% of *E. coli* strains were resistant to more than five antibiotics (therefore considered as multi drug resistant, MDR). The overall resistance pattern of *E. coli* strains was AML, STP, FEP, ATM, AMC, CIP, CRO (Table 3). Likewise, the MIC levels of the antibiotics reported were resistant (Table 4).

Discussion

During the present study much higher levels of resistance were determined among

Table 1: Zoological classification of animal

Code	English name	Local name	Zoological name	Family	Genus
T1	Black buk	Kala Hiran	*A. cervicapra*	Bovidae	*Antilopinae*
T2	Indian desert	Chinkara	*Gazella bennetti*	Bovidae	*Gazella*
T3	Blue bull (M)	Nilgai	*B. tragcamelus*	Bovidae	*Boselaphus*
T4	Jackal	Geedar	*Canis aureus*	Canidae	*Canis*
T5	Hog deer	Parah	*Axis procinus*	Bovidae	*Axis*
T6	Rabbit	Khargosh	*Cuniculus*	Leporidae	*Oryctolagus*
T7	Blue bull (F)	Nilgai	*B. tragcamelus*	Bovidae	*Boselaphus*

Table 2: Characteristics of animal faeces

Code	English name	C°	Length	Shape	Color
T1	Black buk	34	0 mm	Spherical	Dark green
T2	Indian desert	34	16 mm	Pin shaped	Dark green
T3	Blue bull (M)	34	29 mm	Spherical	Dark green
T4	Jackal	34	25 mm	Cigrate shaped	Dark green
T5	Hog deer	34	20 mm	Rod shaped	Dark green
T6	Rabbit	34	11 mm	Pin shaped	Dark green
T7	Blue bull (F)	34	26 mm	Spherical	Dark green

Table 3: Antimicrobial resistance pattern of *E. coli* strains

Animal faeces	Resistance pattern	%age (No.)
T1 Black buk	AML, ATM, AMC, CRO, CIP	50 (2)
T2 Indian deer	AML, FEP, STP, ATM, AMC, CRO	75 (3)
T3 Blue bull (M)	AML, FEP, STP, AMC, CRO	50 (2)
T4 Jackal	AML, STP, AMC, CRO	75 (3)
T5 Hog deer	AML, MEM, STP, FEP, ATM, AMC, CIP, CRO	50 (2)
T6 Rabbit	AML, ATM, AMC, CRO	75 (3)
T7 Blue bull (F)	STP, ATM, AMC, CRO	75 (3)
Antibiogram *E. coli* ATCC 29050	AML, MEM, STP, FEP, ATM, AMC, CIP, CRO	-

AML amoxicillin, MEM meropenem, STP streptomycin, FEP cefepime, OFX ofloxacin, ATM azteronam, AMC amoxicillin/clavulanic acid, CRO ceftriaxone, and CIP ciprofloxacin

Table 4: MIC of *E. coli* against selected antibiotics

Antibiotic	MIC (mg/ml) s						
	T1 Black buk	T2 Chinkara	T3 Nilgai (M)	T4 Jackal	T5 Parah	T6 Rabbit	T7 Nilgai (F)
AML	0.5-0.625	0.5-0.625	1-0.125	0.25-0.0625	0.25-0.0625	1-0.25	-
MEM	0.25-0.125	0.25-0.125	0.25-0.125	0.25-0.125	1-0.5	0.25-0.125	0.25-0.125
FEP	-	1-0.5	0.25-0.0625	1-0.0312	0.25-0.125	-	-
STP	-	1-0.0312	0.25-0.125	1-0.5	0.5-0.625	-	0.25-0.125
ATM	0.25-0.0625	0.5-0.25	0.25-0.0625	-	1-0.0312	0.5-0.25	1-0.5
AMC	0.5-0.625	1-0.25	0.5-0.625	0.25-0.0312	1-0.5	0.25-0.0312	0.5-0.625
CRO	1-0.125	1-0.5	0.25-0.0625	1-0.0312	0.5-0.25	0.25-0.0312	0.5-0.625
CIP	0.25-0.0625	-	0.5-0.625	-	-	-	-

Reference values (resistance mg/L) AML≥2, MEM≥8, FEP≥2, STP≥16, ATM≥16, MC≥16, CRO≥2, and CIP≥4 resistant

faecal samples representative of all farm animals compared to that reported earlier (Sayah *et al*., 2005; Zhao *et al*., 2005). The resistance pattern of all *E. coli* strains revealed nearly similar results. Moreover, significantly elevated resistance levels were reported towards all antibiotic classes, especially towards the cepahlosporins (100%). This fact may be the result of ESBLs production by *E. coli* strains which is regarded as one of the most important resistance factors in gram negative bacteria (Asbel and Levison, 2000).

It was noted that all representative strains exhibited resistance to more than five antibiotics, which confined them as

multidrug resistant as reported earlier (Zhao *et al*., 2005; Rahman and Rahman, 2008). Additionally, the *E. coli* strains isolated from hog dear were also resistant to meropenem, which is an indicator of higher levels of antibiotic resistance.

Likewise, the isolated *E. coli* strains showed significantly elevated levels of corresponding MIC values that were in close agreement with results of disc diffusion assay. These findings confirmed the presence of resistance plasmids in the isolates as reported previously (Sayah *et al*., 2005). This is perhaps due to frequent use of antibiotics in the feed and for prophylaxis that resulted in the development of selective pressure and the ultimate emergence of antibiotic resistance (Vanden *et al*., 2001).

During the present investigation, about 50 to 75% of *E. coli* isolates were observed resistant to more than three antimicrobial agents (MDR). Mainly high resistance levels are indicative of this finding (Vanden *et al*., 2001). There is strong evidence that the excessive use of antimicrobial agents and circulation, and amplification of antimicrobial resistance genes in the environment may result in the emergence of multidrug resistant (MDR) *E. coli* (Asbel and Levison, 2000).

It is concluded that *E. coli* strains isolated from animals in a farm house were highly resistant and a significant majority of isolates were observed as multidrug resistant.

References

Amara, A; Ziana, Z and Bouzoubaa, K (1995). Antibioresistance of *Escherichia coli* strains isolated in Morocco from chickens with colibacillosis. Vet. Microbiol., 43: 325-330.

Asbel, LE and Levison, ME (2000). Cephalosporins, carbapenems, and mono-bactams. Infect. Dis. Clin. N. Am., 14: 435-447.

Bauer, AW; Kirby, WMM; Sherris, JC and Truck, M (1966). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol., 45: 493-496.

Bender, JB and Shulman, SA (2004). Reports of zoonotic disease outbreaks associated with animal exhibits and availability of recommendations for preventing zoonotic disease transmission from animals to people in such settings. J. Am. Vet. Med. Assoc., 224: 1105-1109.

Diarra, MS; Silversides, FG; Diarrasouba, F; Pritchard, J; Masson, L; Brousseau, R; Bonnet, C and Delaquis, P (2007). Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens *Clostridium perfringens* and *Enterococcus* counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Appl. Environ. Microbiol., 73: 6566-6576.

Ewers, C; Janssen, T; Kiessling, S; Philipp, HC and Wieler, LH (2004). Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Vet. Microbiol., 104: 91-101.

Johnson, TJ; Wannemuehler, Y; Doetkott, C; Johnson, SJ; Rosenberger, SC and Nolan, LK (2008). Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol., 46: 3987-3996.

Kasravi, R; Bolourchi, M; Farzaneh, N; Seifi, HA and Barin, A (2010). *In vitro* antimicrobial sensitivity of bovine subclinical mastitis isolates and treatment outcome in lactating dairy cows. Iranian J. Vet. Res., 11: 249-254.

Krier, NR and Holt, JG (1984). *Bergey's manual of systematic bacteriology*. 2nd Edn., Vol. 1, Baltimore: Williams and Wilkins. P: 394.

Levine, M (1987). *Escherichia coli* that cause diarrhea: enterotoxaemia, enter pathogenic, entero invasive, entero hemorrhagic and enter adherent. J. Infect. Dis., 155: 377-390.

Levy, SB and Marshall, B (2004). Antibacterial resistance worldwide: causes, challenges and responses. Natur. Med., 10: 122-129.

National Committee for Clinical Laboratory Standards (2002). Performance standards for antimicrobial susceptibility testing: twelfth informational supplement. M100-S12. NCCLS, Wayne, PA, USA.

O'Brien, T. F. (2002). Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin. Infect. Dis., (Suppl. 3), 34: 78-84.

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

Sayah, RS; Kaneene, JB; Johnson, Y and Miller, R (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.

Vanden, BAE; London, N; Driessen, C and Stobberingh, EE (2001). Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J. Antimicrob. Chemother., 47: 763-771.

Zhao, S; Maurer, JJ; Hubert, S; Villena, JF; McDermott, PF; Meng, J; Ayers, S; English, L and White, DG (2005). Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet. Microbiol., 107: 215-224.