Pakistan J. Zool., vol. 43(2), pp. 303-310, 2011.

Antimicrobial Resistant *Escherichia coli* Strains Isolated From Food Animals in Pakistan

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Abstract.- Antimicrobial resistance among Enterobacteriaceae is an increasing problem in human and veterinary medicine. Resistance profile of Sulphamethoxazole–Trimethoprim combination, Trimethoprim alone, Ciprofloxacin, Nalidixic acid, Cefotaxime, Ceftazedime, Ampicillin and Nitrofurantoin was investigated among *E. coli* isolates on the basis of phenotypic and genotypic evaluation of *Escherichia coli* isolates from food animals. The results showed that *E. coli* isolates resistant to Sulphamethoxazole–Trimethoprim were associated with class I integron of characteristic sizes. In addition the prevalence of antimicrobial resistance genotypes *sul*1 and *sul*2 was studied in Sulphamethoxazole–Trimethoprim resistant isolates from food animals. Prevalence of class 1 Integrons, *sul*1 and *sul*2 in resistant commensal *E. coli* isolates of food animals is a serious public health concern. The changing spectrums of microorganisms in terms of their antibiotic resistance patterns require continuous monitoring of the environment.

Key words: *Escherichia coli,* integrons, antimicrobial resistance, chicken, buffalo.

INTRODUCTION

Escherichia coli (E. coli) like many other bacteria are normal commensal of the warm blooded animals as well as it is a pathogen which causes several types of infections. Among these commensals *E. coli* constitutes an enormous potential reservoir of resistance genes for pathogenic bacteria (Turnidge, 2004; Jakobsen *et al*., 2010). The prevalence of resistance among commensals of food animals may reflect the selective pressure of antimicrobial usage and is a potential indicator of resistance in future infections (Anderson *et al*., 2003). The abundance of *E. coli* in different host species including man, cattle, and poultry, implicates them as likely candidates for the spread of resistance genes and vectors between the bacterial populations of animals and humans (O'Brien, 2002). The largest reservoir of antimicrobial resistance has been thought to reside in the commensal gut microbiota (McDonald *et al.,* 2001). One of the major causes of the spread and transmission of the antibiotic resistance is the transfer of drug resistance from one to other bacteria of the same or different species, within the same host or the different hosts. This transfer of antibiotic resistance genes is facilitated to a great extends through the presence of integrons and plasmids in bacterial communities (Livermore, 2003).

E. coli has been shown to be prolific in the environment as a result of faecal contamination and can survive for weeks in optimal conditions in the environment so the chances of spread of resistant *E. coli* strains from one host (donor) to the other (recipient) are high. The potential for transfer of antimicrobial resistance from enteric zoonotic bacteria of food animals to the human population is a cause of concern. Contact with food animals or their excreta or consumption of foods of animal origin has been suggested to be the main route of dissemination of resistance from food-producing animals into human populations (Helmuth and Hensel, 2004). Slaughtering is potentially the most important stage for bacterial contamination and as a result resistant isolates from the animal gut may readily contaminate carcasses (Aslam *et al.,* 2003; Hassan *et al*., 2010). Thus food of animal origin may serve as a vehicle to transport resistant bacteria and resistance genes between animals and humans since contamination of carcasses with faecal flora inevitably occurs during slaughtering (van den Bogaard and Stobberingh, 2000).

After successful transmission of drug resistant *E. coli* isolates from one host species to the other host species, it is possible to disseminate the

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0030-9923/2011/0002-0303 $ 8.00/0
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antibiotic resistance genes by horizontal transfer which may lead to the rapid emergence of antibiotic resistance among clinical isolates of bacteria. The spread of resistance genes is greatly enhanced when they are a part of a mobile gene cassette, since this provides a chance for horizontal transfer by several mechanisms including integrons. Integron mediated antimicrobial resistance is a major mechanism for transfer of resistance traits within both Gram-negative and Gram-positive bacteria (O'Brien, 2002; Leverstein-van Hall *et al*., 2002). Integrons have been found to harbor the majority of resistance genes within the mobile resistance elements (transposons and plasmids), which allow for transfer of resistance and multi-resistance between bacteria which may include resistance transfer from animal to human microbiota (Leverstein-van Hall, 2002; Guardabassi *et al.,* 2004).

E. coli is considered an indicator of faecal contamination in food and water. The prevalence of resistance in commensal *E. coli* of food animals is a good indicator for the selective pressure by antibiotics use and resistance problems to be expected in pathogenic bacteria. In food animals, a low prevalence and degree of antibiotic resistance in the intestinal flora is considered as a distinguishing quality and safety mark (van den Bogaard and Stobberingh, 2000). Faecal contamination of water is considered a human health risk and there has always been a great deal of concern regarding the level of coliform bacterial counts in water. The sources of these coliforms are thought to be faecal contamination from humans, domestic animals and wildlife in addition to runoff from agricultural lands, inadequate septic systems or sewer overflow (Catry *et al*., 2003). Despite the detailed understanding of zoonotic transmission of *E. coli,* there is a paucity of information on how widespread multidrug resistant faecal isolates are in food animals of Pakistan. In an effort to understand the level of risk to the community, we investigated the drug resistance profiles and detected the presence of integrons and related drug resistance genes in commensal *E. coli* of chicken and buffaloes, the most consumed food animals in Pakistan, as a possible risk factor for the community via environmental contamination or through direct contact.

MATERIALS AND METHODS

Bacterial isolates and Antimicrobial resistance

The study population was composed of 121 Food animals (63 chicken and 58 buffaloes). One buffalo from one herd was randomly selected. Fresh faecal samples were taken using sterile tongue depressors from each animal (buffaloes). Fresh cloacae swabs were collected from chicken (each one randomly selected from separate poultry shops) using sterile swab sticks. Samples were brought back in laboratory in Cary-Blair transport medium and processed onto nutrient agar for growth and Gram negative rods were subsequently streaked on MacConkey agar by using a sterile inoculating loop and the plates were incubated at 37°C for 24 hours. Identification of *E. coli* isolates was performed by standard biochemical methods (Ewing**,** 1986). Susceptibility to Sulphamethoxazole-Trimethoprim combination (SXT: 25 µg), Trimethoprim alone (W: 2.5 µg), Ciprofloxacin (CIP: 10 µg), Nalidixic acid (NAL: 30 µg), Cefotaxime (CTX: 30 µg), Ceftazedime (CAZ: 30 µg), Ampicillin (AMP: 25 µg) and Nitrofurantoin (F: 50 µg) was determined using standard disc diffusion method in accordance with Clinical and Laboratory Standard Institute recommendations (CLSI, 2007). All antimicrobial discs were obtained commercially (Oxoid, UK). Each unique resistance pattern was assigned a resistotype number. Frequencies of each resistotype were determined.

PCR method

Total genomic DNA templates of *E. coli* isolates were extracted by Phenol-Chloroform extraction method. All isolates found to be resistant to Sulphamethoxazole-Trimethoprim were screened for the presence of integrons, *sul*1 and *sul*2. Sulphamethoxazole resistance determinants *sul1* and *sul2* were identified by PCR amplification using gene specific primers (Invitrogen, Carlsbad, USA). The amplification of *sul*1 was performed using the forward primer *sul1*F (5'-CTTCGATGAGAGCCGGCGGC-3') and reverse primer *sul1*R (5'-GCAAGGCGGAAACCCGCGCC-3'). Amplification of *sul*2 was done by using the forward primer *sul*2-F (5'-GCGCTCAAGGCAGATGGCATT-3') and the

reverse primer *sul*2-R (5'-GCGTTTGATACCGGCACCCGT-3') (Blahna *et al.,* 2006).

Screening for the presence of class 1, 2 and 3 integrons (*int*1, *int*2, *int*3) was done with the help of PCR-RFLP method (White *et al*., 2001). The sequences of the degenerate primers to hybridize to conserved regions of integron encoded integrase genes were as follows: *hep35* (5'-TGCGGGTYAARGATBTKGATTT-3') and *hep36* (5'- CARCACATGCGTRTARAT-3'), while B = C or G or T; K = G or T; R = A or G; and Y = C or T. Integrase PCR products were digested using *Hinf*1 restriction enzyme according to the manufacturer's instructions (Fermentas). Classification of integrons was done on the bases of the size of restricted fragments after treatment of *hep* amplified products with *Hinf*1 (491bp fragment for class 1 integrons, 300 bp and 191 bp fragments for class 2 integrons, while 119 bp and 372 bp fragments for class 3 integrons) (White *et al*., 2001).

PCR amplifications were carried out in 50 µl reaction mixtures containing 5 µl DNA templates, 50 pmol of each primer, 1.5 mM $MgCl_2$, 0.2mM of dNTPs and 2.5U Taq polymerase. Thermal cycle reaction conditions for all primer pairs were as follows: 94 ^{o}C for 5 minutes; 30 cycles of denaturation at 94 ^{o}C for 40 seconds, annealing at 55 ^{o}C for 40 seconds, and extension at 72 ^{o}C for 1 minute. A final extension of 72 ^{o}C was run for 7 minutes.

Statistical analysis

A logistic regression analysis was performed on isolates to determine which resistance phenotypes are independent of other resistance phenotypes associated with the presence of an integron. The Bonferroni correction was applied and the p-value < 0.006 was considered significant.

RESULTS AND DISCUSSION

A total of 121 *E. coli* non-repetitive isolates (58 isolates from buffaloes, and 63 isolates from chicken) were screened for evaluation of their antimicrobial sensitivities. It is apparent from the results that reasonable proportion of the isolates has shown resistance to one or other antibiotic tested (Table I). In addition these isolates were grouped into seventeen categories, according to the occurrence of different individual antibiotics resistances as well as co-occurrence of combination of different resistance phenotypes (Table II). The differences observed in the case of sensitivity patterns of *E. coli* isolates from the buffalo and poultry are apparent from the descending order of resistance in poultry isolates (W > SXT = NAL > CIP = AMP > F > CTX = CAZ) as compared to the descending order of resistance in buffalo isolates (SXT = W > F = AMP > NAL > CTX > CIP > CAZ). Sulphamethoxazole and Trimethoprim are the antibiotics to which largest number of *E. coli* isolates from Poultry and Buffaloes were resistant. These isolates were more sensitive to Ceftazedime and Cefotaxime than other drugs to which the sensitivity patterns were observed.

Table I.- Resistance phenotypes in 121 *E. coli* isolates from buffaloes (n = 58) and poultry (n = 63) faecal samples.

Antimicrobials tested	Resistant isolates (%)	
	Poultry (n = 63)	Buffalo (n = 58)
Sulphamethoxazole - Trimethoprim	84 %	28 %
Trimethoprim	88 %	28 %
Ciprofloxacin	44 %	8 %
Nalidixic acid	84 %	20 %
Cefotaxime	16 %	16 %
Ceftazedime	16 %	4 %
Ampicillin	44 %	24 %
Nitrofurantoin	28 %	24 %

We observed that buffalo isolates are more sensitive to the antibiotics as compared to the poultry isolates. This is perhaps due to frequent use of antibiotics in the chicken as feed additives to serve as growth promoters (Diarra *et al*., 2007). Resistant strains of *E. coli* can settle in the intestinal flora of the chickens even in the absence of the selective pressure of antibiotics (Guillot and Boucaud, 1988). The buffaloes under this study were not exposed to the antibiotics as they consumed the natural fodder and grass and did not consume any antibiotic as feed additive or as a growth promoter. However, a considerable number of drug resistant isolates were detected from the

buffalo faeces which may reflect the ingestion of drug resistant bacteria from streams, ingestion of antibiotic traces from streams may have lead to selective pressure (Guardabassi *et al.,* 2004; Samara *et al.,* 2009) and the ultimate emergence of antibiotic resistance.

Table II.- Various antimicrobial resistance patterns in 121 *E. coli* isolates from buffaloes (n = 58) and poultry (n = 63) faecal samples.

Resisto-type #	Antibiotics to which resistance observed[a]	Poultry (n = 63)	Buffalo (n = 58)
I	C[s]	8%	49%
II	CTX	0%	3%
III	F	0%	8%
IV	NAL	0%	5%
V	SXT	0%	4%
VI	W	0%	4%
VII	AMP, F	0%	3%
VIII	SXT, W, NAL	39%	2%
IX	CTX, W, AMP, F	4%	0%
X	CTX, SXT, W, AMP	0%	6%
XI	CTX, NAL, CAZ, F	4%	0%
XII	SXT, W, AMP, CAZ	6%	4%
XIII	SXT, W, AMP, NAL	20%	0%
XIV	SXT, W, AMP, NAL, F	7%	3%
XV	CTX, SXT, W, AMP,	3%	9%
XVI	NAL, F	5%	0%
XVII	CTX, SXT, W. NAL, CAZ, F	4%	0%
	SXT, W, AMP, NAL, CAZ, F		

[a]Abbreviation: SXT, Sulphamethoxazole-Trimethoprim; CTX, Cefotaxime; CIP, Ciprofloxacin; W, Trimethoprim, AMP, Ampicillin; NAL, Nalidixic acid; CAZ, Ceftazedime; F, Nitrofurantoin; C[s], Sensitive to all antibiotics tested

The Sulphamethoxazole-Trimethoprim resistant isolates were screened for the presence of Integrons and the related genes (*sul*1, *sul*2) (Tables III, IV; Fig. 1). Out of 63 *E. coli* isolates from poultry, 53 (84%) were resistant to Sulphamethoxazole-Trimethoprim. Among these 53 Sulphamethoxazole-Trimethoprim resistant isolates, *int*1 was detected in 20 (38%), *int*2 in 1 (2%), *sul*1 in 25 (47%), and *sul*2 was detected in 32 (60%) isolates.

Sixteen (28%) of 58 *E. coli* isolates from buffalo were found to be resistant to Sulphamethoxazole-Trimethoprim. Integron-1 was detected in 4 (25%), *sul*1 in 4 (25%) and *sul*2 was detected in 9 (56%) of these 16 Sulphamethoxazole-Trimethoprim resistant isolates. Although there were some minor differences in prevalence of class 1 Integron, *sul*1 and *sul*2 between the Sulphamethoxazole-Trimethoprim resistant isolates from poultry and buffaloes (Table III), however the co-occurrence patterns of these markers showed some resemblances especially in case of *sul*2 (Table IV).

Table III.- Occurrence of antimicrobial resistance markers *int*1, *sul*1, *sul*2 in 69 Sulphamethoxazole-Trimethoprim resistant *E. coli* isolates.

Origin of resistant isolates	Presence of antimicrobial resistance markers				
	***Int*1**	***Int*2**	***Int*3**	***sul*1**	***sul*2**
Poultry (n=53)	38%	2%	0%	47%	60%
Buffaloes (n=16)	25%	0%	0%	25%	56%

Table IV.- Association of class 1 Integron with *sul*1 and *sul*2 among 69 Sulphamethoxazole-Trimethoprim resistant *E. coli* isolates.

Combinations of genotypes	Poultry (n = 53)	Buffalo (n = 16)
*Int*1 alone	0 %	0 %
*Int*1 + *sul*1 combination	14 %	0 %
*Int*1 + *sul*1 + *sul*2 combination	24 %	25 %
*sul*1 alone	4 %	0 %
*sul*2 alone	31 %	31 %
*sul*1+ *sul*2 combination	5 %	0 %
*Int*2	2%	0%
*Int*3	0%	0%

Logistic regression analysis have shown that in case of poultry isolates, Sulphamethoxazole-Trimethoprim resistance phenotype is associated positively with each of Trimethoprim, Ampicillin and Nalidixic acid resistance phenotypes with the presence of integron while other phenotypes are negatively associated as shown in Table V. Similarly in case of Trimethoprim resistant cases Sulphamethoxazole-Trimethoprim, Nalidixic acid, and Ampicillin resistance in the presence of integron was increased and vice versa is observed. On the other hand while in the presence of integrons all the antimicrobial resistance phenotypes studied

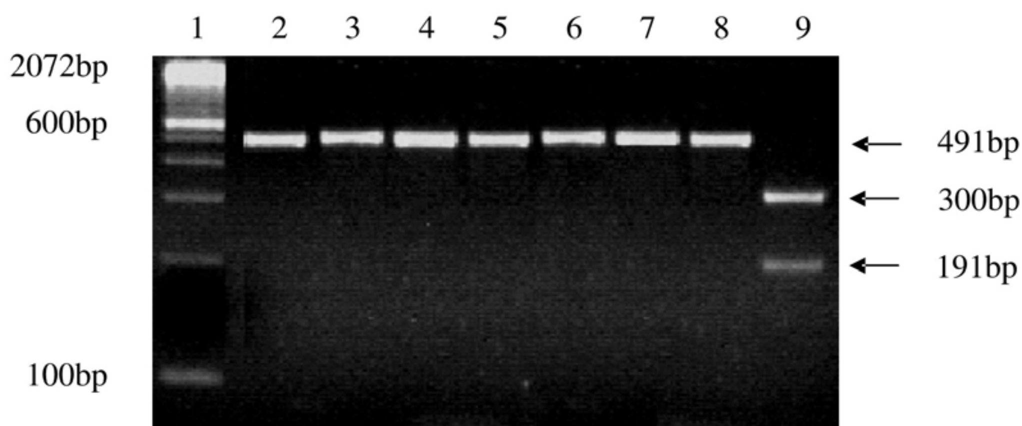

Fig. 1. Sizes of *hep* amplified, *Hinf*1 treated PCR products with 100bp ladder. Lane 1, 100 bp ladder; Lanes 2-8, *int*1 bands (491bp), Lane 9, *int*2 bands (300bp, 191bp).

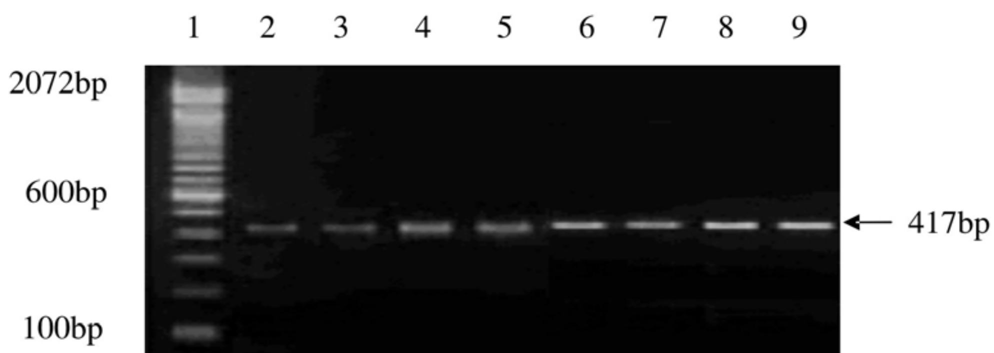

Fig. 2. Sizes of *sul*1 amplification products with 100bp ladder. Lane 1, 100 bp ladder; Lanes 2-9, *sul*1 amplification bands (417bp).

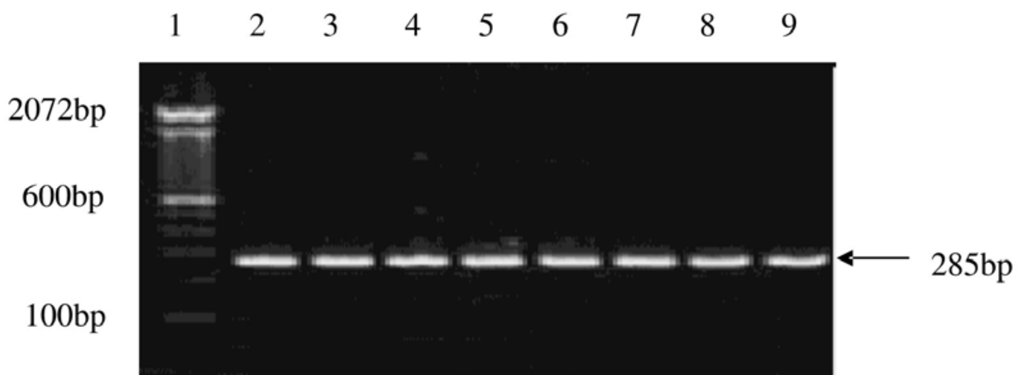

Fig. 3. Sizes of *sul*2 amplification products with 100bp ladder. Lane 1, 100bp ladder; Lanes 2-9, *sul*2 amplification bands (285bp).

were independent of each other in *E. coli* isolates from buffalo samples (Table VI).

Table V.- Associations between antimicrobial resistance phenotypes with the presence of class 1 Integron in *E. coli* isolates from poultry faecal samples (n = 63).

Variable		OR	(95% CI)	P-value
Outcome	Predictor			
SXT	CTX + *int*1	78.18	20.74-327.84	<0.001
	W + *int*1	11.4	4.48-29.73	<0.001
	AMP + *int*1	20.38	7.55-56.97	<0.001
	NAL + *int*1	13.25	5.15-35.04	<0.001
	CAZ + *int*1	50.35	15.45-175.62	<0.001
	F + *int*1	31.8	10.92-97.06	<0.001
CTX	SXT + *int*1	2.78	0.74-11.29	0.08
	AMP + *int*1	5.75	1.11-39.93	0.01
W	SXT + *int*1	14.78	5.49-41.19	<0.001
	CTX + *int*1	101.41	25.65-450.27	<0.001
	AMP + *int*1	26.44	9.27-78.75	<0.001
	NAL + *int*1	17.19	6.31-48.52	<0.001
	CAZ + *int*1	65.31	19.06-242.27	<0.001
	F + *int*1	41.25	13.43-134.02	<0.001
AMP	SXT + *int*1	3.08	1.31-7.31	0.004
	CTX + *int*1	24.4	5.16-157.97	<0.001
	W + *int*1	3.08	1.31-7.31	0.004
	NAL + *int*1	3.78	1.56-9.34	0.001
	CAZ + *int*1	11.8	3.53-43.54	<0.001
	F + *int*1	6.4	2.34-18.15	<0.001
NAL	SXT + *int*1	1	0.43-2.32	1
	CTX + *int*1	5.9	1.71-22.29	0.001
	AMP + *int*1	1.89	0.75-4.82	0.13
	F + *int*1	2.4	0.91-6.46	0.05
CAZ	SXT + *int*1	1.79	0.55-6.02	0.28
	CTX + *int*1	5.75	1.11-39.93	0.01
	AMP + *int*1	2.78	0.74-11.29	0.08
F	SXT + *int*1	2.4	0.91-6.46	0.05
	CTX + *int*1	5.9	1.71-22.29	0.001

Our findings support the previous findings of the several studies in Europe and other countries which have also shown that bacteria, including *E. coli,* from food animals may carry integrons (Goldstein, 2001; Maguire *et al*., 2001; Zhao *et al*., 2001; Adrienne *et al.,* 2005; Song *et al*., 2010). Previous studies by several workers indicate that integrons harbor multiple of resistant genes at one time and they can transfer antimicrobial resistance from commensal to pathogenic strains (Goldstein, 2001; Maguire *et al.,* 2001; Zhao *et al.,* 2001; van Essen-Zandbergen *et al*., 2009) or pathogenic to commensal (Nagachinta and Chen, 2008).

Table VI.- Associations between antimicrobial resistance phenotypes with the presence of class 1 Integron in *E. coli* isolates from buffaloes' faecal samples (n = 58).

Variable		OR	(95% CI)	P-value
Outcome	Predictor			
SXT	CTX + *int*1	10.67	2.16-71.21	<0.001
	W + *int*1	5.14	1.46-19.80	0.003
	AMP + *int*1	6.98	1.75-32.43	0.001
	NAL + *int*1	6.98	1.75-32.43	0.001
CTX	SXT + *int*1	5.14	0.96-36.33	0.02
	AMP + *int*1	10.47	1.28-228.39	0.008
W	SXT + *int*1	5.14	1.46-19.80	0.003
	CTX + *int*1	10.67	2.16-71.21	<0.001
	AMP + *int*1	6.98	1.75-32.43	0.001
AMP	SXT + *int*1	5.83	1.44-27.42	0.003
	CTX + *int*1	8.91	1.78-60.08	0.001
NAL	SXT + *int*1	4.78	1.15-22.86	0.01
	CTX + *int*1	14.87	1.88-317.36	0.001
	AMP + *int*1	7.3	1.43-49.94	0.004
F	SXT + *int*1	8.91	1.78-60.08	0.001
	CTX + *int*1	18.14	2.34-383.55	<0.001

The detection of integrons and related antibiotic resistance genes in this study indicates that there is serious risk of spread of the antibiotic resistance as the gene cassettes can be re-arranged and duplicated within the integrons and the resistance genes can also be transmitted to and/or from these resistant isolates (O'Brien, 2002; Leverstein–van Hall *et al.,* 2002) which are present in both types of food animal species investigated in this study. Integrons represent a significant mechanism for transfer of resistance characteristics from commensal to pathogenic organisms, while integrons have been found to harbor multiple resistance genes at one time (Goldstein, 2001; Maguire *et al*., 2001; Zhao *et al*., 2001) and it is evident that within the Gram-negative bacteria, a substantial portion of the resistance genes present on plasmids and transposons are incorporated into Class 1 integrons (Leverstein-van Hall *et al*., 2002). Moreover, the detection of integrons and the related antimicrobial resistance genes in non-pathogenic *E. coli* from animals (buffaloes and chicken) in this study may represent a considerable reservoir of

antibiotic resistance genes that might be transferable to pathogens (Sunde *et al*., 1998; Sunde and Sorum*,* 1999; Summers, 2002).

Contact with food animals or their excreta or consumption of foods of animal origin has been suggested to be the main route of dissemination of resistance from food-producing animals into human populations (Helmuth and Hensel 2004; Hassan *et al*., 2010). Food of animal origin may serve as a vehicle to transport resistant bacteria and resistance genes between animals and humans (van den Bogaard and Stobberingh, 2000). The prevalence of such multiple resistant *E. coli* in large proportion is a serious concern and transmission mode may include direct contact with live animals, their environment or exposure to contaminated water sources (Manges *et al*., 2007).

Moreover, the prevalence of resistance in commensal *E. coli* in this study is an indicator for the presence of selective pressure by antibiotics use. A high prevalence of antibiotic resistance in the *E. coli* isolates of the food animals (buffaloes and chicken) The presence of various antibiotics resistance in a variety of bacterial species from animal sources supports their global distribution and that cross-species transfer via mobile genetic elements may occur frequently as shown by recent study (Pak-Leung *et al*., 2010).

CONCLUSIONS

In short, it is evident from the results of this study that the *E. coli* isolates of buffalo and poultry origin are highly resistant. In addition the co-occurrence of various antibiotics resistance in such *E. coli* isolates which are circulating in environment, are posing a serious risk to the human health. Moreover, these isolates carry integron-1, *sul*1, *sul*2, and there is a real chance of spread and transmission of highly resistant bacteria as well as the gene cassettes encoding resistance phenotypes from one organism to other organism either commensal or pathogenic by lateral gene transfer.

ACKNOWLEDGEMENTS

H. Bokhari is grateful to COMSATS Institute of Information Technology and Higher Education Commission of Pakistan (HEC), Islamabad, Pakistan for providing research facility and support to carry out this work.

REFERENCES

ADRIENNE, T.A., BOX MEVIUS, D.J., PEPIJN, S., VERHOEF, J. AND FLUIT, A.D.C., 2005. Integrons in *Escherichia coli* from food-producing animals in The Netherlands. *Microb. Drug Resist.*, **11**: 53-57

ANDERSON, A.D., NELSON, J.M., ROSSITER, S. AND ANGULO, F.J., 2003. Public health consequences of use of antimicrobial agents in food animals in the United States. *Microb. Drug Resist.,* **9**: 373-379.

ASLAM, M., NATTRESS, F., GREER, G., YOST, C., GILL, C. AND MCMULLEN, L., 2003. Origin of contamination and genetic diversity of *Escherichia coli* in beef cattle. *Appl. Environ. Microbiol.,* **69**: 2794-2799.

BLAHNA, M.T., ZALEWSKI, C.A., REUER, J., KAHLMETER, G., FOXMAN, B., CARL, F. AND MARRS., 2006. The role of horizontal gene transfer in the spread of trimethoprim–sulphamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. *J. Antimicrob. Chemother.,* **57**: 666-671.

CATRY, B., LAEVENS, H., DEVRIESE, L.A., OPSOMER, G. AND KRUIF, A.D.E., 2003. Antimicrobial resistance in livestock. *J. Vet. Pharmacol. Therap*., **26**: 81-93.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI), 2007. *Performance standards for antimicrobial susceptibility testing*, 17th informational supplement. Clinical and Laboratory Standards. Wayne, PA.

DIARRA, M.S., SILVERSIDES, F.G., DIARRASSOUBA, F., PRITCHARD, J., MASSON, L., BROUSSEAU, R., BONNET, C., DELAQUIS, P., BACH, S., SKURA, B.J. AND TOPP, E., 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.

EWING, W.H., 1986. Edward's and Ewing's identification of Enterobacteriaceae (4th ed). Elsevier Science Publishing Co. Inc., New York.

GOLDSTEIN, F.W., 2001. Antibiotic susceptibility of bacterial strains isolated from patients with community- acquired urinary tract infections in France. *Eur. J. clin. Microbial. Infect. Dis.*, **19**: 112-117.

GUARDABASSI, L., SCHWARZ, S. AND LLOYD, D.H., 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother.*, **54**: 321-332.

GUILLOT, J.F. AND BOUCAUD, J.L., 1988. *In vivo* plasmid

transfer between strains of *Escherichia coli* in the chicken digestive system. *Pathol. Biol.*, 36: 655-659.

HASSAN, A.N., FAROOQUI, A., KHAN, A., KHAN, A.Y. AND KAZMI, S.U., 2010. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan**.** *J. Infect. Dev. Ctries*., **4**: 382-388.

HELMUTH, R. AND HENSEL, A., 2004. Towards the rational use of antibiotics: results of the first international symposium on the risk analysis of antibiotic resistance. *J. Vet. Med.*, **51**: 357-360.

JAKOBSEN, L., KURBASIC, A., SKJOT-RASMUSSEN, L., EJRNAES, K., PORSBO, L.J., PEDERSEN, K., JENSEN, L.B., EMBORG, H.D., AGERSO, Y., OLSEN, K.E., AARESTRUP, F.M., FRIMODT-MOLLER, N. AND HAMMERUM, A.M., 2010. *Escherichia coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. *Foodborne Pathog. Dis*., **7**: 537-547.

LEVERSTEIN-VAN HALL, M.A., PAAUS, A., BOX, T.A., BLOK HEM., VERHOEF, J. AND FLUIT, A.C., 2002. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. *J. clin. Microbiol.*, **40**: 3038-3040.

LIVERMORE, D.M., 2003. Bacterial resistance: origins, epidemiology, and impact. *Clin. Infect. Dis.*, **36**: S11-S23.

MAGUIRE, A.J., BROWN, D.F.J., GRAY, J.J. AND DESSELBERGER, U., 2001. Rapid screening technique for class 1 integrons in Enterobacteriaceae and nonfermenting Gram negative bacteria and its use in molecular epidemiology. *Antimicrob. Agents Chemother*., **45**: 1022-1029.

MANGES, A.R., SMITH, S.P., LAU, B.J., NUVAL, C.J., EISENBERG, J.N., DIETRICH, P.S. AND RILEY L.W., 2007. Retail meat consumption and the acquisition of antimicrobial resistant *Escherichia coli* causing urinary tract infections: a case-control study. *Foodborne Pathog. Dis*., **4**: 419-431.

MCDONALD, L.C., CHEN, F.J., LO, H.J., YIN, H.C., LU, P.L., HUANG, C.H., CHEN, P., LAUDERDALE, T.L. AND HO, M., 2001. Emergence of reduced susceptibility and resistance to fluoroquinolones in *Escherichia coli* in Taiwan and contributions of distinct selective pressures. *Antimicrob. Agents Chemother.*, **45**: 3084-3091.

NAGACHINTA, S. AND CHEN, J., 2008. Transfer of class 1 integron-mediated antibiotic resistance genes from shiga toxin-producing *Escherichia coli* to a susceptible *E. coli* K-12 strain in storm water and bovine feces. *Appl. environ. Microbiol*., **74**: 5063-5067.

O'BRIEN, T.F., 2002. Emergence, spread and environmental effect of antimicrobial resistance: how use of antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clin. Inf. Dis*., **34**: S78-S84.

PAK-LEUNG, H., RIVER, C.W., STEPHANIE, W.L., KIN-HUNG, C., SAMSON, S.W. AND TAK-LUN, Q., 2010. Genetic identity of aminoglycoside-resistance genes in *Escherichia coli* isolates from human and animal sources. *J. med. Microbiol*., **59**: 702-707.

SAMARA, Z.Q., NASEEM, M., KHAN, S.J., DAR, N. AND ATHAR, M.A., 2009. PCR targeting of antibiotic resistant bacteria in public drinking water of Lahore metropolitan, Pakistan. *Biomed. environ. Sci*., **22**: 458-63.

SONG, L., NING, Y., SHEN, J., FAN, X., ZHANG, C., YANG, C. AND HAN, J., 2010. Investigation of integrons/cassettes in antimicrobial-resistant *Escherichia coli* isolated from food animals in China. *Sci. China Life Sci.,* **3**: 613-619.

SUMMERS, A.O., 2002. Generally overlooked fundamentals of bacterial genetics and ecology. *Clin. Inf. Dis.*, **34**: S85-S92.

SUNDE, M. AND SORUM, H., 1999. Characterization of integrons in *Escherichia coli* of the normal intestinal flora of swine. *Microb. Drug Resist.,* **5**: 279-287.

SUNDE, M., FOSSUM, K., SOLBERG, A. AND SORUM, H., 1998. Antibiotic resistance in *Escherichia coli* of the normal intestinal flora of swine, *Microb. Drug Resist*., **4**: 289-299.

TURNIDGE, J., 2004. Antibiotic use in animals**-**prejudices, perceptions and realities. *J. Antimicr. Chemother.,* **53**: 26-27.

VAN DEN BOGAARD, A.E. AND STOBBERINGH, E.E., 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. *Intl. J. Antimicrob. Agents.*, **14**: 327-335.

van ESSEN-ZANDBERGEN, A., SMITH, H., VELDMAN, K. AND MEVIUS, D., 2009. *In vivo* transfer of an incFIB plasmid harbouring a class 1 integron with gene cassettes d*frA1***-***aadA1*. *Vet. Microbiol.,* **137**: 402-407.

WHITE, P.A., MCIVER, C.J. AND RAWLINSON, W.D., 2001. Integrons and gene cassettes in the Enterobacteriaceae. *Antimicrob. Agents Chemother*., **45**: 2658-2661.

ZHAO, S., WHITE, D.G., GE, B., AYERS, S., FRIEDMAN, S., ENGLISH, L., WAGNER, D., GAINES, S. AND MENG, J., 2001. Identification and characterization of integron mediated antibiotic resistance among shiga toxin producing *Escherichia coli* isolates. *Appl. environ. Microbiol.,* **67**: 1558-1564.

(Received 30 March 2010, revised 29 July 2010)