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Study of Multiple-Drug Resistance Transfer Factors from Isolated *E. coli* of Poultry Farms to *Salmonella typhimurium*

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Abstract Different studies on animals in around the world show that there are multiple-drug resistance in strains of *Escherichia coli* (*E. coli*). Such microorganisms are notorious for the extension of colibacillosis in poultry, which great economic losses bring for the poultry industry. This study was carried out to determine drug resistance and resistance factor transfer in *E. coli* strains isolated from poultry farms, in south west of Iran. Than 100 pieces of poultry carcasses from cases of colibacilosis twenty-five *E. coli* resistant to one or more antibiotics were isolated and were analyzed to determine their drug resistance pattern and also transfer of resistance factor via conjugation. The highest rate of resistance was against nalidixic acid and tetracycline. Multiple drug resistance was shown in 80% of strains. By using *Salmonella typhimurium* RITCC1730 (*S. typhimurium*) as a recipient and *E. coli* as donor's resistance factor examined the transfer of drug resistance that the highest rate of transfer was related to neomycin and tiamulin 68% and 64%, respectively. Finally, we concluded that there are a substantially evidence of multiple drug resistance in *E. coli* isolates taken from poultry farms, so in our opinion restricting use of antibiotics and performing antimicrobial susceptibility tests to select suitable antibiotic can decrease developing of resistant strains.

Keywords Drug resistance factor, *Escherichia coli*, Poultry, *Salmonella typhimurium*

1. Introduction

Escherichia coli (*E. coli*) is part of the normal gastrointestinal tract microbial flora in humans, poultry and other animals and in addition, causes disease in both [1]. Howbeit most strains of *E. coli* are nonpathogenic but five pathogenic strains of *E. coli* are causing some of gastrointestinal diseases and diarrhea in humans and animals [2]. *E. coli* is intended as index of stool pollution in food. This bacteria to makes approximately 10 to 15% of gastrointestinal coliforms which are opportunistic and pathogenic serotype and is cause the types of damages in poultry and also in immunocompromised hosts that these injuries are include yolk sac infection epidemic and diarrhea of adults and children, urinary tract infection, meningitis, septicemia, omphalitis, endocarditis, swollen head syndrome, cellulitis, colibacillosis and coligranuloma [3].

After the discovery and use of antibacterial agents, the complication and deaths caused by bacterial infections were markedly reduced but nowadays bacterial resistance to antibiotics is a global problem and emerging antimicrobial resistance has been considered as a public health problem throughout the world [4]. Antibiotics are widely used for control infectious disease or growth enhancers in poultry production, for this reason the number of bacteria resistant to antibiotics have increased in recent years [5]. Horizontal gene transfer is widely recognized as the bacterial mechanism responsible for the widespread distribution of drug resistance genes that this mechanism outset is needy cell-to-cell contingence and then act by DNA replication resulting one-way transfer of genetic material from a donor to a recipient cell [6]. Horizontal transfer of plasmids occurs within the gastrointestinal tract which is known to be a reservoir of natural plasmids. When plasmid transfer occurs in the gastrointestinal tract, the resulting transconjugants are generally unable to become established in that native bacterial community [6]. Bacteria receives resistance factor genes through mobile elements like integrons, plasmids and transposons which is looking for mutations in genes responsible for antibiotics absorption or activation of portions of bacterial chromosomes or binding sites, and after obtain, resistance genes can be transferred between bacteria and this ability of *E. coli* to transfer antibiotic resistant is

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completely known [7]. There are specific genes that control the four general mechanisms of resistance which these resistances include the enzymatic inactivation or modification of the antibiotic itself, impermeability of the membrane or bacteria cell wall, decreased drug accumulation and alteration in target receptors [8]. Multiple-drug resistance in strains of *E. coli* are common in both animals and humans in around the world. Non pathogenic *E. coli* are part of the normal intestinal flora that is likely an important reservoir of resistance genes, furthermore some of drug-resistant *E. coli* of animal origin can be deployed in the human intestine therefore resistant strains of *E. coli* with animal origin due to the overuse of antibiotics may possibly become infectious organisms in humans [5]. The aim of the present study was investigation of antimicrobial drug resistance and transfer of resistance factor from *E.coli* isolated of poultry farms in Chaharmahal va Bakhtiari province in south west of Iran to *Salmonella typhimurium* (*S. typhimurium*).

2. Methods

In present study 100 pieces of carcasses from cases of colibacilosis during September to December 2013 examined from industrial poultry farms in Chaharmahal va Bakhtiari province (southwest of Iran). After collection, the samples were transported to the laboratory immediately in an insulating foam box with ice. Standard methods were used for the cultivation and identification of *E. coli* isolates. Twenty five *E. coli* strains isolated from 100 cases of colibacilosis from poultry farms were tested for determine resistance or susceptibility to 10 antibiotics and as well as their ability to transfer the resistance factor. For drug resistance testing was used standard Bauer-Kirby disk diffusion method. Diameters of the inhibition zones were interpreted based on the Clinical and Laboratory Standards Institute (CLSI). A total of 10 antibiotic discs (Padtanteb, Iran) include Ampicillin (AM, 10 µg), Flumequine (FM, 30 µg), Linkospectin (LS, 200 µg), Nalidixic Acid (NA, 30 µg), Gentamicin (Gm, 10 µg), Neomycin (N, 30 µg), Tiamulin (TM, 30µg), Chloramphenicol (C, 30 µg), Tetracycline (TE, 30 µg) and Entrofloxacin (NFX, 5µg) were used. These antibiotics were chosen based on of their importance in treating animal or human *E. coli* infections and also their use as a food supplement to promote growth in poultry. For to consider the transfer of antimicrobial agent, conjugation was performed by mixed culture method. Twenty five *E. coli* isolated from cases of colibacilosis from poultry farms as donors of resistance factor and *S. typhimurium* RITCC1730 as acceptor of resistance factor were used. Lyophilized *S. typhimurium* RITCC1730 strain was obtained from Razi Research Institute of Iran. Donor strains were *E. coli* strains isolated from samples and were resistant to one or more antibiotics but acceptor strain (*S. typhimurium* RITCC1730) was quite sensitive to all antibiotics. Donor and recipient strains were cultured on Mac Conkey agar at 37°C overnight, then one colony from recipient strain was inoculated in 5 mL Trypticases Soy Broth (TSB) and one colony from donor strains was inoculated in 2 mL TSB and both incubated at 37°C overnight. In the next stage 0.1 mL from soluble of donor cells were mixed with 0.9 mL from soluble of recipient cells and were mixed in a final volume of 10 ml fresh TSB and incubated at 37°C for 24 h and then donor and recipient strains were centrifuged at 5000 rpm for 10 minute. The bacteria were sediment at the bottom of the tube, then solution was poured out of over sediment and 10 mL streptococcus faecalis medium broth (SF medium broth) were added to the sediment. SF medium broth contains bacteria was incubated at 37°C overnight and then were centrifuged at 5000 rpm for 10 minute. The supernatant was removed and then bacterial pellet were cultured using a sterile swab from the tube bottom on selective media containing antibiotics (50 µg/ml) and incubated at 37°C for 24 h. Also separate cultures of donor and recipient were treated in parallel as control, and after counting the grown colonies on the surface of selective medium and confirming that these colonies were *Salmonella*, the rate of transfer of resistance for each plate was calculated.

All data were analyzed by using SPSS version 17.0 computer software (SPSS, Chicago, IL) and p value was calculated using Chi-square and Fisher's exact tests to find any significant relationship. The level of statistical significance was set at $P<0.05$.

3. Results and Discussion

3.1. Determining of Bacterial Resistance against Antibiotics

Table 1. Resistance of *E. coli* against antibiotics

Antibiotic	N	Efficiency (%)
AM (10µg)	5	20
FM (30µg)	9	36
NA (3 µg)	20	80
TE (30µg)	11	44
C (30µg)	8	32
N (30µg)	10	40
TM (30µg)	7	28
NFX (5µg)	3	12
LS (200µg)	3	12
GM (10 µg)	0	0

About 80% strains that are representatives of poultry farms elucidated resistance to antimicrobial agents. A total of 25 *E. coli* strains were analyzed from poultry farms in Chaharmahal Va Bakhtiari province and processed conjugative R-plasmid transfer and antimicrobial resistance pattern. The results of areas of inhibition (mm) were articulated as ratio of isolates resistant, susceptible and intermediate to each antibiotic. The highest rate of resistance was against NA (80%) followed by TE (44%) and N (40%) (Table 1). All the strains were resistant to at least one

antibiotic. Four different patterns of resistance to antibiotics were acquired which triple pattern and more than triple pattern were most frequently encountered. Resistance to NA was maximum among the entire agent used. The most common antimicrobial resistance pattern of these isolates was NA/TE/ N/FM /C (Table 1).

3.2. Conjugation

S. typhimurium was used as a recipient for twenty five *E. coli* resistant to one or more antibiotics. The highest rate of transfer was for N (68%) followed by TM (64%), LS (60%), C (37.5%), AM (25%), TE (20%), FM (14%) and NA & NFX (4%) (Figure 1).

E. coli is a rod-shaped bacteria, gram-negative, non-spore forming and facultatively anaerobic from the family Enterobacteriaceae [9]. Strains of *E. coli* that are inhabiting the intestinal tract of warm-blooded organisms contribute in the supply of vitamin K for their hosts. While many of *E. coli's* are non-pathogenic but some particular species of this bacterium that are pathogenic in humans, are the most common cause of urinary tract infections and lots of digestive diseases [10]. Development of antibiotic resistance in *E. coli* serotypes has been considered as a public health problem throughout the world. This resistance is transferable among *E. coli* serotypes and as well as other species of bacteria [11]. In this study *E. coli* strains showed high percentage of resistance against antibiotics and resistance factor transfer.

Ngeleka et al. (1996) found that *E.coli* was resistant to entrofloxacin and chloramphenicol and recorded a high level of resistance to antimicrobial drugs among pathogenic *E. coli* strains [12]. In a NARMS summary of antibiotic resistance in *E. coli* during 1998 to 2003, different species of bacteria collected showed tetracycline resistance ranged from 36 to 79.9%, streptomycin resistance ranged from 35.4 to 62.2%, and gentamicin resistance ranged from 14.7 to 26.5 [4]. Ahmadi (2004) in Iran, announced that the resistance factor can be transfer among the gram negative bacteria specially in the family of Enterobacteriaceae [13]. In Tricia et al. (2006) investigation, 43% isolates of *E. coli* were resistant to ampicillin but no isolate was found resistant to gentamicin, which is in agreement with this present study [3]. In Apun et al. (2008) investigation in Malaysian, Resistance of *E. coli* isolates of chickens to tetracycline, gentamicin and ampicillin with 11-95% range was reported [14]. Daini and Adesemowo (2008) in Nigeria, announced the resistance of *E. coli* 88% and 54% strains against tetracycline and gentamicin respectively [15]. In Rahman et al. (2008) study in Bangladesh, reported *E. coli* isolates from chickens in Bangladesh were found resistant to chloramphenicol, ampicillin, ciprofloxacin, streptomycin and tetracycline in 37-87.5% cases; and 50-66.6% of isolates were susceptible to gentamicin and chloramphenicol. 66-100% *E. coli* strains from poultry in Bangladesh showed resistance to penicillin, chloramphenicol, erythromycin and tetracycline [16]. Lindsey et al. (2009) showed that R-plasmids are as important agents in emergence of antimicrobial resistance [17]. Obviously in our study and other studies throughout the world *E.coli* strains show high rate multi drug resistance, and as well as the result of this study and other researches indicate that drug resistance factor can be transfer from *E. coli* strains to *Salmonella* strains.

The high prevalence of resistance to antibiotics, among *E. coli* strains in the present study is sizeable difference in antimicrobial resistance pattern. These results are supported by many reports. Many studies have examined antibiotic resistance profiles of *E. coli* strains isolated from humans, different species of animals and foods of animal origin [7]. In many cases that antibiotic compounds are added to chicken feed in sub therapeutic dose for prophylaxis helps to a significantly higher population of resistant bacteria in these animal species by exerting a potent selective pressure for emerging resistant strains which already pre–existed in the bacterial population. Indiscriminate use of antibiotics in humans and animals is cause for great concern. The substantial increase in the prevalence of resistance in *E. coli* strains is due to antimicrobial agents and high rate transfer of resistance factor.

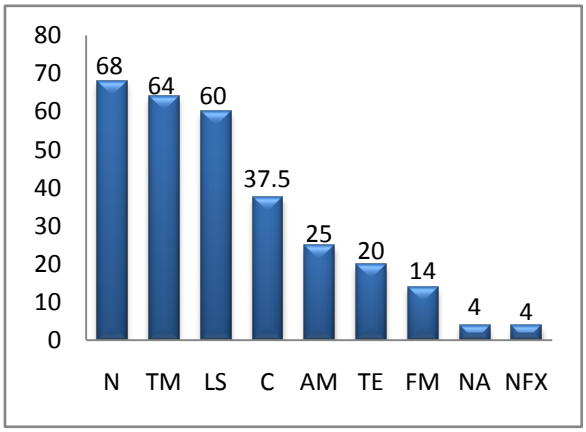


Figure 1. Resistance factor transfer from *E. coli* strain to *S. typhimurium*

4. Conclusions

Our studies shows a high percentage of antibiotic resistance in the *E. coli* which is a result of the indiscriminate use of antibiotics in the poultry industry; therefore we suggest restricting use of antimicrobial drugs in humans and animals, further

studies for detect any changes in resistance pattern, performing antimicrobial susceptibility tests to select suitable antimicrobial agent, application of recommended dosage of antibiotic and following duration of therapy can help to decrease developing of resistant strains.

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REFERENCES

[1] Amin, A., Ayaz Khan, M., Haroon, U., Azam, S. M. F., Andaleeb, S., Ghumro, P. B., 2012, Determination of Antibiotic Resistance Prototype and in vitro R Plasmid Conjugation of *Escherichia coli* Isolates from Broiler Feces., Thai J Vet Med., 42, 87-93.

[2] Wose Kinge, C. N., Ateba, C. N., Kawadza, D. T., 2010, Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, North-West Province, South Africa., S Afr J Sci., 106, 44-49.

[3] Akond, M. A., Alam, S., Hassan, S. M. R., Shirin, M., 2009, Antibiotic Resistance of *Escherichia coli* Isolated from Poultry and Poultry Environment of Bangladesh., Internet J Food Safety., 11, 19-23.

[4] Musgrove, M. T., Jones, D. R., Northcutt, J. K., Cox, N. A., Harrison, M. A., Fedorka-Cray, P. J., Ladely, S. R., 2006, Antimicrobial Resistance in *Salmonella* and *Escherichia coli* Isolated from Commercial Shell Eggs., Poult Sci Association Inc., 85, 1665-1669.

[5] Al-Ghamdi, M. S., El-Morsy, F., Al-Mustafa, Z. H., Al-Ramadhan, M., Hanif, M., 1999, Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia., Trop Med Int Health., 4, 278-283.

[6] Leite, A. A. M., Nardi, R. M. D., Nicoli, J. R., Chartone-Souza, E., Nascimento, A. M. A., 2005, Carica papaya seed macerate as inhibitor of conjugative R plasmid transfer from *Salmonella Typhimurium* to *Escherichia coli* in vitro and in the digestive tract of gnotobiotic mice., J Gen Appl Microbiol., 51, 21-26.

[7] Sayah, R. S., Kaneene, J. B., Johnson, Y., Miller, R. A., 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.

[8] Braoudaki, M., and Hilton, A. C., (2004), Adaptive Resistance to Biocides in *Salmonella enterica* and Escherichia coli O157 and Cross-Resistance to Antimicrobial Agents., J Clin Microbiol., 42, 73-78.

[9] Cabral, J. P. S., 2010, Water Microbiology, Bacterial Pathogens and Water., Int J Environ Res Public Health., 7, 3657-3703.

[10] Ronald, A., 2003, The etiology of urinary tract infection: traditional and emerging pathogens., Dis Mon., 2003, 49, 71-82.

[11] Rafiei Tabatabaei, R., Yazadni Moghaddam S., Pourbakhsh, S. A., 2011, R-Factor Transfer from *Salmonella* Strains to *Escherichia coli*., Middle-East Journal of Scientific Research., 9, 404-409.

[12] Ngeleka, M., Kwaga, J. K., White, D. G., Whittam, T. S., Riddell, C., Goodhope, R., Potter, A. A., Allan, B., 1996, *Escherichia coli* cellulitis in broiler chickens: clonal relationships among strains and analysis of virulence associated factors of isolates from diseased birds., Infect Immun., 64, 3118-3126.

[13] Ahmadi, M., 2005, Study on transfer of drug resistance factors among *Escherichia coli* isolated from chicken farms of Urmia., J Fac Vet Med Univ Tehran., 60, 71-77.

[14] Apun, K., Chong, Y. L., Abdullah, M. T., Micky, V., 2008, Antimicrobial Susceptibilities of *Escherichia coli* Isolates from Food Animals and Wildlife Animals in Sarawak, East Malaysia., Asian Journal of Animal and Veterinary Advances., 3, 409-416.

[15] Daini, O. A., Adesemowo, A., 2008, Antimicrobial Susceptibility Patterns and R-Plasmids of Clinical Strains of *Escherichia Coli*., Australian Journal of Basic and Applied Sciences., 2, 397-400.

[16] Rahman, M., Rahman, B. M., Rahman, B., 2008, Antibiogram and Plasmid Profile Analysis of Isolated *Escherichia coli* from Broiler and Layer., Research Journal of Microbiology., 3, 82-90.

[17] Lindsey, R. L., Fedorka-Cray, P. J, Frye, J. G., Meinersmann, R. J., 2009, Inc A/C Plasmids Are Prevalent in Multidrug-Resistant *Salmonella enterica* Isolates., Appl Environ Microbiol., 75, 1908-1915.