Original Article

Evaluation of antimicrobial resistance patterns of biofilm forming *Escherichia coli* isolated from humans and chickens

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(Received 28 February 2017; Accepted 17 May 2017)

Summary

There is strong evidence that the use of antimicrobials can lead to the appearance and rise of bacterial resistance both in human and animals. *Escherichia coli (E. coli)* isolates from human and chicken samples were examined for their biofilm formation ability and antibiotic resistance patterns in this study. A total of 100 *E. coli* samples, isolated from humans and chickens were examined to determine the biofilm forming properties by tube test, cover slip test and microtitre plate method. After which the prevalence of antimicrobial resistance among the organisms was determined. Among avian isolates, tissue culture plate method, cover slip test and tube assay detected 68%, 54% and 60% antimicrobial resistance, respectively. In human isolates 72%, 56% and 66% antimicrobial resistance were evidence by tissue culture plate method, cover slip test and tube assay, respectively. The resistance pattern of these isolates showed that *E. coli* from chicken samples was resistant to Nalidixic acid (100%), Ciprofloxacin (80%), Doxycycline (80%), Tetracycline (76%), Cefotaxime (30%), Ceftriaxone (30%), Amikacin (28%), Nitrofurantoin (24%), Ceftazidime (22%), Furazolidone (20%), Cefixime (10%), and Gentamicin (0%). *E. coli* from human clinical samples was resistant to Tetracycline (62%), Doxycyclie (58%), Ciprofloxacin (58%), Nalidixic acid (50%), Ceftazidime (40%), Cefotaxime (36%), Ceftriaxon (24%), Cefixime (20%), Amikacin (16%) and gentamicin (8%), Furazolidone (4%) and Nitrofurantoin (0%). Furthermore multi resistant *E. coli* isolates were common in human and chicken samples. However, the percentages of multi resistant *E. coli* were higher in chicken than in human isolates. The results of this study suggested that chickens can act as reservoirs for transfer of antimicrobial resistant bacteria to humans**.** Furthermore, all of the *E. coli* biofilm producers from human and avian origins had multidrug resistance patterns and biofilm formation ability can increase the antibiotic resistance profile of *E. coli* isolates.

Keywords: E. *coli*, biofilm, Antimicrobial resistance.

Introduction

Biofilms are defined as microbial derived sessile communities which attach themselves to solid surfaces or to each other by using their sticky appendages and employing a rolling motion. So they could show continuous attachments to the surface and forming micro aggregation (Taj et al., 2012). Previous investigations have shown that biofilms are resistant to antimicrobial agents (Mohammad and Shalakany, 2015). Bacteria growing in a biofilm are 1000 fold resistant than planktonic cells to antibiotics. Therefore, higher concentrations of antibiotics are needed to inactivate bacteria grown on a biofilm (Hassan et al., 2011).

Antimicrobial agents have a significant role in decreasing the percentage of infectious diseases in both animals and

humans (Tadesse et al., 2012). However the constant use of antimicrobials over a period of time is one of the important reasons leading to prevalence of antibiotic-resistant bacteria (Van den Bogaard et al., 2001). The most significant factor for the development of antibiotic resistance is the exposure of different antibiotics with different concentrations into the microflora of human and animal guts. Over a period of time under the selective pressure, resistant bacteria will have overgrowth (Sayah et al., 2005). In veterinary medicine, antimicrobial agents are commonly used as growth promoter which leads to the high resistance toward antibiotics in normal and pathogenic bacterial flora of poultry (Romanus et al., 2012). Since resistant bacteria from food animals may colonize the humans through the food chain, contact via occupational exposure or waste run off from animal production facilities, it is possible that antibiotic-resistant bacteria could be transferred from food animals to man (Schroeder et al., 2002).

E. coli is known for its ability to cause different types of infections. Among them, gastrointestinal infections leading to the diarrhea are important. Additionally, a variety of diseases outside of the gut of humans and animals such as urinary tract infections, sepsis, meningitis, abdominal infections, osteomyelitis, cellulitis, wound infections and colibacillosis are significant (Kazemnia et al., 2014). *E. coli* colibacillosis usually causes the considerable damages to poultry production all around the world (Rafiei Tabatabaei and Nasirian, 2003). Surveillance data indicated that *E. coli* have strong ability for biofilm formation and emergence of resistance in *E. coli* is one of the highest for antimicrobial agents that have been in use the longest time in human and animals (Tadesse et al., 2012).

In this study, *E. coli* strains isolated from chicken and human clinical samples were analyzed to determine their biofilm producing properties and their susceptibilities to antimicrobial agents.

Materials and Methods

Bacterial strains

A total of 100 strains of *E. coli* from humans and chicken samples; including 50 samples from human urine and 50 samples from chicken muscles, were isolated from September to December 2013 from Urmia, Iran.

Bacteriology

All samples were macroscopically and microscopically examined by gram staining (Abdul Rahaman Shariff et al., 2013). Then cultured on MacConkey and Eosin methylene blue agar plates (Sigma Aldrich, USA) and incubated at 37^{o}C for 24h. The colonies suspected to be *E. coli* were identified by other bacteriological assays (Hammerum and Heuer, 2009).

All human and chicken isolates were frozen in Nutrient broth containing 30% glycerol at -70^{o}C until further processing (Tadesse et al., 2012).

Molecular identification of E. coli

All human and avian *E. coli* isolates were sub cultured overnight in Nutrient broth media (Sigma Aldrich, USA) and genomic DNA was extracted using bacterial genomic DNA purification kit (Intron, Korea). Then the presence of *E. coli* 16s rRNA gene was determined by polymerase chain reaction (PCR) method using forward primer:

5'-GTATAGATACCCTGGTAGTCCA-3' and reverse primer:
5'-CCCGGGAACGTATTCACCG-3' (Sharma et al., 2013). The PCR assay was done in a total volume of 25 µl using Intron premix (Korea). The PCR was performed in a DNA thermo cycler (MWG AG Biotech Thermal Cycler, USA) and the PCR conditions were as follow: an initial template denaturation at 95°C for 3 mins, 26 cycles followed with DNA denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 10 mins (Sharma et al., 2013). The PCR products were separated electrophoretically in 1% agarose gel.

Detection of biofilm formation

Biofilm formation by *E. coli* isolates was assayed by microtitre plate, tube and cover slip assays. In microtitre plate assay 1×10^7 cfu of *E. coli* in 100 microliter LB Broth medium was inoculated into the wells of 96 well flat bottom polystyrene plates (Sigma Aldrich, USA). After growing bacteria at 37°C for 48h, the planktonic cells in media were discarded. The plate was washed and the attached biofilms were stained with 0.1% crystal violet (20 min). After rinsing the plates with distilled water, all stains associated with the attached biofilms were dissolved with 95% ethanol and OD595 absorbance was measured (Nakao et al., 2009). An optical density of 0.240 was chosen to distinguish biofilm producers from non-biofilm formers (Van den Bogaard et al., 2001).

In the tube method, a loop full of bacteria was cultured in 10 ml trypticase soy broth with 1% glucose. After incubation at 37°C for 24h, tubes were decanted and washed with phosphate buffer saline. Then tubes were stained with 0.1% crystal violet stain. Each tube was then gently rotated to ensure uniform staining and then the contents were gently decanted. The tubes were placed upside down to dry. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube (Hassan et al., 2011).

In coverslip method, sterile culture flasks were filled with 50 ml Brain heart infusion broth media and 18mm glass microscope cover slip added to it. Then defined volume of overnight culture of bacteria was inoculated into the flasks. After incubating the flasks at 37°C for 48h, glass cover slip containing biofilm was removed and rinsed with phosphate buffer saline then stained with 0.5% crystal violet stain for 5min. Stained biofilms were then microscopically screened (Sharma et al., 2013).

Antimicrobial drug susceptibility testing

While a single *E. coli* isolated and identified from each collected sample, the antimicrobial susceptibility tests were done on Muller-Hinton agar plates using Kirby Bauer disk diffusion method. A 150mm Muller- Hinton medium plate was swabbed with Nutrient broth inoculated with *E.* coli, and incubated to a turbidity of 0.5 McFarland standard medium. Twelve prepared antimicrobial agent disks were place on the inoculated plate. Then these plates were incubated at 35°C for 18 to 20h. The diameter zone of growth inhibition around each disk was measured (Sayah et al., 2005). Selected disks for this study were: Tetracycline(30 µg), Ceftriaxone(30 µg), Doxycycline(30 µg), Amikacin(30µg), Cefixime(5 mcg), Gentamicin(10µg), Furazolidone(100 mcg), Amikacin(30µg), Ceftazidime(30-µg), Cefotaxime(30 µg), Nalidixic

acid(30µg), Nitrofurantoin(300 mcg), Ciprofloxacin(5µg) (Padtan Teb, Iran). The diameters of the zones of inhibition were interpreted by referring to the Performance Standard for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement – January 2013.

Results

Bacteria detection

All 100 isolated bacteria from human and avian samples had the cultural, morphological and biochemical characteristics of *E. coli* (Fig. 1). Moreover, molecular identification of *E. coli 16s rRNA* gene using PCR method confirmed the *E. coli* isolates. Fig. 2 shows the product of 16s rRNA gene found at 612bp using 100bp DNA marker.

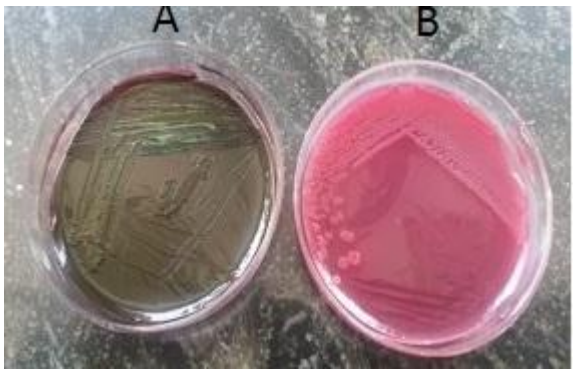

Fig. 1. Cultural characteristics of *E. coli*. A) ***E. coli*** colonies have a characteristic green sheen on Eosin methylene blue agar plate. B) ***E. coli*** appeared as pink colonies on MacConkey Agar plate.

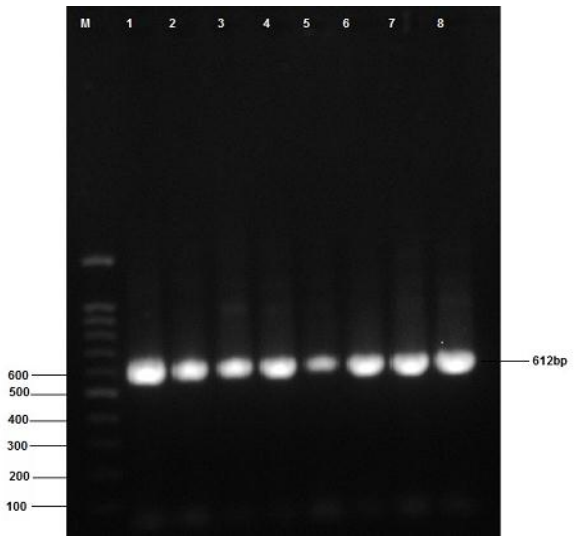

Fig. 2. Gel electrophoresis of PCR products of *16s rRNA* gene. Lane M- 100bp ladder marker, Lane 1; positive control *(E. coli* 25922), Lanes 2-5; human *E. coli 16s rRNA* gene found at 612bp, Lanes 6-8; human *E. coli 16s rRNA* gene found at 612bp.

Biofilm formation

Among 50 human *E. coli* isolates, microtitre plate method detected 72% as biofilm producers, which followed by tube test (66%) and cover slip assay (56%). Thirty two percent of human *E. coli* strains were confirmed to have biofilm forming ability by all three methods. Thirty four percent were positive biofilm producers by two assays. Thirty two percent were detected as biofilm producers by only one method. Two percent of isolates did not have any ability in biofilm formation.

Among 50 avian *E. coli* isolates, microtitre plate method detected 38% biofilm producers which followed by tube test (60%) and cover slip assay (54%). Eighteen percent of human *E. coli* strains were confirmed to have biofilm forming ability by all three methods. Fifty four percent were positive biofilm producer by two assays and 28% were detected as biofilm producers by only one method.

Two percent of isolates did not have any ability in biofilm formation (Fig. 3, 4, 5).

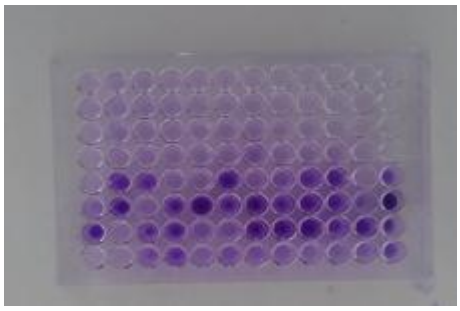

Fig. 3. Microtitre assay plate for *E. coli* biofilm.

Fig. 4. Biofilm formation of *E. coli* by tube assay.

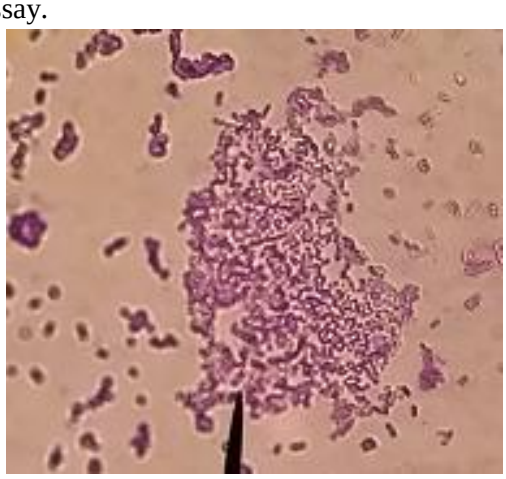

Fig. 5. Microscopic view of biofilm formation of *E. coli* by cover slip assay.

Antibiotic susceptibility results.

Fifty samples from chickens and fifty samples from humans *E. coli* isolates were compared for antimicrobial resistance prevalence (Table 1). Table 1 shows the percentage of susceptible, intermediate and resistant isolates to each antimicrobial agent. The most frequent antimicrobial resistance among avian isolates was with Nalidixic acid (100%), Ciprofloxacin (80%), Doxycycline (80%) and Tetracycline (76%).

Avian *E. coli* expressed resistance to Nitrofuran derivatives, Nitrofurantoin and Furazolidone at frequencies to 24% and 20%, respectively. Amikacin resistance occurred at a frequency of 28%. The resistance for Cephalosporins, Cefotaxime, Ceftazidime, Cefixime and Ceftriaxon among the isolates was 30%, 22%, 10% and 30%, respectively. No Gentamicin resistant *E. coli* was isolated from avian samples (Table 1). However, the majority of human *E. coli* isolates were resistant to Tetracycline (62%), Ciprofloxacin (58%), Doxycycline (58%) and Nalidixic acid (50%). The next most frequent resistance phenotypes were resistance to Cefotaxime (36%) and Ceftazidime (40%). Twenty four and 20 percent of human *E. coli* isolates were resistant to Ceftriaxon and Cefixime, respectively.

Resistance to aminoglycosides; Amikacin and Gentamicin was seen in 16% and 8% *E. coli* samples, respectively. No Nitrofurantoin resistant *E. coli* was isolated from human samples (Table 1).

Table 2 shows the prevalence of multiple resistance patterns in avian and human *E.coli* isolates. The most frequently observed resistance pattern in avian isolates was resistance to Nalidixic acid in combination with Ciprofloxacin, Tetracycline and Doxycycline (22%).

Table 1. The percentages of susceptible (S), intermediate (I) and resistant (R) *E. coli* isolates from human and avian sources to individual antibiotics.

Antimicrobial agent	Avian isolates (n=50)			Human isolates (n=50)		
	S	I	R	S	I	R
Cefotaxime	60	10	30	20	44	36
Gentamicin	96	4	-	72	20	8
Nalidixic acid	-	-	100	48	2	50
Ciprofloxacin	4	16	80	34	8	58
Ceftazidime	72	6	22	20	40	40
Furazolidone	70	10	20	94	2	4
Amikacin	12	60	28	30	54	16
Nitrofurantoin	26	50	24	96	4	-
Ceftriaxone	68	2	30	64	12	24
Doxycycline	20	-	80	32	10	58
Tetracycline	24	-	76	32	6	62
Cefixime	84	6	10	32	48	20

The next most frequent resistant phenotypes were resistance to Nalidixic acid, Ciprofloxacin and Tetracycline (18%), and Nalidixic acid, Ciprofloxacin, Ceftazidime, Cefotaxime, Cefixime, Cefriaxon and Tetracycline (8%). Six percent of avian *E. coli* isolates exhibited resistance to Nalidixic acid, Ciprofloxacin, Amikacin, Tetracycline and Doxycycline. Moreover, 6% of avian *E. coli* isolates was resistant to Nalidixic acid, Ciprofloxacin, Nitrofurantoin, Tetracycline and Doxycycline. Six percent of avian *E. coli* isolates exhibited resistance to Nalidixic acid, Ciprofloxacin, Amikacin and Tetracycline while 4% of them exhibited resistance to Nalidixic acid, Ciprofloxacin, Amikacin and Tetracycline, Doxycycline and Nitrofurantoin.

Resistance to Nalidixic acid, Ceftazidime, Cefotaxime and Ceftriaxon were exhibited in 4% avian *E. coli* isolates. The remaining 26% of avian *E. coli* isolates exhibited a single unique phenotypic pattern (Table 2). Among human *E. coli* isolates the most multiple resistance patterns were for Doxycycline and Tetracycline (10%).

The next resistant phenotype was Doxycycline, Tetracycline and Nalidixic acid (8%). Six percent of human *E. coli* isolates were resistance to Cefotaxime, Doxycycline, Tetracycline, Ciprofloxacin, Ceftazidime and Nalidixic acid. Resistance to Cefotaxime, Doxycycline, Tetracycline and Ceftazidime was seen in 4% of *E. coli* samples. In addition, 4% of isolates showed resistance to Cefotaxime, Doxycycline, Tetracycline, Ciprofloxacin and Ceftazidime. Fourteen percent of human *E. coli* samples exhibited a single unique phenotype pattern. The remaining isolates showed resistance to only one antibiotic (Table 2).

Since the biofilm forming isolates from both human and avian sources showed multidrug resistance, their antibiotic resistance patterns was identified. A total of 37.5% avian biofilm forming *E. coli* showed resistance to 6 antibiotics. Among them, 31.25%, 80.75% and 6.25% had resistance to 5, 3 and 1 antibiotics, respectively.

Among human biofilm formers 55% of biofilm producers showed resistance to four antibiotics, while 22% of them revealed resistance to two drugs. The same amount (22%) was evident for five antibiotics.

Table 2. Prevalence of multiple resistant patterns in avian and Human *E. coli* isolates

Antimicrobial resistance pattern Number		Antimicrobial resistance pattern	Antimicrobial resistance (%)
Avian isolates	1	NA, CP, TE, D	22
	2	NA, CP, TE	18
	3	NA , CP, CAZ, CFM, CTX, CRO, TE	8
	4	NA, CP, AN, TE, D	6
	5	NA, CP, AN, FM, TE,D	6
	6	NA, CP, AN, TE	6
Human isolates	1	TE, D	10
	2	NA, D, TE	8
	3	CTX, D, TE, CP,CAZ, NA	6
	4	CTX, D, TE, CAZ	4
	5	CTX, D, TE, CP, CAZ	4

Key: Doxycycline=D, Amikacin=AN, Cefixime=CFM, Gentamicin= GM, Furazolidone=FR, Ceftazidime=CAZ, Cefotaxime=CTX, Nalidixic acid=NA, Nitrofurantoin=FM, Ciprofloxacin=CP, Tetracycline=TE.

Discussion

Biofilms are the community of bacteria attached to the solid surfaces by cell surface components such as flagella, type 1 fimbriae and outer membrane proteins (Wood, 2009). It demonstrated that formation of bacterial biofilms is a process that contributes to microorganism's pathogenicity (Rinser et al., 2006).

Antimicrobial resistance is one of the important problems in both human and veterinary medicine and antibiotic administration is recognized as the most significant factor for the prevalence of antimicrobial agent resistant bacteria (Sayah et al., 2005). The microbial ecosystems of humans and poultries are connected to each other, so that it is not easy to determine the antimicrobial resistance (Schroeder et al., 2002). Emergence of antimicrobial resistance may decrease the time that these drugs will be useful for effective treatment of infections (Hammerum et al., 2009).

E. coli was chosen for this study because it's a ubiquitous bacterium both in the nature and in the intestine of both animal and human. Although, resistant strains from the intestine may contaminate chicken carcasses at slaughter causing multi resistant *E. coli* emergence in poultry meats. The mechanism of the spread of antibiotic resistance from food animal to humans is not clear however, the colonization of human gut with resistant *E. coli* from chicken has been shown and it seems that animals act as a reservoir for *E. coli* found in humans (Nakao et al., 2012).

In 2000, Edland and Nord examined the effect of oral antibiotics for treatment of urinary infections on the normal human microflora and observed the overgrowth of resistant Enterobacteriacea (Wood, 2009).

Sayah et al. (2005) identified antimicrobial resistance patterns in *E. coli* isolated from domestic and wild animal fecal samples. Multidrug resistance was seen in a variety of sources (Sayah et al., 2005).

In a study conducted by Raum et al. (2007), the changes in *E. coli* resistance patterns during and after antibiotic therapy were investigated concluding that there was a significant increase in the prevalence of *E. coli* isolates during antibiotic treatment.

In addition, Momtaz et al. (2012) studied the distribution of antibiotic resistant genes in *E. coli* isolated from slaughtered commercial chickens by PCR. All isolates showed one or more antibiotic resistance genes (Miles et al., 2006). Looking to the association between biofilm production and antimicrobial resistance, Sharma et al. (2013) indicated that resistant strains of *E. coli* isolated from sewage water had strong biofilms forming ability which is in accordance to the finding of this study.

Evaluating different biofilm detection methods, Hassan et al. (2011) concluded that microtitre plate method is reliable than the tube test for detection of biofilm forming microorganisms. Their finding is in accordance of the results of this study indicating that microtitre plate method is the superior one.

Mohamad and Shalakany (2015) also found that antibiotic resistance was higher among biofilm producers to antimicrobial agents in comparison with non-biofilm producers. They also have reported that microplate method was reliable than the tube test in biofilm producers detection, which is again in accordance to the present findings.

In another study, the biofilm production of *Staphylococcus aureus* was comparatively evaluated by three conventional methods (Taj et al., 2012). According to their findings, the tube test was more sensitive than coverslip assay in screening of biofilm producers which was similar to the present study. To unveil the evolution of drug resistance in *E. coli*, the existing strain collections of these bacteria for their resistance to a common panel of 12 antibiotics were tested in the present study. After testing 100 *E. coli* isolates from human and chicken, the results suggested that the resistant strains of *E. coli* were common among chickens and human samples. These results are consistent with the findings of previous studies. Multi-drug resistance was observed in *E. coli* from both animal and human sources however, it was higher in frequency and proportion in *E. coli* isolated form chicken samples. This is consistent with the reports of other studies performed elsewhere (Kibert et al., 2011). In conclusion, antimicrobial resistance is more common in biofilm forming *E. coli* and chicken can be a source of transferring antimicrobial resistant bacteria to human.

Ethics

It is declared that all ethical standards have been respected in preparation of this article.

Acknowlegement

This work was financially supported by the University of Tabriz.

References

Abdul Rahaman Shariff AR., Shenoy S., Taruna Y. and Radhakrishna M. (2013). The

Antibiotic susceptibility patterns of uropathogenic *Escherichia coli*, with special reference to the fluoroquinolones. *Journal of Clinical*

*and Diagnostic Res*earch, 7(6), pp. 1027-1030.

Edlund C. and Nord CE. (2000). Effect on the human normal micro flora of oral antibiotics for treatment of urinary tract infections. *Journal of Antimicrobial chemotherapy*, 46, pp. 41-48.

Hammerum AM. and Heuer OE. (2009). Human Health Hazards from Antimicrobial- Resistant *Escherichia coli* of animal origin. *Clinical infectious Dis*eases, 48, pp. 916-921.

Hassan A., Usman J., Kaleem F., Omair M., Khalid A. and Iqbal M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *Brazilian Journal of Infectious Diseases*, 15(4), pp.305-3011.

Kazemnia A., Ahmadi M. and Dilmaghani M. (2014). Antibiotic Resistance pattern of different *Escherichia coli* phylogenetic groups isolated from Human Urinary Tract infection and Avian Colibacillosis. *Iranian Biomedical Journal*, 18(4), pp. 219-224.

Kibert M. and Abera B. (2011). Antimicrobial susceptibility of *E. coli* from clinical sources in northeast Ethiopia. *African Health Science*, 11(1), pp. 40-44.

Mohamad EA. and Shalakany H. (2015) Detection of biofilm formation in uropathogenic bacteria, *Egyptian Journal of Medical Microbiology*, 24(1), pp. 49-58.

Miles TD., McLaughlin W. and Brown PD. (2006). Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *MBC Veterinary Research*, 2, pp. 7.

Momtaz H., Rahimi E. and Moshkelani. (2012). Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinary Medicina-CZECH*, 57(4), pp. 193-197.

Nakao R., Ramstedt M., Wai SN. and Uhlin BE.(2012). Enhanced biofilm formation by *Escherichia coli* LPS Mutans Defective in help biosynthesis, *PLOS ONE*, 7(12), pp. 1-12.

Romanus I., Chinyere OE., Amobi NE., Antonia OE., Ngozi AF., Chidiebube A. and Eze AT. (2012). Antimicrobial resistance of *Escherichia coli* isolated from animal and human clinical sample. *Global Research Journal of Microbiology*, 2(1), pp. 85-89.

Rafiei Tabatabaei R. and Nasirian A. (2003). Isolation, Identification and Antimicrobial resistance patterns of *E. coli* isolated from chicken flocks. *Iranian Journal of Pharmacology & Therapeutics*, 2, pp. 39-42.

Raum E., Lietzau S., Von Baum H., Marre R. and Brenner H. (2008). Changes in *Escherichia coli* resistance patterns during and after antibiotic therapy: a longitudinal study among outpatients in Germany. *Clinical Microbiology and* In*fection*, 14(1), pp. 41-48.

Rinser A., Krogfelt KA ., Klein BM., Zechner EL. and Molin SW. (2006). In vitro biofilm formation of Commensal and pathogenic *Escherichia coli* strains: Impact of Environmental and genetic factors, *Journal of Bacteriology*, 188(10), pp. 3572-3581.

Sayah S., Kaneene JB., Johnson Y. and Miller Rose A. (2005). Patterns of Antimicrobial Resistance observed in *Escherichia coli* isolates obtained from

Domestic and wild animal fecal samples, human septage and surface water. *Applied and Environmental Microbiol*ogy, 71(3), pp.1394-1404.

Schroeder CM., Zhao C., Debroy CH., TOrcolini J., Zhao SH., White DG., Wagner DD., Mcdermott PF., Walker RD. and Meng J. (2002). Antimicrobial Resistance of *Escherichia coli O157* Isolated from Humans, Cattle, Swine and Food. *Applied and Environmental Microbiology,* 68(2), pp. 576- 581.

Sharma S., Mahajan B., Patidar RK ., Sahare KN. and Khare M. (2013). Molecular Detection of antimicrobial resistance genes in biofilm forming *Escherichia Coli. American* J*ournal of Pharmacy and Health Research,* 1, pp.2321-3647.

Tadesse D., Zhao SH., Tong E., Ayers SH., Singh A., Bartholomew MJ. and McDermott PF. (2012). Antimicrobial drug resistance in Escherichia coli from Humans and food animals, united states, 1950-2002. *Emerging Infectious Diseases Journal,* 18(5), pp. 741- 749.

Taj Y., Essa F., Aziz F. and Kazmi SH. (2012). Study on biofilm forming properties of clinical isolates of *Staphylococcus aureus, The Journal of Infection in Developing Countries,* 5(6), pp. 403-409.

Van den Bogaard AE., London N., Driessen C. and Stobbering H. (2001). Antibiotic Resistance of fecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial chemotherapy,* 47, pp. 763-771.

Wood TH. (2009). Insights on *Escherichia coli* biofilm formation and inhibition from whole transcriptome profiling, *Environmental Microbiology,* 11(1), pp. 1-15.