ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken

Ismail Abd- El-hafeez Radwan [1], Hala Sayed Hassan Salam [1, *], Soad Abd-El Aziz Abd-Alwanis [2], Marwa Ahmed Yahia Al-Sayed [3]

1. Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62551, Egypt
2. Animal Health Research Institute Dokki, Egypt
3. Animal Health Research Institute Beni-Suef, Egypt

Manuscript Info

Manuscript History:

Received: 15 October 2014
Final Accepted: 22 November 2014
Published Online: December 2014

Key words: Antimicrobial**,** Broiler, Egypt, Escherichia coli, Serogroup,Virulence.

Corresponding Author

Hala Sayed Hassan Salam

Abstract

Avian pathogenic *E. coli* (APEC), the causal organism of infection in chickens, is responsible for large economic losses in the poultry industry in Egypt and worldwide. In this study 83 *E. coli* isolates were recovered from 200 broiler chicken suffering from colibacillosis. The disc diffusion method was used to determine antibiotic susceptibility of the isolates to 10 antibiotics comprising 6 different antimicrobial classes. Many isolates 75 (90.4%) exhibited resistance to 3 or more of the tested compounds and were defined as multiple drug resistant (MDR). Antibiogram profiles indicated maximum resistance to ampicillin (100%), high frequency of resistance to amoxicillin (97.6%), sulfamethoxazole/trimthoprim (94%), streptomycin (92.8%) and ciprofloxacin (89.2%). Conversely, the aminoglycoside amikacin was still effective against 97.6% of the isolates. Some MDR isolates (n = 40) were selected for serogrouping and 9 different serogroups were detected among them. The previously selected isolates (n = 40) were screened for presence of some virulence associated genes (*iutA*, *hlyF*, *iss*, *iroN* and *ompT*) using a pentaplex PCR. There was a high prevalence for such genes among the tested isolates except for *iutA* gene, which was harbored by only 5% of the tested isolates.

Introduction

Escherichia (E.) coli typically colonize the gastrointestinal tract of warm-blooded animals within a few hours after birth. However, a large number of highly adapted *E. coli* pathovars have acquired specific virulence attributes (kaper et al., 2004). Some pathotypes of *E. coli* are capable of causing intestinal diseases, while others referred to as extraintestinal pathogenic *E. coli* (ExPEC), are responsible for extraintestinal infections. Avian pathogenic *E. coli* (APEC), fall under the category of ExPEC (Mellata, 2013) that induces different syndromes in poultry including, systemic and localized infections such as respiratory colibacillosis, acute colisepticemia, salpingitis, yolk sac infection, and swollen-head syndrome (Dho-Moulin and Fairbrother, 1999).

Colibacillosis is a widespread disease, which is responsible for severe economic losses for the world's poultry industries. The most common form of colibacillosis is characterized by an initial respiratory disease, which is usually followed by a systemic infection with characteristic fibrinous lesions (airsacculitis, perihepatitis and pericarditis) and fatal septicemia. The infection is generally initiated or enhanced by predisposing agents, such as mycoplasmal, viral infections and environmental factors (Dho-Moulin and Fairbrother, 1999; Barnes et al., 2008).

The long-term use of antimicrobials for therapy and growth promotion in poultry resulted in drug resistance in Gram-negative pathogens (Singer and Hofacre, 2006).

As *E. coli* acquires resistance easily and found more frequently in a wide range of hosts, it usually used as sentinel for monitoring antimicrobial drug resistance in fecal bacteria (Erb et al., 2007).
Usually, commensal *E. coli* isolates harbor no or only very few virulence factors (VFs), while ExPEC isolates have specialized VFs enabling them to colonize host surfaces, injure host tissues, and avoid or subvert host defense systems (Johnson and Stell, 2000).
APEC strains are very diverse, which is related to the diversity of their virulence factors and serotypes (Mellata et al., 2009). The virulence mechanisms of APEC have been continually studied and are believed to be multifactorial.
Little literature is available on APEC isolated from Egypt, therefore, the current work was carried out to determine some phenotypic virulence properties and antimicrobial susceptibility of *E. coli* isolated from colibacillosis in broilers. Serogrouping and detection of some virulence associated genes in randomly selected multiple drug resistant (MDR) isolates using a previously designed multiplex PCR (Johnson et al., 2008).

Material and methods

Samples

The samples (n = 200) were obtained from chickens of different ages (3-5weeks), suspected to harbor *E. coli* including liver, heart, air sac and kidney which transferred immediately on ice packs to the research laboratory.

Isolation and identification of E. coli

It was carried out as outlined by Collee et al. (1996).

Phenotypic detection of virulence factors

Congo red binding activity

This assay was carried out according to Berkhoff and Vinal (1986) to test the isolated *E. coli* strains for its binding ability with Congo red dye. Congo red-positive (CR+) *E. coli* were indicated by growth of red colonies, while Congo red-negative *E. coli* (CR-) did not bind the dye and appeared as white colonies.

Hemagglutination test

The presence of fimbriae was detected by the ability of strains to agglutinate chicken erythrocytes in presence or absence of of 2% D-Mannose (Evans et al., 1977).

Haemolytic reaction

All the isolates were assessed for their haemolytic activity on 5% sheep blood agar plates using standard methods (Forbes et al., 1998).

Antimicrobial susceptibility

Antimicrobial susceptibility testing using the Kirby-Bauer disk difusion method following the Clinical and Laboratory Standards Institute, CLSI (CLSI, 2010) guidelines was performed. All isolates were tested for 10 different antimicrobial agents (Oxoid, Basingstoke, UK): amoxicillin (30 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (20+10 µg), ceftriaxone (30 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), sulfamethoxazole/ trimethoprim (1.25 + 23.75 µg), doxycycline (30 µg) and streptomycin (10 µg).
The phenomenon of multiple drug resistance was defined as resistance to three or more antimicrobials of different tested classes, with trimethoprim plus sulfamethoxazole considered as one unit since the testing was in combination.

Serogrouping of some randomly selected multidrug-resistant isolates

Serological identification of selected *E. coli* isolates (n = 40) was carried out according to Ewing (1986).

DNA extraction

DNA was extracted by using bacterial DNA extraction kits (Qiagen) according to the manufacturer instructions.

Screening of APEC virulence genes by multiplex PCR

The previously selected isolates for serogrouping were examined for the presence of five ColV-associated genes (*iutA*, *hlyF*, *iss*, *iroN*, and *ompT*) known for their association with APEC virulence. The pentaplex PCR was carried out as previously described (Johnson et al., 2008). Targeted genes and their primer sequences are listed in Table 1.

Table 1. Primer sequences and amplified products for the targeted virulence genes

Target gene		Primer sequence	Amplicon size
iroN	F	5- AATCCGGCAAAGAGACGAACCGCCT -3	553 bp
	R	5- GTTCGGGCAACCCCTGCTTTGACTTT -3	
ompT	F	5- TCATCCCGGAAGCCTCCCTCACTACTAT -3	496 bp
	R	5- TAGCGTTTGCTGCACTGGCTTCTGATAC -3	
hlyF	F	5- GGCCACAGTCGTTTAGGGTGCTTACC -3	450 bp
	R	5- GGCGGTTTAGGCATTCCGATACTCAG -3	
iss	F	5- CAGCAACCCGAACCACTTGATG -3	323 bp
	R	5- AGCATTGCCAGAGCGGCAGAA -3	
iutA	F	5- GGCTGGACATCATGGGAACTGG -3	302 bp
	R	5- CGTCGGGAACGGGTAGAATCG -3	

Results

Isolation and biochemical identification of *E. coli*

Out of 200 diseased chickens clinically diagnosed with colibacillosis, 83 isolates were biochemically identified as *E. coli* with a prevalence of 41.5%.

Phenotypic detection of virulence factors

Congo red binding activity

All the studied isolates (n = 83) were able to bind congo red dye and were regarded as CR+.

Haemolytic activity

Haemolysis of sheep blood agar was detected by 34 (41%) out of 83 isolates.

Detection of haemagglutination and mannose resistance haemagglutination using chicken RBCs

All isolated *E. coli* (n = 83) were haemagglutinating (100%) for chicken RBCs. Out of them, 64 (77%) isolates were mannose resistant while the remaining 19 isolates (22.9%) were mannose sensitive.

Antimicrobial susceptibility testing

There were no isolates that exhibited resistance to all the tested antimicrobials concurrently whereas out of 83 *E. coli*, 75 (90.4%) isolates were resistant to three or more antimicrobials, which were defined as MDR isolates. All the tested isolates were resistant to ampicillin with a frequency of 100% followed by amoxicillin, sulfamethoxazole/trimthoprim, streptomycin, ciprofloxacin and docycycline in a frequency of 97.6%, 94%, 92.8%, 89.2% and 51.8% respectively while moderate resistance was detected against gentamicin, amoxicillin-clavulanic acid and ceftriaxone in a frequency of 36.1 %, 30.1% and 21.7% respectively.

Conversely, the aminoglycoside amikacin was still effective against 97.6% of the tested isolates. The detailed antimicrobial susceptibility results are shown in Table 2.

Serogrouping of the selected isolates

Nine serogroups were detected amongst the examined isolates (n = 40), O115 (n = 6), O125 (n = 6), O158 (n = 5), O20 (n = 5), O1 (n = 2), O78 (n = 2), O6 (n = 2), O157 (n = 1), O148 (n = 1) while 10 isolates (25%) could not be serogrouped by available antisera used in this study.

Prevalence of some virulence genes among APEC

The targeted genes in the current study were all detected in 32 (80%) out of the investigated isolates (n = 40) but with different distribution whereas 8 isolates were negative for all. The highest prevalence was for *iroN, ompT, hylF* genes that were equally detected in 32 (80%) out of 40 isolates followed by *iss* gene, which was harbored by 30 (75%) isolates. In contrast, the *iutA* gene was only detected in 2 (5%) isolates.

There was a higher prevalence of virulence associated genes among the typable as compared to the untypable isolates, 29 out of all the typable isolates (n = 30) regardless for their serogroup were carriers for 4 genes (*iroN*,

ompT, *hylF* and *iss*) except one isolate serogrouped as O148 was negative for them with a prevalence of 96.7% while the *iutA* gene was not detected in any of the typable isolates with prevalence of 0%.
Regarding the untypable isolates (n = 10), the prevalence of *iroN*, *ompT*, *hylF* genes were 40% whiles the prevalence of *iss* and *iutA* genes were 20%.
In general, the most prevalent co existence for the tested genes was for *iroN*, *ompT*, *hylF* and *iss*, which were co detected in 30 (75%) out of 40 isolates.

Table 2. Antimicrobial susceptibility of *E. coli* isolated from diseased chicken.

Antimicrobial and class	Investigated isolates n = 83					
	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Cephalosporins						
Ceftriaxone	18	21.7	11	13.3	54	65
Penicillins						
Ampicillin	83	100	0	0	0	0
Amoxicillin	81	97.6	2	2.4	0	0
Amoxicillin-clavulanic acid	25	30.1	10	12	48	57.9
Aminoglycosides						
Amikacin	2	2.4	0	0	81	97.6
Gentamicin	30	36.1	3	3.6	50	60.3
Streptomycin	77	92.8	0	0	6	7.2
potentiated sulfonamides						
Sulfamethoxazole trimthoprim	78	94	3	3.6	2	2.4
Fluoroquinolones						
Ciprofloxacin	74	89.2	3	3.6	6	7.2
Tetracycline						
Docycycline	43	51.8	33	39.8	7	8.4

Discussion

In the current study, all the isolated *E. coli* (n = 83) were tested for some phenotypic virulence traits as *E. coli* strains that cause infections usually possess one or more virulence properties. Out of them was the congo red binding activity, all the tested isolates (100%) were CR+, This high percentage is due to isolation of *E. coli* from diseased chickens, which go parallel to some studies proved a direct correlation between the Congo red binding activity of isolated *E. coli* strains and their ability to cause septicaemic infection in chickens (Berkhoff and Vinal, 1986, Corbett et al., 1987).
Among APEC strains, Type 1 fimbriae are related to the adhesion to the avian upper respiratory tract. The adhesive properties of Type 1 fimbriae are inhibited by specific antiserum and by D-mannose, a carbohydrate that is its cellular receptor on the eurocariotic cell membrane (Gyimah and Panigraphy, 1988). These characteristics are used for its characterization Such as in haemagglutinating pattern with and without D-mannose. Our isolates were all haemagglutinating for chicken RBCs (100%). Out of them 64 (77%) isolates contained mannose resistant haemagglutinin.
Haemolysis of sheep blood agar was exerted by 34 (41%) out of 83 isolates, which is a rare virulence phenotype possessed by some APEC attributed to presence of a haemolysin gene (*hlyE*), which was first identified by Reingold et al. (1999). Haemolysis of erythrocytes is considered as an important virulence factor for some strains of *E. coli* to overcome host defense mechanism through haemolysis production, which leads to the release of iron into the bacterial environment and cytotoxic effect on neutrophils (Cavalieri et al., 1984).
Our evaluation of the multi-antimicrobial resistance patterns of the studied isolates against 10 compounds out of 6 antimicrobial classes revealed that no isolate was resistant to all of the compounds while 90.4% of our isolates

exhibited multi drug resistance with high level of resistance against penicillin, amoxicillin, sulfamethoxazole-trimthoprim, streptomycin, ciprofloxacin and docycycline while moderate levels of resistance was detected against gentamicin, amoxicillin-clavulanic acid and ceftriaxone, although ceftriaxone is not used in poultry production in Egypt.

Sulfonamides, penicillins, tetracyclines and streptomycin are the oldest drugs used in infectious disease (FAO, 2007) and it is not surprising that high level of resistance would have emerged over time.

Generally, antimicrobial agents are used extensively in poultry production and are usually administered in the feed or drinking water. The spread of multidrug resistance among avian *E. coli* is usually endorsed to the selective pressure exerted by the antimicrobials included in broiler feed for the past years (Singer and Hofacre, 2006).

The current resistance phenotypes with MDR are similar to data reported from other regional study (Ahmed et al., 2013) as well as global studies (Zaho et al., 2005; Jiang et al., 2011; Saidi et al., 2013).

Predominant serogroups all over the world of APEC are O1, O2, and O78, but they account for only 15 to 60% of isolates depending on the study (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2004; Rodriguez-Siek et al., 2005). In the current work only 10% of the serogrouped isolates were O1 and O78 while 65% were assigned to other infrequently occurring serogroups (O115, O125, O158, O20, O6, O157, O148); these serogroups have been associated with *E. coli* pathotypes other than ExPEC, including intestinal diseases in mammals including humans (Wolf, 1997; WHO, 1998; Bai et al., 2008). Moreover, one of such APEC strains among this collection was identified as serogroup O157, the serogroup associated with outbreaks and sporadic cases of human haemolytic uremic syndrome and haemorrhagic colitis (Gould et al., 2009). Some of such serogroups have been previously isolated from chickens (Heuvelink et al., 1999; Rezk et al., 2010; Kalin et al., 2012 and Hussein et al., 2013), suggesting that poultry could be considered as a reservoir of *E. coli* having zoonotic potential. Some of the studied isolates (25%) could not be serogrouped by available antisera used in this study. High percentage of untypable isolates in APEC was previously recoreded by numerous studies regardless of their geographic location (Zhao et al., 2005; Ewers et al., 2009; Hussein et al., 2013). This highlights the need for molecular characterization of *E. coli* of chicken origin for their virulence associated factors as several authors propose serotying fails to distinguish APEC and avian faecal *E. coli* (Ewers et al., 2009).

The mechanisms of APEC pathogenesis remain remarkably ambiguous, however, a number of virulence factors have been implicated in these extraintestinal diseases in avian species, including adhesins and genes related to bacterial adhesion, iron acquisition systems, toxins, haemolysins and invasion genes (Ewers et al., 2004; Mokady et al., 2005; Ewers et al., 2009).

Johnson et al. (2008) designated and validated a pentaplex PCR panel targeting for five virulence genes were identified as being the most significantly associated with highly pathogenic APEC isolates.

These genes (*iutA, hlyF, iss, iroN* and *ompT*) are carried by plasmids: The outer membrane siderophore receptor gene *iroN* ,which facilitates iron chelation in the host, aids in the resistance to host serum and is associated with APEC complement resistance (Pfaff-McDonough et al., 2000; Dozois et al., 2003), A new class of avian haemolysin gene, *hlyF* was identified in *E. coli* strains from broilers and supposedly influences the virulence of APEC (Morales et al., 2004; Johnson et al., 2006), *ompT*, which cleaves the antimicrobial peptides protamine and plasminogen encodes for the episomal outer membrane protease that cleaves colicins (Stumpe et al., 1998; Johnson et al., 2006) and *iutA* gene one of the five genes of the aerobactin operon, which encodes an outer membrane protein involved in the high binding affinity of Fe3 (Johnson et al., 2006; Mellata et al., 2009). In the current study, we used this pentaplex PCR for screening of *E. coli* isolates from septicemic broiler chicken.

Three to four genes were detected in 80% of the investigated isolates. The most prevalent co existence for the tested genes was for *iroN, ompT, hylF* and *iss*, which were co detected in 75% of them.

This finding agreed with other regional study, where 80.2% of *E. coli* isolates carried three or more of the virulence genes (Ahmed et al., 2013), while Hussein et al. (2013) reported that more than 90% of the total APEC examined possessed *iroN, ompT, hlyF, iss,* and *iutA.*

On the other hand a study carried by Kobayashi et al. (2011) from Brazil confirmed the importance of the current Pentaplex PCR in the diagnosis of APEC as they detected 4 or 5 virulence genes in the majority of APEC strains (65%) using such PCR.

In our finding the highest prevalence was for *iroN, ompT* and *hylF* genes, which were equally detected in 80% of the isolates followed by *iss* and *iutA* genes, which were harbored by 75% and 5% of the isolates respectively.

Nearer percentages were reported by other regional and global studies (Rodriguez-Siek et al., 2005; Johnson et al., 2008; Kobayashi et al., 2011; Ahmed et al., 2013; Hussein et al., 2013) except for *iutA* gene, which was detected in a low frequency in the current isolates (5%) whiles detected in a higher frequency between 78% up to 100% by the other studies.

Highly pathogenic APECs lead to primary infections while less pathogenic strains only cause disease when the poultry are under severe stressful conditions such as other diseases and environmental stress factors (Dho-Moulin and Fairbrother, 1999), therefore comparing the screening results of potential APEC isolates revealed that not all the tested isolates are equally virulent.

It was noticed that in the current virulence associated genes still detected in the untypable isolates while one isolate serogrouped as O148 was negative for all of them, Scouler et al. (2012) stated that serotyping does not reflect the virulence traits while genotyping methods allowed the identification of more APEC isolates with greater reliability.

In conclusion, there was a high prevalence of multidrug resistance and some virulence associated genes among APEC examined in this study as well as poultry could be considered as a reservoir of *E. coli* having zoonotic potential.

References

Ahmed, A.M., Shimamotob, T. and Shimamotob, T. (2013): Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int. J. Med. Microbiol., 303 (8): 475-483.

Bai, L., Schüller, S., Whale, A., Mousnier, A., Marches, O., Wang, L., Ooka, T., Heuschkel, R., Torrente, F., Kaper, J.B., Gomes, T.A., Xu, J., Phillips, A.D. and Frankel, G. (2008): Enteropathogenic *Escherichia coli* O125: H6 triggers attaching and effacing lesions on human intestinal biopsy specimens independently of Nck and TccP/TccP2. Infect. Immun., 76 (1): 361-368.

Barnes, H.J., Nolan, L.K. and Vaillancourt, J.F. (2008): Colibacilliosis. In Diseases of Poultry (Saif, Y.M. and Fadly, A.M., eds.) Blackwell Publishing, Ames, IA, 691–732.

Berkhoff, H.A. and Vinal, A.C. (1986): Congo red medium to distinguish between invasive and non-invasive *Escherichia coli* pathogenic for poultry. Avian Dis., 30 (1): 117-121.

Cavalieri, S.J., Bohach, G.A. and Synder, I.S. (1984): *E. coli* alpha haemolysin characteristics and probable role in pathogenicity. Microbiol. Rev., 48: 326-343.

Clinical and Laboratory Standard Institute (CLSI) (2010): Performance standards for antimicrobial susceptibility testing, 20 th Ed. CLSI document M100-S20,Wayne, Pennsylvania, USA, 30: 1-45.

Collee, J.G., Fraser, A.G., Marmion, B.P. and Simmons, A. (1996): Practical Microbiology, 14th Ed. (Mackie and McCartney). The English langue book society and Churchill living stone, Edinburgh and New York.

Corbett, W.T., Berkhoff, H.A. and Vinal, A.C. (1987): Epidemiological study of the relationship between Congo red binding *Escherichia coli* and avian colisepticemia. Can. J. Vet. Res., 51**:** 312-315.

Dho-Moulin, M. and Fairbrother, J. M. 1999. Avian pathogenic *Escherichia coli* (APEC). Vet. Res., 30**:** 299-316.

Dozois, C.M., Daigle, F. and Curtiss, R.3rd. (2003): Identification of pathogen-specific and conserved genes expressed in vivo by an avian pathogenic *Escherichia coli* strain. Proc. Natl. Acad. Sci. USA, 100: 247-252.

Erb, A., Stürmer, T., Marre, R. and Brenner, H. (2007): Prevalence of antibiotic resistance in *Escherichia coli*: overview of geographical, temporal, and methodological variations. Eur. J. Clin. Microbiol. Infect. Dis., 26: 83–90.

Evans, D.G., Evans, D.J. and Tjoa, W. (1977): Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun., 18: 330-337.

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

Ewers, C., Antao, E.M., Diehl, I., Philipp, H.C. and Wieler, L.H. (2009): Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. Appl. Environ. Microbiol., 75: 184-192.

Ewing, W.H. (1986): Edwards and Ewing's. Identification of Enterobacteriaceae. Elsevier Science Publishing Company, New York.

Food and Agricultural Organization (FAO) (2007): Joint FAO/WHO/OIE meeting on critically important antimicrobials. November 26–30 Report of the FAO/WHO/OIE expert meeting. Rome, Italy.

Forbes, B.A., Sahm, D.F. and Weissfeld, A.S. (1998) Bailey and Scott's Diagnostic Microbiology. St Louis, MO, Mosby.

Gould, L.H., Demma, L., Jones, T.F., Hurd, S., Vugia, D.J., Smith, K., Shiferaw, B., Segler, S., Palmer, A., Zansky, S. and Griffin, P.M. (2009): Hemolytic uremic syndrome and death in persons with *Escherichia coli* O157:H7 infection, foodborne diseases active surveillance network sites, 2000-2006. Clin. Infect. Dis., 49 (10): 1480-1485.

Gyimah, J.E. and Panigraphy B. (1988): Adhesin-receptor interactions mediating the attachment of pathogenic *Escherichia coli* to chicken tracheal epithelium. Avian Dis.**,** 32: 74-78.

Heuvelink, A.E., Zwartkruis-Nahuis, J.T., van den Biggelaar, F.L., van Leeuwen, W.J. and de Boer, E. (1999): Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. Int. J. Food Microbiol., 52: 67–75.

Hussein, A.H., Ghanem, I.A., Eid, A.A., Ali, M.A., Sherwood, J.S., Li, G., Nolan, L.K. and Logue, CM. (2013): Molecular and phenotypic characterization of *Escherichia coli* isolated from broiler chicken flocks in Egypt. Avian Dis., 57 (3): 602–611.

Jiang, H.X., Lü, D.H., Chen, Z..L., Wang, X.M., Chen, J.R., Liu, Y..H., Liao, X.P., Liu, J.H. and Zeng, Z.L. (2011): High prevalence and widespread distribution of multi-resistant *Escherichia coli* isolates in pigs and poultry in China. Vet. J., 187 (1): 99–103.

Johnson, J.R. and Stell, A.L. (2000): Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis., 181: 261-272.

Johnson, T.J., Siek, K.E., Johnson, S.J. and Nolan, L.K. (2006): DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J. Bacteriol., 188: 745-758.

Johnson, T.J., Wannemuehler, Y., Doetkott, C., Johnson, S.J., Rosenberger, S.C. and Nolan, L.K. (2008): Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol., 46: 3987–3996.

Kalin, R., Ongor, H. and Cetinkaya, B. (2012): Isolation and molecular characterization of *Escherichia coli* O157 from broiler and human samples. Foodborne Pathog. Dis., 9 (4): 313-318.

Kaper, J.B., Nataro, J.P. and Mobley, H.L. (2004): Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., 2: 123-140.

Kobayashi, R.K., Aquino, I., Ferreira, A.L. and Vidotto, M.C. (2011): EcoR phylogenetic analysis and virulence genotyping of avian pathogenic *Escherichia coli* strains and *Escherichia coli* isolates from commercial chicken carcasses in southern Brazil. Foodborne Pathog. Dis. 8 (5): 631-634.

Mellata, M., Touchman, J.W. and Curtiss III, R. (2009): Full sequence and comparative analysis of the plasmid pAPEC-1 of avian pathogenic *E. coli* chi7122 (O78: K80:H9). PLoS One., 4 (1): e 4232.

Mellata, M. (2013): Human and Avian Extraintestinal Pathogenic *Escherichia coli*: Infections, Zoonotic Risks, and Antibiotic Resistance Trends. Foodborne Pathog. Dis., 10 (11): 916-932.

Mokady, D., Gophna, U. and Ron, E.Z. (2005): Virulence factors of septicemic *Escherichia coli* strains. Int. J. Med. Microbiol., 295: 455-462.

Morales, C., Lee, M.D., Hofacre, C. and Maurer, J.J. (2004): Detection of a novel virulence gene and a Salmonella virulence homologue among *Escherichia coli* isolated from broiler chickens. Foodborne Pathog. Dis., 1: 160-165.

Pfaff-McDonough, S.J., Horne, S.M., Giddings, C.W., Ebert, J.O., Doetkott, C., Smith, M.H. and Nolan, L.K. (2000): Complement resistance-related traits among *Escherichia coli* isolates from apparently healthy birds and birds with colibacillosis. Avian Dis., 44: 23-33.

Reingold, J., Starr, N., Maurer, J. and Lee, M.D. (1999): Identification of a new *Escherichia coli* She hemolysin homolog in avian E. coli. Vet. Microbiol., 66 (2): 125-134.

Rezk, M.M., Enany, M.E. and Hanafy, M.S. (2010): Relationship between o-serogroup, virulence and plasmid profile in *Escherichia coli* isolated from diseased chickens. J. Food Safety, 30: 679–698.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J. and Nolan, L.K. (2005): Characterizing the APEC pathotype. Vet. Res., 36: 241–256.

Saidi, B., Mafirakureva, P., and Mbanga, J., (2013): Antimicrobial resistance of *Escherichia coli* isolated from chickens with colibacillosis in and around Harare, Zimbabwe Avian Dis., 57 (1): 152-154.

Schouler, C., Schaeffer, B., Brée, A., Mora, A., Dahbi, G., Biet, F., Oswald, E., Mainil, J., Blanco, J. and Moulin-Schouleur, M. (2012): Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. J. Clin. Microbiol., 50 (5): 1673-1678.

Singer, R.S. and Hofacre, C.L. (2006): Potential impacts of antibiotic use in poultry production. Avian Dis., 50: 161–172.

Stumpe, S., Schmid, R., Stephens, D.L., Georgiou, G. and Bakker, E.P. (1998): Identification of *OmpT* as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*. J. Bacteriol., 180: 4002-4006.

Wlof, M.K. (1997): Occurrence, Distribution, and Associations of O and H Serogroups, Colonization Factor Antigens, and Toxins of Enterotoxigenic *Escherichia coli*. Clin. Microbiol. Rev., 10 (4): 569-584.

World Health Organization (WHO) (1998): Zoonotic non-O157 shiga toxin-producing *Escherichia coli* (STEC). WHO/CSR/APH/98.8 World Health Organization. Geneva, Switzerland.

Zhao, S., Maurer J.J., Hubert, S., De Villena, J.F., McDermott, P.F., Meng, J., Ayers, S., English, L. and White, D.G. (2005): Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet. Microbiol., 107 (3-4): 215-224.