P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

CLEANACTIV® Combatting Crisis of Multidrug Resistant Avian Pathogenic *E. coli* in Broiler Chickens

Raheel IAR[1], Orabi A[2]*, Shimaa Hassan[3] and Ahmed El Masry[4]

[1]Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suief University, Egypt; [2]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt;
[3]Animal Health Research Institute, Fayoum, Egypt
[4]Director of Scientific Office of PROVET -EGY Company
***Corresponding author:** drorabi2012@yahoo.com; orabi.vet@cu.edu.eg

Article History: Received: May 18, 2019 Revised: July 05, 2019 Accepted: July 13, 2019

ABSTRACT

The dramatic increase in antimicrobial resistance for avian pathogenic *E. coli* constitutes a key threat to Poultry industry. With the misuse and overuse of antibiotics to treat diseases, resistance to the drugs has begun to appear and has become more serious. In this paper, we studied the antibacterial effects of CLEANACTIV® essential oil (CEO) both alone, using a twofold dilution method, and combined with antibiotics, using a microtitre assay, against multidrug resistant avian pathogenic *Escherichia coli*. The result indicated that multiple drug-resistant APEC was very sensitive to CEO. The antibacterial effects of CEO in combination with fluoroquinolones, doxycycline, lincomycin, and florfenicol displayed synergism against *E. coli*, so this combination used to treat infections caused by MDR-APEC which may lower, to a great extent, the effective dose of these antibiotics and thus minimize the side effects of antibiotics.

Key words: CLEANACTIV®; Antibiotic resistance; Avian Pathogenic, *E. coli*

INTRODUCTION

Avian Pathogenic *Escherichia coli* is a pathogen of the chickens and other avian species especially broilers, it is considered as a member of the extra-intestinal pathogenic *E. coli* (Anatao *et al*., 2008). In the broiler chickens, Avian Pathogenic *Escherichia coli* (APEC) infections lead to respiratory tract infection, air sacculitis, pericarditis, peri-hepatitis, and swollen head syndrome. APEC is amongst the greatest health threats to the developed poultry industries with evidence of zoonotic risk (Rodriguez *et al*., 2005). Recently, APEC has become accepted as a primary pathogen rather than a consequence of immunosuppressive infections, so *E. coli* found in the intestinal tract are likely to form a reservoir of potential infection, as it has a great genomic diversity and plasticity (Moulin-Schouleur *et al*., 2006).Although virulence genes associated with pathogenicity are common in APEC including *iss*, *iroN*, *hly*, *tsh* and *iuc*, certain *E. coli* serotypes such as O78 and O1 are more frequently associated with infections (Johnson *et al*., 2006). It is difficult to control APEC bacteria that are normally a part of the chickens microbiota and this represent as a crisis in broilers industries as it is considered to be one of the principal causes of morbidity and mortality in poultry worldwide (Lagragione *et al*., 2013). During the last years, APEC becomes one of the microorganisms that are commonly resistant to antimicrobials (Zhao *et al*., 2012), as in the poultry sector antimicrobial agents not only used for therapy but also for growth promotion (Van den Bogaard and Stobberingh, 2000), which further imposes selective pressure on the microorganisms leading to development of drug resistance and this has serious public health implications (Szmolka *et al*., 2012). ESβLs is one of microbial drug resistance and infections caused by ESBL-producing bacteria isolated from poultry have become a clinical and therapeutic problem (Velasco *et al*., 2007). A current approach to limiting the drug resistant APEC is to use essential oils as alternative agents in disease control. It was reported that oregano oil has antibacterial activity, However There is a little knowledge about its mechanism against ESBL-producing *E. coli* isolated from poultry especially in combination with antibiotics, it may be in its principal components, i.e. thymol and carvacrol, which have antimicrobial properties (Baydar *et al*., 2004). Garlic oil has generated a lot of interest for decades as it has antimicrobial activities against many pathogens, which related to related to a non-protein amino acid "alliin" and

Cite This Article as: Raheel IAR, A Orabi, S Hassan and A El Masry, 2019. CLEANACTIV® combatting crisis of multidrug resistant avian pathogenic *E. coli* in broiler chickens. Inter J Vet Sci, 8(4): 283-288. www.ijvets.com

an enzyme "alliinase" (Mnayer *et al*., 2014). Hesperidin and naringin is a flavonoids found mainly in citrus fruits and it has a great variety of biopharmaceutical activities, e.g. antioxidant and antimicrobial Consequently, it is widely used in the treatment of many diseases, and serve as a raw ingredient for different drugs in pharmaceutical industry (Sosa *et al*., 2002; Devi *et al*., 2015). *Urtica dioica* L., commonly known as nettle, globally known as a medicinal plant as it has antibacterial properties (Kiaei *et al*., 2010). Saponins is the active principle of quillaya Extract which is are glycoside compounds with many biological and antibacterial activities differ according to type of the bacteria (Hassan *et al*., 2010), also one of the most proved criteria of cinnamon extracts as cassia oil is the antibacterial activity against Gram-positive and Gram-negative bacteria (Keskin *et al*., 2011),so the current study aimed to analyze the ability of CLEANACTIV® essential oil (CEO), which is a mixture of volatile oils such as; nettle extract 3.3%, cassia oil 1.2%, oregano oil 0.5%, carvacrol 0.3%, garlic oil 0.3%, quillaya Extract 0.15%, hesperidin 0.1% and naringin 0.05% in resolving the problem of ESβLs–MDR-APEC and in broiler chickens.

MATERIALS AND METHODS

Bacterial strains

Escherichia coli isolates were recovered from broilers chickens suffered from chronic respiratory disease. Strains were isolated, purified identified using an API 20E system (bioM´erieux, France) and serotyped according to (Quin *et al*., 2011), while the antibiogram disk diffusion technique was adapted according to (CLSI, 2018).

Screening for ESBLs by Combined Disc Diffusion Test (CDD)

Using disk of Cefotaxime and Ceftazidime, alone and in combination with Clavulanic acid. The organisms were considered to be producing ESβL when A≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus the zone diameter of the agent when tested alone equals EsβL (CLSI, 2018).

Identification of Virulence and ESβLs genes

Different sets of primers used for detection of ESβLs *E. coli* and some of its virulence genes showed in (Table 1) according to (Hasman *et al*., 2005 and Johnson *et al*., 2008) respectively.

CLEANACTIV® essential oil (CEO)

It is a mixture of essential volatile oils as; nettle extract 3.3%, cassia oil 1.2%, oregano oil 0.5%, carvacrol 0.3%, garlic oil 0.3%, quillaya Extract 0.15%, hesperidin 0.1% and naringin 0.05% manufactured by phytosynthese company, France.

CEO minimal inhibitory concentration (MIC)

E. coli adjusted in sterile saline with 0.5% Tween-80 to enhance oil solubility to match the 0.5 McFarland. MIC of CEO alone or both with antibiotics as enrofloxacin 5µg, lincomycin 30µg, florfenicol 30µg and doxycycline 20µg were examined by broth dilution method in 96-well, microtitre plates. The MICs of the agents against the isolated strain were determined and interpreted in according to (CLSI, 2018).

CEO Fractional inhibitory concentration (FIC)

Determined by dividing the MIC of the combination of antibacterial and essential oil by the MIC of antibacterial or essential oil alone. The FIC index, obtained by adding both FICs, was interpreted as synergistic when it was ≤0.5, as additive (indifferent) when it was >5 and ≤2.0 and as antagonistic when it was >2 (White *et al*., 2000).

Experimental design

Three hundred commercial Ross broiler chicks were used and vaccinated with common vaccines as IB, ND and IBD, also they divided in three groups each one consists of 100 chicks, Gp1: CLEANACTIV® treated, Gp2, enrofloxacin treated, Gp3: treated with CLEANACTIV® plus enrofloxacin, All groups challenged with MDR-APEC in concentration $1.5x10^8$ cfu/ml by eye dropping and treating begin after 48hr from challenge, livability, clinical signs, postmortem changes were detected (Barnes *et al*., 2003), while the re-isolated challenged strains is screened by using RT-qPCR for determination of down-regulation and fold change of *E.coli* virulence and ESβLs genes (in vivo) compared with its (in vitro) record when the challenged isolates exposed to 0.001% conc. of CLEANACTIV® (Sun *et al*., 2011). Intestinal samples from each group were admitted to Electron Microscope for Histomorphological study according to (Bozzola and Russell, 1991).

RESULTS

E. coli identification, serotyping, ESβL and virulence genes screening

E. coli isolates were recovered from broilers chickens CRD disease with an incidence of 22% which confirmed biochemically by API 20E and serotyped by using specific antisera to O78 (35%), O8 (30%), O158 (25%), O111 (10%), these isolates tested against 10 different chemotherapeutic: amoxicillin, floraphenicol, colistinsulphate, enrofloxacin, doxycycline, gentamycin, lincomycin, ciprofloxacin, Cefotaxime, Sulfamethxazole / Trimethoprim. The multidrug resistance isolates were in percentage of 92% (Fig. 1), with phenotypically ESβL positive in an incidence of 85%, while molecular screening in Table 2 for some ESβL genes as (bla_{TEM}, bla_{CTX}, and bla_{SHV}) showed that these genes detected in percentage of 66% regarding the characterization of some virulence genes (*iss*, *hlyF* and *iutA*) these genes detected in a percentage of 37%.

CEO; minimal and fractional inhibitory concentration

CLEANACTIV® essential oils has MIC and FIC against selected MDR–APEC illustrated in Table 3 at which CEO MIC is 0.72 µl/ml and has synergetic effect with enrofloxacin, florafenicol, doxycycline, also has additive effect with lincomycin.

Table 1: ESβL and virulence genes primers sequence for MDR-APEC

Primer		Sequence	bp	Reference
hlyF	F	GGC CAC AGT CGT TTA GGG TGC TTA CC	450	Johnson *et al*., 2008
	R	GGC GGT TTA GGC ATT CCG ATA CTC AG		
iss	F	CAG CAA CCC GAA CCA CTT GAT G	323	
	R	AGC ATT GCC AGA GCG GCA GAA		
iutA	F	GGC TGG ACA TCA TGG GA ACT GG	302	
	R	CGT CGG GAA CGG GTA GAA TCG		
blaTEM	F	GCGGAACCCCTATTTG	964	Hasman *et al*., 2005
	R	TCTAAAGTATATATGAGTAAACTTGGTCTGAC		
blaCTX	F	ATGTGCAGYACCAGTAARGTKATGGC	593	
	R	TGGGTRAARTARGTSACCAGAAYCAGCGG		
bla_{SHV}	F	TTCGCCTGTGTATTATCTCCCTG	854	
	R	TTAGCGTTGCCAGTGYTCG		

Table 2: ESβL and virulence genes patterns in MDR-APEC

Patterns	virulence genes			ESβL		
	hlyF	*iss*	*iutA*	*blaTEM*	*blaCTX*	bla_{SHV}
Incidence	35%	52%	25%	90%	82%	25%
Total average		37%			66%	

Table 3: MIC and FIC of CEO (µL/mL) and of antibacterial (µg/mL) against MDR-APEC

Active principles	MIC		FIC	Interpretation
	Single	Combined		
CEO	0.72	-	0.38	Independence
Enrofloxacin	0.5	0.24	0.35	Synergism
Lincomycin	0.56	0.32	0.72	Additive effect
Florfenicol	0.44	0.12	0.32	Synergism
Doxycycline	0.53	0.133	0.34	Synergism

MIC; minimal inhibitory concentration, FIC; fractional inhibitory concentration,MDR; multi drug resistance, APEC; avian pathogenic *E.coli*, *CEO*; CLEANACTIV® essential oils.

Table 4: RT-qPCR for determination of down-regulation and fold change of virulence and ESBLs genes in MDR-APEC used in challenged study

Experimental challenge		Virulence genes						ESβL					
		hlyF		*iss*		*iutA*		*blaTEM*		*blaCTX*		bla_{SHV}	
		CT	FC	CT	FC	CT	FC	CT	FC	CT	FC	CT	FC
MDR, ESβL,	In vitro	12.3	0.34	11.6	0.56	10.7	0.4	16.3	0.92	14.8	0.45	17.3	0.16
Virulent, APEC	In vivo	13.6	0.57	13.2	0.52	12.4	0.7	18.5	1.3	16.3	0.75	18.3	0.54

CT; Cycle Threshold, FC; Fold Change.

Experimental challenge with MDR-APEC and RT-qPCR for determination of down-regulation and fold change of *E. coli* virulence and ESBLs genes

Livability in Gp3 **(**treated with CLEANACTIV® plus enrofloxacin) were 96% with mild respiratory and digestive signs and mild enteritis, while Gp1 (CLEANACTIV® treated) livability were 90% with moderate respiratory and digestive signs and moderate enteritis, in the other hand Gp2 (enrofloxacin treated), livability was 92%, with rales, sneezing and diarrhea signs, postmortem changes were detected moderate enteritis. Down-regulation and fold change of *E. coli* virulence were recorded in Table 4 as *hlyF*, *iss*, *iutA* cycle threshold were 12.3,11.6,10.7 and their fold change were 0.34,0.56,0.43 respectively, while ESBLs genes CT were bla_{TEM}, bla_{CTX}, bla_{SHV} were 16.3,14.8,17.3 and their fold change were 0.92,0.45,0.16 respectively compared with value of in vitro study.

Histomorphology

In Gp3 **(**treated with CLEANACTIV® plus enrofloxacin) intestinal Histomorphology in (Fig. 2) showed intestinal villi healthiness with immune cells infiltration mainly plasma cells and macrophages and, while increase proliferation of lymphoid cells, endocrine cells, goblet cell and in Gp1 (Fig. 3), unfortunately in Gp 2 edematous and destructive microvilli with massive heterophiles was detected (Fig. 4).

DISCUSSION

Avian colibacillosis causes significant economic losses, either as a primary disease or as a secondary infection, to broilers chicken(Lutful Kabir., 2010), as the presence of resistance plasmids in avian *E. coli* strains could facilitate horizontal transfer of virulence gene between pathogenic and non-pathogenic strains (Barros., 2012). Several factors have been shown to contribute to the virulence of avian *E. coli* ,and many of the genes encoding these factors have been found on large conjugative plasmids, so in the current study characterization of some virulence genes (*iss*, *hlyF* and *iutA*) these genes detected in a percentage of 37% (Table 2). Because of the occurrence of antimicrobial resistance genes on these same plasmids, it is possible that the use of antimicrobial agents may select for persistence of E *.coli* containing such plasmids (Johnson *et al*., 2004). In the present study phenotypically investigate incidence of APEC in broilers chickens which is 22% with high isolation rate for O78, O8, O158 and O111 (10%) serotypes

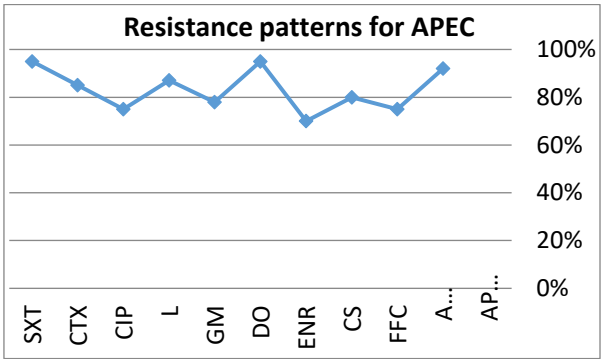

Fig. 1: showing resistance patterns for MDR-APEC. AMX; Amoxicillin, FFC; Flor phenicol, CS ;Colistin sulphate ENR; Enrofloxacin, DO; Doxycycline GM ;Gentamycin, L; Lincomycin, CIP; Ciprofloxacin, CTX; Cefotaxime, SXT; Sulfamethxazole/Trimethoprim.

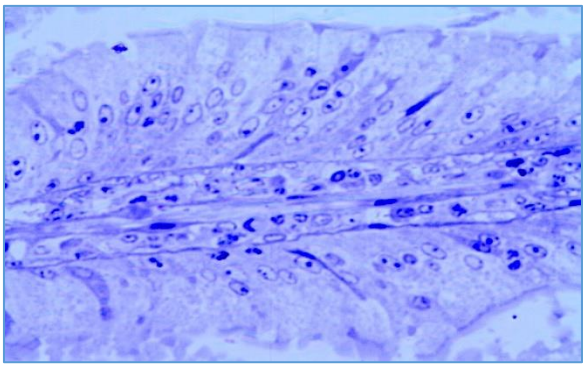

Fig 2: Micrograph of the Gp3 treated intestinal tissue showing healthy villi with plasma and macrophages cells infiltration.

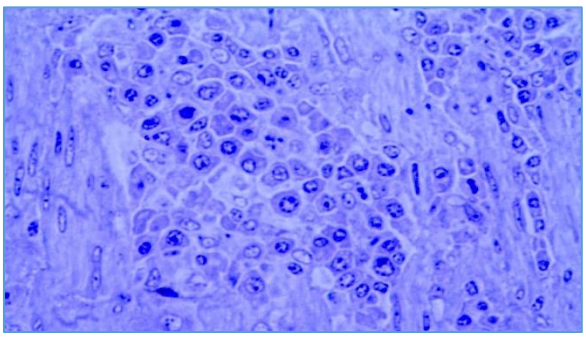

Fig. 3: Micrograph of the Gp1 treated intestinal tissue showing healthy microvilli with proliferation of lymphoid cells, endocrine cells, and goblet cell.

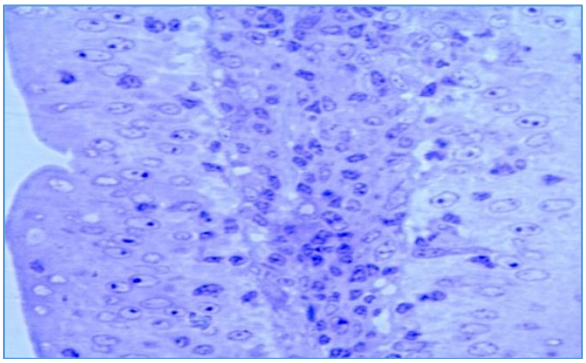

Fig. 4: Micrograph of the Gp2 treated intestinal tissue showing edematous and destructive microvilli with heterophiles infiltration.

respectively, also the antibiogram profile for the recovered isolates showed high level of MDR serotypes in rate of 92%. Production of beta-lactamases represents the most important contributing factor to resistance against beta-lactam antibiotics in Gram-negative bacteria (Liebana, 2013). ESβLs can hydrolyze the newer beta-lactam antibiotics, including 3rd and 4th generation of cephalosporins (WHO, 2017). The current study explore the incidence of ESβLs incidence between the recovered MDR-APEC which revealed that phenotypically ESβL positive present in an incidence of 85%, while molecular screening in Table 2 for some ESβL genes as (*bla*$_{TEM}$, *bla*$_{CTX}$ and *bla*$_{SHV}$) showed that these genes detected in percentage of 66%Multiple antimicrobial resistances may be acquired through mobile genetic elements which contributing in the creation of multi drug-resistant phenotype, and the widespread use of extended-spectrum cephalosporins creates a reservoir of resistant bacteria, also continuous emergence of resistance to antimicrobial agents among the prevalent pathogens is the most dangerous threat for the treatment of infectious diseases (Roca *et al*., 2015). There are many edible and medicinal plants with high antimicrobial effects, such as thyme (*Thymus vulgaris* L.), tea (*Camellia sinensis* L.), garlic (*Allium sativum* L.), turmeric (*Curcuma longa* L.), berries belonging to Rosaceae family, and cinnamon (species belonging to Cinnamomun genus) (Nabavi *et al*., 2013). The present work MIC and FIC For CLEANACTIV® essential oils against VIRULENT-MDR –APEC illustrated in Table 3 at which CEO MIC is 0.72 µl/ml and has synergetic effect with enrofloxacin, florafenicol, doxycycline, also has additive effect with lincomycin, and in the experimental challenge with VIRULENT-MDR – APEC in broilers chickens Livability was high in Gp3 (treated with CLEANACTIV® plus enrofloxacin) were 97% with mild signs which mainly refer to Down-regulation and fold change of *E.coli* virulence and resistance genes as showed in Table 4, *hlyF*, *iss*, *iutA* CT were 12.3, 11.6, 10.7 and their fold change were 0.34, 0.56, 0.43 respectively, while ESBLs genes CT were *bla*$_{TEM}$, *bla*$_{CTX}$, *bla*$_{SHV}$ were 16.3, 14.8, 17.3 and their fold change were 0.92, 0.45, 0.16 respectively, this may related to that various essential oils can disrupt the Quorum sensing of pathogenic bacteria (Zhou *et al*., 2013), which referred to a regulatory system in bacteria that regulates gene expression (De Kievit and Iglewski, 2000).

Conclusions

Combination between antibiotic and CLEANACTIV® essential oils act as lifeline in control of multidrug resistant avian pathogenic *E. coli* and avoidance emergence of ESβL *E. coli* in broilers chickens.

REFERENCES

Antao EM, S Glodde, G Li, R Sharifi, T Homeier, and C Laturnus, 2008. The chicken as a natural model for extra-intestinal infections caused by avian pathogenic Escherichia coli (APEC). Microb Pathog, 45: 361.

Barnes HJ, JP Vaillancourt and WB Gross, 2003. Colibacillosis, pp: 631-652. In Saif YM, *et al* (ed),

Diseases of poultry, 11th ed. Iowa State Press, Ames, IA.

Baydar H and G Sagdic OZkan, 2004. Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. Food Control, 15: 169–172.

Bozzola J and L Russell, 1991. Electron microseopy principles and techniques for biologists: Jones and Bartlitt publishers 20 park plasa Boston Ma o 2116.

CLSI, 2018. Clinical and Laboratory Standards Institute (Formerly NCCLS), Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. Approved Standard M100-S24, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

De Kievit TR and BH Iglewski. Bacterial quorum sensing in pathogenic relationships, 2000. Infect Immun; 68(9): 4839e49.

Devi PK, T Rajavel, FS Nabavi, NW Setzer, A Ahmadi, K Mansouri and MS Nabavi, 2015. Hesperidin: A promising anticancer agent from nature, Indus Crops Prod, 76: 582-589.

Liebana EL, C Garcia, FA Migura, M Clifton Hadley, RH Breslin, Davies. Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of Salmonella Enteritidis infection in layer farms, 2003.

Hasman H, D Mevius, K Veldman, I Olesen and FM Aarestrup, 2005. Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemoth, 56: 115–121.

Hassan SM, AU Haq, JA Byrd, MA Berhow, AL Cartwright and CA Bailey, 2010. Haemolytic and antimicrobial activities of saponin-rich extracts from guar meal. Food Chem, 119: 600-605.

Johnson JR, C Clabots and MA Kuskowski, 2008a. Multiple-host sharing, long-term persistence and virulence of Escherichia coli clones from human and animal household members. J Clin Microbiol, 46: 4078–4082.

Johnson JR, MA Kuskowski, A Gajewski, DF Sahm and JA Karlowsky, 2004. Virulence characteristics and phylogenetic background of multidrug-resistant and antimicrobial-susceptible clinical isolates of Escherichia coli from across the United States, 2000–2001.

Johnson TJ, KE Siek, SJ Johnson and LK Nolan, 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian Escherichia coli strains. J Bacteriol, 188: 745-58.

Keskin, D and S Toroglu, 2011.Studies on antimicrobial activities of solvent extracts of different spices. J Environ Biol, 32: 251–256.

Kiaei E, M Mazandarani and E Ghaemi, 2010. Antibacterial activity of 7 species of medicinal plants on bacteria isolated from UTI patients in Golestan province. J Med Plants, 9(34): 74-83.

LaRagione RM, Woodward MJ, KumarM, Rodenberg J, Fan H and Wales AD, 2013. Efficacy of a live attenuated Escherichia coli O78:K80 vaccine in chickens and turkeys. Avian Dis, 57: 273-9.

Lutful Kabir SM, 2010. Avian Colibacillosis and Salmonellosis. A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int J Environ Res Public Health, 7: 89-114.

Mércia R, D Barros, Wanderley, da Silveira, M Janete de Araújo, P Elizianne. F Andrea Alice da. Oliveira, Ana Paula da SF Santos, Vanessa Anny S Silva e Rinaldo A, 2012. Mota Resistência antimicrobiana e peril plasmidial de Escherichia coli isolada de frangos de corte e poedeiras comerciais no Estado de Pernambuco. Pesq Vet Bras, 32(5): 405-410.

Mnayer D, AS Fabiano-Tixier, E Petitcolas, T Hamieh, N Nehme and C Ferrant, 2014. Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae Family. Molecules; 19: 20034-53.

Moulin-Schouleur M, C Schouler, P Tailliez, Kao MR, A Bree and P Germon, 2006. Common virulence factors and genetic relationships between O18:K1:H7 Escherichia coli isolates of human and avian origin. J ClinMicrobiol, 44: 3484–92.

Nabavi SF, SM Nabavi, S Habtemariam, AH Moghaddam, A Sureda, M Jafari and AM Latifi, 2013. Hepatoprotective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress. Ind Crop Prod, 44: 50–55.

Quinn PJ, BK Markey, ME Carter, WJC Donnelly, F.C Leonard, and D Maguire, 2011. Veterinary Microbiology and Microbial Diseases. 1st published Blackwell Science ltd.

Roca M, F Akova, J Baquero, Carlet, M, S Cavaleri, J Coenen, D Cohen, I Findlay, Gyssens, OE Heure, G Kahlmeter, H Kruse, R Laxminarayan, E Liébana, L López-Cerero, A MacGowan, M Martins, J Rodríguez-Baño, JM Rolain, C Segovia, B Sigauque, E Taconelli, E Wellington and J Vila, 2015. The global threat of antimicrobial resistance: science for intervention New Microbes New Infect, 6: 22–29.

Rodriguez-Siek KE, CW Giddings, C Doetkott, TJ Johnson, MK Fakhr and LK Nolan, 2005. Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology, 151: 2097–110.

Sosa S, MJ Balick, R Arvigo, 2002. Screening of the topical anti-inflammatory activity of some central American plants, J Ethnopharmacol, 81: 211–215.

Sun YM Dai, H Hao, Y Wang, L Huang, YA Almofti, Z Liu and Z Yuan, 2011. The role of RamA on the development of ciprofloxacin resistance in Salmonella enterica serovar Typhimurium. PLoS One 6: e23471.

Szmolka A, MF Anjum, RM La Ragione, EJ Kaszanyitzky and B Nagy, 2012. Microarray based comparative genotyping of gentamicin resistant Escherichia coli strains from food animals and humans. Vet Microbiol, 156: 110-118.

Van de Bogaard AE and EE Stobberingh, 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents, 14: 327-335.

Velasco C, L Romero, JMR Mart´ınez, J Rodr´ıguez-Ba˜no and A Pascual, 2007. Analysis of plasmids encoding extended-spectrum b-lactamases (ESBLs) from Escherichia coli isolated from non-hospitalized patients in Seville. Int J Antimicrob Agents, 29: 89–92.

White DG, LJ Piddock, JJ Maurer, S Zhao, V Ricci and SG Thayer, 2000. Characterization of fluoroquinolone resistance among veterinary isolates of avian Escherichia coli. Antimicrob Agents Chemother, 44: 2897–2899.

WHO. WHO Critically important antimicrobials for human medicine – 5th rev. ISBN 978-92-4-151222-0 available under the Creative Commons Attribution-Non-Commercial-Share Alike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; https://creativecommons.org/licenses/by-nc-sa/3.0/igo), WHO Press, WHO, Geneva, Switzerland (2017).

Zhao S, K Blickenstaff, S Bodeis-Jones, SA Gaines, E Tong, P.F McDermott,2012. Comparison of the Prevalence and antimicrobial resistances of Escherichia coli isolates from different retail meats in the Unites States, 2002 to 2008. Appl Environ Microbiol, 78: 1702-1707.

Zhou L, H Zheng, Y Tang, W Yu, Q Gong, 2013. Eugenol inhibits quorum sensing at subinhibitory concentrations. Biotechnol Lett, 35(4): 631e7.