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Original Research Article

Bacterial Species Associated with Broiler Proventriculitis and Antimicrobial Resistance of Clinical Important Species

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ABSTRACT

Bacteria could adhere and invade various tissues result in diverse pathologic lesions in concordance to the localization site. Proventriculitis reduces growth rate ends with huge economic losses. Microbiological investigation of 99 proventriculitis specimens revealed the recovery of diverse bacterial species of clinical impact on poultry industry. As far as we know, *P. aeruginosa* was isolated as a first record and with the highest prevalence amongst the recovered bacterial species (39.4%). *C. perfringens* and members of *Enterobacteriaceae* (*P. mirabilis*, *Citrobacter* spp., *E. coli*, *E. aerogenes* and *K. pneumoniae*) were isolated with variable prevalence. *E. coli* represented 8.1% of the overall bacterial species isolated and they were serogrouped in *E. coli* O158 (75%) and O146 (25%). Regarding antimicrobial resistance 100% of the examined *P. aeruginosa* and *E. coli* isolates were multidrug resistance. Extended Spectrum Beta-Lactamase (ESBLs) and AmpC detected in *P. aeruginosa* with 12.8 and 97.9% respectively and in *E. coli* they were detected in 37.5 and 12.5% respectively. The current study indicates that the bacterial proventriculitis not only influences broilers economy but also could threaten human health via bacterial species of zoonotic potential and probability of transferring their antimicrobial resistance determinants.

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1. INTRODUCTION

Bacteria could adhere and invade various tissues result in diverse pathologic lesions in concordance to the localization site. Various bacterial species were claimed to cause proventriculitis including *Clostridium perfringens*, *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. (Birchard and Sherding 2005 and Karki *et al*., 2009). Proventriculitis results in passage of undigested feed that consequently leads to poor feed conversion and ended by reduced growth rate (Dormitorio *et al*., 2007) leads to huge economic losses. Additionally, these bacterial species may use it as a gate to reach birds' blood causing septicemia with great impact on broiler farms.

In context, bacterial species regarded to associate proventriculitis are confirmed to associate various pathological conditions in broilers. *C. perfringens* cause various lesions likewise gizzard lesions, necrotic enteritis and hepatitis in broiler flocks (Timbermont *et al*., 2009). Moreover, it induces necrotic dermatitis and cholangiohepatitis (Hafez, 2011). Avian Pathogenic *Escherichia coli* (APEC) strains cause diversity of diseases in birds resulting in great economic losses in the avian industry (LeStrange, 2013). *Klebsiella* spp. is amongst the bacterial etiology of early chick mortality (Venkanagouda and Upadhye, 1996).

Regarding the public health perspective, proventriculitis could result in damaged proventriculi end by its rupture during evisceration and contaminate broilers meat with various bacterial species that are of zoonotic potential which mostly showed high antimicrobial resistance to antimicrobial agents of human concern (Bayyari *et al*., 1995).

Antimicrobial resistance returns human to the pre-antimicrobial era in the face of superbugs. Extended Spectrum Beta-Lactamase (ESBL)/plasmid-mediated AmpC beta-lactamase are of vital contribution in the overall causes behind antimicrobial resistance. ESBLs/AmpC are of both livestock and human concern as they cause treatment failure (Carmo *et al*., 2014). So this study aimed at investigating the bacterial species incriminated with proventriculitis in broiler and investigating their antimicrobial resistance with special regard to the ESBLs in the isolated bacterial species of clinical impact.

2. MATERIALS AND METHODS

Isolation and Identification of bacterial species associated with proventriculitis (Collee *et al*., 1996)

Samples were collected from Fayoum and Beni-Suef Governorate farms, these farms suffered digestive and/or respiratory manifestations. From these farms, proventriculi showed gross lesions were examined bacteriologically from 99 broiler chicks up to four weeks of age. Each sample represented a distinct farm. Aerobic bacteria (*P. aeruginosa*); facultative anaerobic bacteria (*Enterobacteiaceae* including different species likewise *E. aerogenes*, *K. pneumoniae*, *Citrobacter* spp., *E. coli* and *P. mirabilis*) and anaerobic bacteria as *C. perfringens* were bacteria of concern in the current study.

Serological Identification of *E. coli*

The recovered *E. coli* were serologically investigated (Edwards and Ewing, 1972).

Antimicrobial susceptibility testing (Clinical and Laboratory Standards Institute, 2013)

P. aeruginosa and *E. coli* recovered from the examined specimens were subjected to antimicrobial testing using 18 antimicrobial disks representing various antimicrobial classes of veterinary and human concern in concordance to the guidelines of CLSI (2013). Twelve antimicrobial classes were used: aminoglycosides (amikacin, AK 30 µg; gentamicin, CN 10 µg and tobramycin, TOB 10 µg); β-lactam/β-lactamase inhibitor combinations (piperacillin-tazobactam, TZP 100/10 µg); cephamycins (cefoxitin, FOX 30 µg); first generation cephalosporin (cefazolin, KZ 30 µg); third generation cephalosporins (ceftazidime, CAZ 30 µg; cefotaxime, CTX 30 µg; and ceftriaxone, CRO 30 µg); fourth generation cephalosporins (cefepime, FEP 30 µg); glycylcycline (tigecycline, TGC 15 µg); phenicols (chloramphenicol, C 30 µg); penicillins (ampicillin, AMP 10 µg); monobactam (aztreonam, ATM 30 µg); fluoroquinolones (ciprofloxacin, CIP 5 µg; norfloxacin, NOR 10 µg; and ofloxacin, OFX 5 µg) and carbapenems (ertapenem, ETP 10 µg). Antimicrobial disks were purchased from Oxoid, UK.

Detection of Extended spectrum β-lactamases

Bacterial isolates of *P. aeruginosa* and *E. coli* showed resistant and/or intermediate response to the monobactams, third and/or the fourth generation cephalosporins were tested to confirm the ESBLs production using modified double disk diffusion test in accordance to Garrec *et al*. (2011). Briefly, Muller Hinton agar (MHA) plate was swabbed by a lawn of 18 h fresh bacterial culture under test adjusted to contain 1.5X108 CFU by its matching to McFarland tube (no. 0.5). Then, disks of cefotaxime, ceftazidime, aztreonam and cefepime were manually placed around amoxicillin-clavulanic disk with 20 mm center to center and incubated at 37°C for 18 h. ESBLs confirmed when the inhibition zone around one or more of the four antibiotic disks was enhanced facing the side of the clavulanic acid containing disk (Garrec *et al*., 2011).

Detection of AmpC

P. aeruginosa and *E. coli* isolates showed intermediate and/or resistant pattern to cefoxitin were tested to confirm AmpC production (Edquist *et al*., 2013) by addition of 0.75 mg of cloxacillin to the cefoxitin disks. Briefly, cloxacillin 750 mg was dissolved in 10 mL distilled water and 10 µL of the solution was added to cefoxitin disk and kept to dry for 10 min. before use. Cefoxtin and cefoxitin containing cloxacillin were manually placed onto MHA plate previously swabbed by a lawn of fresh culture of the isolate under test. Inhibition zone difference 5 mm or more between both disks indicated the AmpC enzyme production (Edquist *et al.*, 2013) by the investigated isolates.

3. RESULTS

Isolation and biochemical identification

The overall prevalence of bacterial infection was 75.8%. Various bacterial species were identified with the investigated 99 provetriculus specimens. The most prevalent bacterial species was *P. aeruginosa* with a prevalence of 39.4% followed by *P. mirabilis* (26.3%), *C. perfringens* (18.2%), *E. coli* (8.1%), *Citrobacter* spp. (6.1%) and ended by *E. aerogenes* and *K. pneumoniae* (1% each)(Table 1).

Table 1. Prevalence of different bacterial species associated with proventriculitis in broiler chicken

Bacterial species	Positive samples (No.)	Prevalence (%)**
P. aeruginosa	39*	39.4
P. mirabilis	26	26.3
C. perfringens	18	18.2
E. coli	8	8.1
***Citrobacter* spp.**	6	6.1
E. aerogenes	1	1
K. pneumoniae	1	1

*: *P. aeruginosa* strains were recovered from 39 samples and eight out of them give rise to two different strains with 47 isolates.

**: Mixed infection of the same sample with more than one bacterial species was recorded.

Serogrouping of *E. coli*

Serogrouping of the biochemically identified *E. coli* alienated the eight isolates into two serogoups, *E. coli* O158 and O146 with a prevalence of 75 and 25% respectively.

Antimicrobial susceptibility profile of *P. aeruginosa* isolated from proventriculitis in broiler chicks

Ceftazidime was the most active antimicrobial agent against *P. aeruginosa* with 93.6% followed by amikacin, tobramycin, piperacillin/tazobactam and cefepime with 78.7, 76.6, 76.6 and 74.5% respectively. All the recovered isolates (100%) resisted cefazolin, chloramphenicol and tigecycline give rise to the 100% MDR. *P. aeruginosa* strains showed marked resistance against variable antimicrobial classes which started with at least four classes and reached to 11 classes (Table 2). This investigation alarms for an outstanding record of non-susceptibility against ertapenem with 89.4% (76.6% resistant and 12.8% intermediate).

Table 2. Antimicrobial susceptibility profile of *P. aeruginosa* isolated from broiler proventriculitis

Antibacterial agents	Disk content (µg/disk)	Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No	%
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS • Piperacillin/tazobactam (TZP)	100/10	36	76.6	3	6.4	8	17.0
AMINOGLYCOSIDES • Amikacin (AK)	30	37	78.7	3	6.4	7	14.9
• Gentamicin (CN)	10	31	66.0	9	19.1	7	14.9
• Tobramycin (TOB)	10	36	76.6	4	8.5	7	14.9
Glycylcycline • Tigecycline(TGC)	15	0	0.0	0	0.0	47	100.0
PHENICOLS • Chloramphenicol (C)	30	0	0.0	0	0.0	47	100.0
PENICILLINS • Ampicillin (AMP)	10	1	2.1	0	0.0	46	97.9
CEPHEMS(Cephamycin) • Cefoxitin (FOX)	30	1	2.1	0	0.0	46	97.9
First generation cephalosporins • Cefazolin (KZ)	30	0	0.0	0	0.0	47	100.0
Third generation cephalosporins • Cefotaxime (CTX)	30	1	2.1	13	27.7	33	70.2
• Ceftazidime (CAZ)	30	44	93.6	3	6.4	0	0.0
• Ceftriaxone (CRO)	30	3	6.4	9	19.1	35	74.5
Fourth generation cephalosporins • Cefepime (FEP)	30	35	74.5	0	0.0	12	25.5
MONOBACTAM • Aztreonam (ATM)	30	3	6.4	23	48.9	21	44.7
FLUOROQUINOLONES • Ciprofloxacin (CIP)	5	23	48.9	0	0.0	24	51.1
• Norfloxacin (NOR)	10	23	48.9	1	2.10	2	48.95
• Ofloxacin (OFX)	5	20	42.5	1	2.13	2	55.32
CARBAPENEMS • Ertapenem (ETP)	10	5	10.6	6	12.8	36	76.6

Antimicrobial susceptibility profile of the investigated *E. coli*

Table (3) illustrates the antimicrobial susceptibility patterns of *E. coli* isolated from proventriculitis in broiler chickens against the most clinically used antimicrobials in veterinary and human medicine.

None of the investigated isolates was sensitive to every antimicrobial agent. Additionally, 100% of the inspected *E. coli* resisted ampicillin and cefazolin.

Variable degrees of resistance were noted against many antimicrobials, and the reported resistance patterns in a descending manner were 87.5, 62.5, 62.5 and 62.5% for gentamicin, ceftriaxone, chloramphenicol, and ciprofloxacin respectively. Additionally, growing resistance (12.5%) was reported against third generation cephalosporins (cefotaxime and ceftazidime), cephamycin (cefoxitin) and monobactam (aztreonam). MDR (resistance to at least three antimicrobial classes) was noted in 100% of *E. coli* strains under investigation.

Table 3. Antimicrobial susceptibility profile of *E. coli* isolated from broiler proventriculitis

Antibacterial agent of *E. coli*	Disk content (µg/disk)	Susceptible No.	Susceptible %	Intermediate No.	Intermediate %	Resistant No.	Resistant %
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS • Piperacillin/tazobactam(TZP)	100/10	7	87.5	0	0.0	1	12.5
AMINOGLYCOSIDES • Amikacin (AK)	30	3	37.5	4	50	1	12.5
• Gentamicin (CN)	10	1	12.5	0	0.0	7	87.5
• Tobramycin (TOB)	10	5	62.5	2	25.0	1	12.5
Glycylcycline • Tigecycline (TGC)	15	4	50.0	4	50.0	0	0.0
PHENICOLS • Chloramphenicol (C)	30	1	12.5	2	25.0	5	62.5
PENICILLINS • Ampicillin (AMP)	10	0	0.0	0	0.0	8	100.0
CEPHEMS (Cephamycin) • cefoxitin (FOX)	30	7	87.5	0	0.0	1	12.5
First generation cephalosporins • Cefazolin (KZ)	30	0	0.0	0	0.0	8	100.0
Third generation cephalosporins • Cefotaxime (CTX)	30	5	62.5	2	25.0	1	12.5
• Ceftazidime (CAZ)	30	7	87.5	0	0.0	1	12.5
• Ceftriaxone (CRO)	30	3	37.5	0	0.0	5	62.5
Fourth generation cephalosporins • Cefepime (FEP)	30	7	87.5	1	12.5	0	0.0
Monobactam • Aztreonam (ATM)	30	7	87.5	0	0.0	1	12.5
Fluoroquinolones • Ciprofloxacin (CIP)	5	1	12.5	2	25.0	5	62.5
• Norfloxacin (NOR)	10	4	50.0	1	12.5	3	37.5
• Ofloxacin (OFX)	5	3	37.5	1	12.5	4	50.0
Carbapenems • Ertapenem (ETP)	10	7	87.5	0	0.0	1	12.5

Detection of β-lactamases production in the isolated *E. coli* and *P. aeruginosa*

ESBLs

ESBLs was asserted in 6/47 (12.8%) out of the explored *P. aeruginosa* and 3/8 (37.5%) out of the studied *E. coli* isolates phenotypically using double disk synergy test utilizing aztreonam, cefepime, cefotaxime and ceftazidime antibiotics around the amoxicillin-clavulanic acid disk.

AmpC

Antimicrobial disk diffusion test of *P. aeruginosa* showed 46 (97.9%) out of 47 recovered from proventriculitis to be resistant to cefoxitin and by cefoxitin-cloxacillin combined disk confirmed the presence of AmpC with in the investigated isolates. Regarding *E. coli*, only one isolate (12.5%) resisted cefoxitin and confirmed to contain AmpC using cefoxitin-cloxacillin combined disk test.

4. Discussion

Microbiological investigation of 99 proventriculitis specimens revealed the recovery of diverse bacterial species of clinical impact in poultry industry. As far as we know, *P. aeruginosa* was isolated as a first record and with the highest prevalence amongst the recovered bacterial species (39.4%). Additionally, *C. perfringens* was recovered in concordance with previous report of Karki *et al*. (2009) who report *C. perfringens* as a secondary bacterial invader proventriculitis in broiler chicken while Huff *et al*. (2001) reproduced proventriculitis experimentally in chicken by *C. perfringens*. Several species of family *Enterobacteriaceae* likewise *E. coli, Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. and *Proteus* spp. were isolated from the specimens under investigation. *E. coli* was isolated in a prevalence of 8.1% and this conceded with previous results (Birchard and Sherding, 2005 and Karki *et al*., 2009) who investigated bacterial species associated with proventriculitis in replacement pullets and broiler chickens. Serogrouping of the recovered *E. coli* revealed the prevalence of two serogroups O158 (75%) and O146 (25%) that were previously noted to associate various clinical cases. *E. coli* serogroup O146 was implicated in respiratory affections and septicemia (Ashraf *et al*., 2014) and *E. coli* O158 was reported to cause cellulitis (Ahmed, 2014) and various degrees of mortality (0.07-6.8%) in different broiler breeds (Khelfa and Morsy, 2015). The isolation of *Klebsiella* spp. and *E. aerogenes* from proventriculitis was previously reported by Birchard and Sherding (2005). Moreover, *Citrobacter* spp. and *P. mirabilis* were isolated for the first time from proventriculitis in the present study.

The present study focused on *P. aeruginosa* and *E. coli* antimicrobial susceptibility behavior since they represent risk for broiler farm (Kebede, 2010 and Hassan, 2013) and public health (Walker *et al*., 2002 and Osman *et al*., 2010).The antimicrobial susceptibility/resistance patterns of *P. aeruginosa* diverge greatly when compared with the results of other scholars; Tartor and El-Naenaeey, 2016 reported higher sensitivity of *P. aeruginosa* to ciprofloxacin and carbapenems (imipenem and meropenem) while the present results revealed higher resistance to the same antimicrobial classes (51.1 and 89.4% non-susceptible pattern respectively). Similar result was reported for gentamicin by Walker *et al*., 2002 who recorded 100% sensitivity versus 34% non-susceptible pattern of the isolated *P. aeruginosa* in the present study; and it could be attributed to the intense and misuse of antimicrobial agents in different regions (Lee *et al*., 2015). The antimicrobial susceptibility testing exhibited ceftazidime with 93.6% effect against *P. aeruginosa* followed by amikacin (78.7%). On the contrary, 100% of the investigated isolates were resistant to tigecycline (glycycline), chloramphenicol (phenicols),) and cefazolin (first generation cephalosporins) that indicates 100% MDR of the recovered *P. aeruginosa* from broiler chicks suffered proventriculitis. Additionally, the reported resistance against carbapenem (76.6%) was closely matched to the results of Kamel *et al*. (2011) who reported 72.5% resistance pattern against carbapenems and this may be attributed to the use of different members of the carbapenem group since ertapenem (in the present study) is recommended by CLSI (2013) for screening of carbapenem resistance for its higher sensitivity. Growing pattern of resistance was reported against various antimicrobials including fluoroquinolones (ofloxacin, 55.32%; ciprofloxacin, 51.1%; and norfloxacin 48.95%) and monobactams (aztreonam, 44.7%). Limited data about ESBLs prevalence in *P. aeruginosa* of chicken origin directed the study to investigate the condition. AmpC was confirmed in 46 (97.9%) which exceeded the recently detected prevalence of AmpC (68.6%) in *P. aeruginosa* of human origin (Rafiee *et al*., 2014). Additionally, ESBLs was confirmed in 6/47 (12.8%) out of the investigated *P. aeruginosa* and co-existed with AmpC and this conceded with Rafiee *et al*. (2014).

Investigating the antimicrobial susceptibility of *E. coli* isolated from proventriculitis in broiler chicks revealed variable degrees of resistance against not only the antimicrobial agents of animal concern but also against those of human concern. The highest resistance rates were noted against the frequently used antimicrobials in poultry industry including ampicillin, gentamicin, chloramphenicol and ciprofloxacin (100, 87.5, 62.5 and 62.5% respectively). Low but growing antimicrobial resistance was reported against third generation cephalosporins (cefotaxime and ceftazidime), cephamycin (cefoxitin) and monobactam (aztreonam) and this could be attributed to the reduced usage of these antimicrobials in poultry industry (Landers *et al*., 2012). In the concern of MDR, all (100%) the investigated *E. coli* strains resisted antimicrobials corresponding to at least three antimicrobial classes. The variation of the present study results concerning antimicrobial susceptibility as it lower than Sharada *et al.* (2010) and higher than those reported by Li *et al*. (2015) could be attributed to the variation in the prevention and control scheme applied in various regions as certain areas substituted antibiotic growth promoters and anticoccidial drugs by a drug-free program that is usually associates higher productivity in broiler chicken farms (Gaucher *et al*., 2015). In addition to the regime applied in the treatment of bacterial infection as the propagation of resistance to several antimicrobial classes were attributed to the intensive use of the same antibiotic in different cycle (Schwaiger *et al*., 2013). What is more, bacteria gain resistance by time; in 1985 resistance of *E. coli* against amikacin was 11.43% (Gyurov, 1985) and reached to 62.5% non-susceptible (12.5 resistance and 50% intermediate pattern) in the present study.

Reporting resistance against carbapenems (ertapenem, 12.5%), the last treatment of Gram-negative bacteria in intensive care unit is a worrisome and this result conceded with the previously published results of Carissa *et al*. (2013). MDR of the isolates under test reversed to detection of ESBLs and AmpC characters and all *E. coli* under test were MDR against at least three antimicrobial classes of antibiotics that in concordance with De Jong *et al*. (2012).

Concerning to ESBLs/AmpC detection, our study revealed the presence of ESBLs in 3/8 (37.5%) of the *E. coli* isolates under test and this lower than previously published by Smet *et al*. (2008) who detected ESBL in 45% of the investigated *E. coli*, whereas, Carissa *et al*. (2013) detected lower prevalence of ESBLs in *E. coli* strains among different species of poultry (22.2%). On the other hand, one *E. coli* isolate only showed AmpC among eight investigated isolates (12.5%) that lower the results of Nahla *et al*. (2014) and Smet et al. (2008) who detected AmpC among 86.7 and 43% of *E. coli* isolates they tested. The public health hazard is granted by the probability of transferring ESBL-producing *E. coli* strains from chicken to man and other animals (Carissa *et al*., 2013).

The results of the current study revealed the association of many bacterial species with broiler proventriculitis and those of public health concern showed multidrug resistance with 100%, so we have to keep an eye on the condition and manage it correctly to prevent their influence on both broiler sector and public health.

REFERENCES

Ahmed, R. M. A. (2014). Molecular detection of *E. coli* virulence genes causing broiler cellulitis in Ismailia Governorate. Thesis (M.S.), Suez Canal University - Faculty of Veterinary Medicine, Department of Bacteriology, Immunology and Mycology.

Ashraf, A. A.; Ahmed, A. A. M.; Samir, A. A.; El Hofy, F. I. and El Mougy E. E. A. (2014). Detection of some virulence genes of avian pathogenic *E. coli* by polymerase chain reaction. Benha Veterinary Medical Journal, 26(2):159-176.

Bayyari, G. R.; Huff, W. E.; Balog, J. M.; Rath, N. C. and Beasley, J. N. (1995). Experimental reproduction of proventriculitis using homogenates of proventricuiar tissue. Oxford Journals, Science & Mathematics, Poultry Science, 74(11): 1799-1809.

Birchard, Stephen J., and Robert G. Sherding (2005). Saunders Manual of Small Animal Practice. Elsevier Health Sciences.

Carissa, D.; Edward, N.; Michael, A.; Chika, E.; Charles, E. (2013). Extended-spectrum Beta-lactamase-producing *Escherichia coli* strains of poultry origin in Owerri, Nigeria. World Journal of Medical Sciences, 8(4):349-354.

Carmo, L.P.; Nielsen, L.R.; da Costa, P.M. and Alban, L. (2014). Exposure assessment of extended-spectrum beta-lactamases/AmpC beta-lactamases-producing Escherichia coli in meat in Denmark. Infect. Ecol. Epidemiol. 4: 22924.

Clinical and Laboratory Standards Institute (CLSI, 2013). Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. M100-S23, pp: 1-61.

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.

De Jong, A.; Thomas, V.; Simjee, S.; Godinho, K.; Schiessl, B.; Klein, U.; Butty, P.; Valle, M.; Marion, H. and Shryock, T. R. (2012). Pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food-producing animals. The Journal of Antimicrobial Chemotherapy, 67(3): 638–651.

Dormitorio, T.V.; Giambrone, J.J. and Hoerr, F.J. (2007). Transmissible proventriculitis in broilers. Avian Pathology, 36 (2): 87-91.

Edquist, P.; Ringman, M.; Liljequist, B.O. and Wisell, K.T. (2013). Phenotypic detection of plasmid-acquired AmpC in *Escherichia coli* evaluation of screening criteria and performance of two commercial methods for the phenotypic confirmation of AmpC production. Eur. J. Clin. Microbiol. Infect. Dis., 32: 1205-1210.

Edwards, P. R. and Ewing, W. H. (1972). Identification of Enterobacteriaceae. Minneapolis, Burgess Publishing Co., pp. 709.

Garrec, H.; Drieux-Rouzet, L.; Golmard, J.; Jarlier, V. and Robert, J. (2011). Comparison of nine phenotypic methods for detection of extended-spectrum β-lactamase production by Enterobacteriaceae. J. Clin. Microbiol., 49: 1048-1057.

Gaucher, M. L.; Quessy, S.; Letellier, A.; Arsenault, J. and Boulianne M. (2015). Impact of a drug-free program on broiler chicken growth performances, gut health, *Clostridium perfringens* and *Campylobacter jejuni* occurrences at the farm level. Poultry Science, 94(8): 1791–1801.

Gyurov, B. (1985). Sensitivity to drugs of *Escherichia coli* strains isolated from poultry with colisepticemia. Veterinarno-Meditsinski Nauki, 22(5): 16–24.

Hafez, M. H. (2011). Enteric diseases of poultry with special attention to *Clostridium perfringens*. Pak. Vet. J., 31(3): 175-184.

Hassan, H. M. (2013). Molecular studies on the distribution of associated virulence genes with avian pathogenic *Escherichia coli* (APEC). Thesis (Ph.D.) – Cairo University – Faculty of Veterinary Medicine – Department of Microbiology.

Huff, G. R.; Zheng, Q.; Newberry, L. A.; Huff, W. E.; Balog, J. M.; Rath, N. C.; Kim, K. S.; Martin, E. M.; Goeke, S. C. and Skeeles, J. K. (2001). Viral and bacterial agents associated with experimental transmission of infectious proventriculitis of broiler chickens. Avian Disease, 45(4):828-843.

Kamel, G. M.; Ezz eldeen, N. A.; El-Mishad, M. Y. and Ezzat R. F. (2011). Susceptibility pattern of *Pseudomonas aeruginosa* against antimicrobial agents and some plant extracts with focus on its prevalence in different sources. Global Veterinaria, 6 (1): 61-72.

Karki, K; Manandhar, P. and Koirala, P (2009). Clinical laboratory epidemiological investigation of hemorrhagic proventriculitis and gizzard erosion in Nepal. Veterinary World, 2 (2): 54-56.

Kebede, F. (2010). *Pseudomonas* infection in chickens. Journal of Veterinary Medicine and Animal Health, 2(4): 55-58.

Khelfa D.G. and Morsy, E. A. (2015). Incidence and distribution of some aerobic bacterial agents associated with high chick mortality in some broiler flocks in Egypt. Middle East Journal of Applied Sciences, 05(02): 383-394.

Landers, T. F.; Cohen, B.; Wittum, T. E. and Larson E. L. (2012). A review of antibiotic use in food animals "Perspective, policy, and potential". Public Health Reports, 127(1): 4-22.

Lee, S.H.; Loh, Y.X.; Lee, J.J.; Liu, C.S. and Chu, C. (2015). Antimicrobial consumption and resistance in five Gram-negative bacterial species in a hospital from 2003 to 2011. J. Microbiol. Immunol. Infect., 48(6): 647-654.

LeStrange, K. (2013). Correlating genetic and phenotypic characteristics in avian pathogenic *Escherichia coli* as a model environmental pathogen. Thesis (M S), Science in Food Science, University of Delaware. http://udspace.udel.edu/handle/19716/12956.

Li, Y.; Chen, L.; Wu, X. and Huo, S. (2015). Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Poultry Science, 94:601–611.

Nahla, M.S.; Eman, T.A. and Rana, M.A. (2014): Study of beta-lactamase and extended-spectrum beta-lactamase production by *Escherichia coli* in broiler farms in Sulaimania province. Applied Science Reports, 7 (1): 19-24.

Osman, K. M.; Alabady, M. S.; Ata, N. S. S. M.; Ezzeldin, N. A. and Aly, M. A. K. (2010). Genotypic characterization of *Pseudomonas aeruginosa* isolated from human and animal sources in Egypt. Zoonoses Public Health, 57: 329-338.

Rafiee, R.; Eftekhar, F.; Tabatabaei, S.A. and Minaee Tehrani, D. (2014): Prevalence of extended-spectrum and metallo β-lactamase production in AmpC β-lactamase producing *Pseudomonas aeruginosa* isolates from burns. Jundishapur J. Microbiol., 7(9): e16436.

Schwaiger, K.; Bauer, J. and Hölzel, C. S. (2013). Selection and persistence of antimicrobial-resistant *Escherichia coli* including extended-spectrum β-lactamase producers in different poultry flocks on one chicken farm. Microbial drug resistance (Larchmont, N.Y.), 19(6): 498–506.

Sharada, R.; Wilfred ruban, S. and Thiyageeswaran, M. (2010). Isolation, characterization and antibiotic resistance pattern of *Escherichia coli* from poultry. American-Eurasian journal of scientific research 5(1): 18-22.

Smet, A.; Martel, A; Persoons, D.; Dewulf, J.; Heyndrickx, M.; Catry, B.; Herman, L.; Haesebrouck, F. and Butaye P. (2008). Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. Antimicrobial Agents and Chemotherapy, 52(4): 1238–1243.

Tartor, Y.H. and El-Naenaeey, E.Y. (2016). RT-PCR detection of exotoxin genes expression in multidrug resistant *Pseudomonas aeruginosa*. Cell Mol. Biol., 62(1):56-62.

Timbermont, L.; Lanckriet, A.; Gholamiandehkordi, A. R.; Pasmans, F.; Martel, A.; Haesebrouck, F.; Ducatelle, R. and Van Immerseel, F. (2009). Origin of *Clostridium Perfringens* isolates determines the ability to induce necrotic enteritis in broilers. Comparative Immunology, Microbiology and Infectious Diseases; 32(6): 503–512.

Venkanagouda, G. K. and Upadhye A. S. (1996). Bacterial etiology of early chick mortality. Indian Vet., J., 73: 253-256.

Walker, S. E.; Sander, J. E.; Cline, J. L. and J. S. Helton (2002). Characterization of *Pseudomonas aeruginosa* isolates associated with mortality in broiler chicks. Avian Diseases, 46(4): 1045–1050.