International Journal of Microbiology and Biotechnology
2020; 5(2): 41-47
http://www.sciencepublishinggroup.com/j/ijmb
doi: 10.11648/j.ijmb.20200502.11
ISSN: 2578-9678 (Print); ISSN: 2578-9686 (Online)

Emergence of Multi-Drug Resistant *Pseudomonas aeruginosa* in Broiler Chicks

Jihan Mostafa Badr[1], Fawzy Reyad El Saidy[2], Amal Abdelwahed Abdelfattah[1, *]

[1]Department of Poultry Diseases, Animal Health Research Institute, Dokki, Giza, Egypt

[2]Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Bani Sweif University, Bani Sweif, Egypt

Email address:

jihanbdr@yahoo.com (J. M. Badr), Dr_amalabdelwahwed2007@yahoo.com (A. A. Abdelfattah)

[*]Corresponding author

To cite this article:

Jihan Mostafa Badr, Fawzy Reyad El Saidy, Amal Abdelwahed Abdelfattah. Emergence of Multi-Drug Resistant *Pseudomonas aeruginosa* in Broiler Chicks. *International Journal of Microbiology and Biotechnology*. Vol. 5, No. 2, 2020, pp. 41-47.
doi: 10.11648/j.ijmb.20200502.11

Received: November 2, 2019; **Accepted**: April 8, 2020; **Published**: April 17, 2020

Abstract: *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium responsible for serious problems in poultry farms and is one of the most relevant pathogens causing human opportunistic infections. Poultry has been suggested to be a reservoir for antibiotic resistance bacteria that may aggravate the problem of pseudomonas infection. The present work was applied to investigate the drug resistance among *P. aeruginosa* isolated from chicks in different poultry farms and its hazard to human health. A total of 460 broiler chicks constituted 46 private farms were examined for the presence of *P. aeruginosa* infection. Samples were collected from internal organs of broiler chicks subjected to bacteriological examination and identification. Thirty two *P. aeruginosa* isolates were recovered from 183 broiler chicks (39.78%) were positive for isolation of *P. aeruginosa* constituted 32 positive farms with a prevalence of (69.57%). Antimicrobial drug assay was applied against 14 different antimicrobial agents constituted 10 antibiotic genera. The majority of the isolates were sensitive to 3rd generation quinolones (levofloxacin, Enrofloxacin and Danofloxacin) in incidences 81.25%, 59.375% and 46.875% respectively. The sensitivity to Aminoglycosides (Gentamycin and Tobramycin) ranged from 37.5% to 43.75% while Polymyxins showed 34.375%. The least sensitivity was towards Phenicoles (Florfenicol) and Tetracyclines (Doxycycline), 9.375% for each. Antibiotic resistant pattern of the isolated *P. aeruginosa* revealed that all the isolates were multidrug resistant with MAR*indices* for most isolates was determined to be > 0.6 indicating the misuse of antibiotics in poultry farms. *P. aeruginosa* isolates showed complete resistance towards cefotaxime, cefradine, nalidixic acid, and spectinomycin (100% for each) with high resistance rates among sulfamethxazole/ trimethoprim, amoxicillin (96.875, 93.75, respectively), doxycycline and florfenicol (90.625 for each), followed by colistine sulphate, gentamycin, tobramycin, danofloxacin, and enrofloxacin with percentages of 68.75, 62.5,53.1 56.25 3, 40.6% respectively, which all posing a significant threat to public health. In conclusion poultry farms should take strict measures to improve the management of animal nutrition and production hygiene to overcome possible sources of *pseudomonas* infection. The misuse of antibiotics leads to the development of resistant bacteria that may transfer from poultry to humans. Strict supervision and enforcement of laws to control antibiotic usage in food chain within established safe levels must be done.

Keywords: *Pseudomonas aeruginosa*, Poultry Farms, Drug Resistance, Public Health Hazard

1. Introduction

Pseudomonas aeruginosa is Gram-negative, aerobic, motile, non-capsulated and non-spore forming bacteria. The organisms are ubiquitous, often associated with soil, water and humid environments [1]. *P. aeruginosa* is the most predominant pseudomonas species causing mortality among birds specially chickens. *P. aeruginosa* in chickens is associated with respiratory manifestations, diarrhea and septicemia [2]. The organism is ubiquitous, often associated with soil, water, and humid environments. Generally, it is considered to be an opportunistic organism that produces respiratory infections, septicemia and other forms when

introduced into tissues of susceptible birds with greatest losses in very young birds [3]. *P. aeruginosa* is the most common pseudomonad causing infections. Virulent strains can cause diarrhea, dehydration, dyspnea, septicemia and death to newly hatched chicks. The infection may occur through skin wounds or contaminated vaccines, egg dipping or egg inoculation or through contamination of needles used for injection, infection can also spread from infected to susceptible flocks on the same premises under conditions.

P. aeruginosa is capable of infecting/colonizing a wide range of ecological niches, including aquatic and soil habitats, animals and plants [4]. *P. aeruginosa*, is a ubiquitous microorganism, is one of the most relevant pathogens causing human opportunistic infections [5]. Also, *P. aeruginosa* is one of the most frequent and severe causes of acute nosocomial infections, particularly affecting immunocompromised patients or those admitted to the Intensive Care Unit (ICU) [6]. Likewise, *P. aeruginosa* is the most frequent and severe driver of chronic respiratory infections in patients suffering from cystic fibrosis (CF) or other chronic underlying diseases [7].

On the other hand, Resistance to antimicrobials is a global public health concern that is impacted by both human and non-human usage. Antimicrobial resistance is an emerging concern to public health, and food-producing animals are known to be a potential source for transmission of resistant bacteria to humans. Recently, the importance of poultry as a source of foodborne diseases and antimicrobial-resistant organisms was highlighted [8, 9, 10]. With the global increase in antibiotic resistance, there is the need for all countries to preserve the effectiveness of essential antibiotics, especially those that are of critical importance [11, 12] for human health. The present work was applied to investigate the drug resistant among *P. aeruginosa* isolated from chicks as food animal and its hazard to human health.

2. Materials and Methods

2.1. Sample Collection

A total of 460 broiler chicks (320 freshly dead and 140 living ailing (were collected from 46 poultry farms from different governorates in Egypt (Giza, Dakahlia, Kaliobia, Monofia, Kena and Aswan). Samples were taken from liver, heart, lung, yolk and bone marrow were collected under complete aseptic conditions and brought to the bacteriology unit of poultry diseases department- Animal Health Research Institute, Dokki, Giza and submitted to bacteriological examination.

2.2. Isolation of Pseudomonas

Isolation and identification of pseudomonas from collected samples was done according to Koneman et al., 1997 and Quinn et al., 2011 [13, 14]. In brief, A loopful from tested samples were directly taken and inoculated into nutrient broth then a loopful from the previously incubated tubes was streaked on to the surface of nutrient agar, pseudomonas cetrimide ager and MacConkey agar, and incubated at 37°C for 24 hours. Then a loop full of inoculated nutrient broth was streaked on to pseudomonas cetrimide ager (selective media), nutrient agar and MacConkey agar, incubated aerobically for 24hrs at 37°C. The suspected colonies were identified biochemically according to [14].

2.3. Antimicrobial Drug Assay

Antimicrobial in-vitro susceptibility testing of the isolated pseudomonas strains against various chemotherapeutics was screened for susceptibility against 14 antibiotics by disc diffusion method and the interpretation was assigned as sensitive, intermediate and resistant performed according to the recommendations of The Clinical and Laboratory Standard Institute [15]. The antibiotics used for the susceptibility testing are classified by the WHO according to importance to human medicine [16, 17] (table 1) as critical important drugs (Category I) included: Penicillins: amoxicillin (20 µg); 3rd generation Cephalosporins: Cefotaxime (30 µg); Fluoroquinolones: Enrofloxacin (10 µg), Danofloxacin (10 µg), Levofloxacin (5 µg) and Nalidixic acid (30 µg) and Polymyxins: Colistin (10 µg). The High Important (Category II) included: Aminoglycosides: gentamycin (10 µg); and Tobramycin (10 µg), 1st generation Cephalosporins: Cephradine (30 µg), Sulfonamid & Trimethoprim: Sulphamethoxazole /Trimethoprim (25 µg), Tetracyclines: doxycycline (30 µg) and Phenicols: florfenicol (30 µg); and the important (Category III) included: aminocyclitols: spectinomycin (10 µg).

Table 1. *Classification of antibiotics categorized their important in human and veterinary medicine.*

Antibiotic	Disc conc.	Antimicrobial group	Antimicrobial category	Medical importance
Gentamicin (CN)	10 µg	Aminoglycosides	II	High Important
Tobramycin (TOB)	10 µg	Aminoglycosides	II	High Important
Cephradine (CE)	30 µg	1st generation Cephalosporins	II	High Important
Cefotaxime (CTX)	30 µg	3rd generation Cephalosporins	I	critical important
Enrofloxacin (ENR)	10 µg	Fluoroquinolones	I	critical important
Danofloxacin (DFX)	10 µg	Fluoroquinolones	I	critical important
Levofloxacin (LEV)	5 µg	Fluoroquinolones	I	critical important
Nalidixic acid (NA)	30 µg	Fluoroquinolones	I	critical important
Amoxycillin (AMX)	20 µg	Penicillins	I	critical important
Florfenicol (FFC)	30 µg	Phenicoles	II	High Important
Colistin sulphat (CT)	10 µg	Polymyxins	I	critical important
Sulphamethoxazole/Trimethoprim (SXT)	25 µg	Sulfonamid & Trimethoprim	II	High Important
Doxycycline (DO)	30 µg	Tetracyclines	II	High Important
Spectinomycin (SH)	10 µg	aminocyclitols	III	Important

2.4. Determination of Multi-drug Resistance Index (MDRI)

MDR index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed [18]. Isolates with MDRI values of more than 0.2 were considered highly resistant.

$$\text{MDR index} = \frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotics used}}$$

3. Results

3.1. Prevalence of Isolation of P. aeruginosa

In this investigation, the examination of 460 chicks collected from 46 private farms (10 chick per farm) revealed that 32 farms (69.57%) were positive for pseudomonas isolation from which 183 chicks (39.78%) were positive for isolation of *P. aeruginosa* (table 2).

3.2. Antibiogram of the Isolated P. aeruginosa

Sensitivity testing of the isolated *P. aeruginosa* revealed that most of the isolates were sensitive to Levofloxacin (81.25%) followed by Enrofloxacin (59.375%), Danofloxacin (46.875%) and Tobramycin (43.75%) while, sensitivity to Gentamycin and Colistin sulphate were 37.5%, 34.375% respectively and the least sensitivity was towards Florfenicol and Doxycycline (9.375% for each), table 3.

3.3. Multi-drug Resistance Index (MDRI)

The investigation of antibiotic resistant of the isolated *P. aeruginosa* revealed that all the isolates were multidrug resistant to at least 1 agent in at least 5 antimicrobial categories used recording a multidrug resistant index ranged of 0.4 to 0.9 (table 4).

Table 2. *Prevalence of isolation of Pseudomonas aeruginosa from chick's samples.*

no. of Farms	No. of chicks/ farm	Total no. of chicks	positive farms		positive chicks	
			No.	%	No.	%
46	10	460	32	69.57%	183	39.78

Table 3. *Sensitivity of Pseudomonas aeruginosa isolated from chicks.*

Antibacterial agent	Disc Conc.	Pseudomonas isolates (n=32)					
		Resistance		Intermediate		Sensitive	
		NO.	%	NO.	%	NO.	%
Amoxycillin (AMC)	20 µg	28	87.5	2	6.25	2	6.25
Cefotaxime (CTX)	30 µg	11	34.375	21	65.625	0	0
Cephradine (CE)	30 µg	32	100	0	0	0	0
Colistin sulphate (CT)	10 µg	21	65.625	0	0	11	34.375
Danofloxacin (DFX)	10 µg	12	37.5	5	15.625	15	46.875
Doxycycline (DO)	30 µg	24	75	5	15.625	3	9.375
Enrofloxacin (ENR)	10 µg	8	25	5	15.625	19	59.375
Florfenicol (FFC)	30 µg	29	90.625	1	3.125	3	9.375
Gentamycin (CN)	10 µg	18	56.25	2	6.25	12	37.5
Levofloxacin (LEV)	5 µg	0	0	6	18.75	26	81.25
Nalidixic acid (NA)	30 µg	32	100	0	0	0	0
Spectinomycin (SH)	10 µg	32	100	0	0	0	0
Tobramycin (TOB)	10 µg	14	43.75	4	12.5	14	43.75
Trimethoprim/Sulphamethoxazole (SXT)	25 µg	26	81.25	5	15.625	1	3.125

3.4. Drug Resistant P. aeruginosa in Accordance to Public Health Hazard

Studying Antibiotic resistance to different antibiotics among *P. aeruginosa* isolated from broiler chicks in accordance to their importance to human health (table 4), showed complete resistance towards cefotaxime, and nalidixic acid (with percentages 100% for each) followed by amoxicillin, colistine sulphate, danofloxacin, enrofloxacin (93.75, 65.625, 53.125, %40.625 respectively) which all classified according to WHO as a critical Important antibiotics for human use (Category I). Also, complete resistance were recorded among highly important antibiotics (Category II) as cefradine, and high resistance rates among sulfamethxazole/ trimethoprim, doxycycline, florfenicol, gentamycin and tobramycin (96.88, 90.63, 62.5,%56.25 respectively). Complete resistance was found in all isolates towards spectinomycin which included as an important drug for human medicine which all classified according to WHO as critical important antibiotics for human use (Category I). Also, complete resistance were recorded among highly important antibiotics (Category II) as cefradine, and high resistance rates among sulfamethxazole/ trimethoprim, doxycycline, florfenicol, gentamycin and tobramycin (96.88, 90.63, 62.5,%56.25 respectively Complete resistance was found in all isolates towards spectinomycin which included as an important drug for human medicine (CategoryIII).

Table 4. *Drug resistant patterns in Pseudomonas aeruginosa isolated from chicks.*

isolate No.	Resistant Antibiotic	No. of resistant antibiotic	No. of resistant antibiotic categories	MDR_{INDEX}
1C	AMC, CE, SXT, CTX, FFC, DO, NA, SH	8	7	0.6
2C	AMC, CE, TOB, SXT, CTX, FFC, DO, NA, CN, SH	10	9	0.7
3C	AMC, CE, CTX FFC, NA, SH	6	5	0.4
4C	AMC, CE, CT, SXT, CTX, FFC, DO, NA, CN, SH	10	9	0.7
5C	AMC, CE, SXT, CTX, FFC, DO, NA, SH	8	7	0.6
6C	AMC, CE, CT, SXT, CTX, FFC, DO, NA, SH	9	8	0.6
7C	AMC, CE, DFX CTX, FFC, DO, NA, CN, SH	9	7	0.6
8C	AMC, CE, DFX, TOB, CT, SXT, CTX, ENR, FFC, DO, NA, CN, SH	13	9	0.9
9C	AMC, CE, TOB, CTX FFC, SH	7	5	0.5
10C	AMC, CE, DFX, TOB, CTX, ENR, FFC, DO, NA, CN, SH	11	7	0.8
11C	AMC, CE, CT, SXT, CTX, FFC, DO, NA, CN, SH	10	9	0.7
12C	AMC, CE, DFX, TOB, CT, SXT, CTX, ENR, FFC, DO, NA, CN, SH	13	9	0.9
13C	AMC, CE, DFX, TOB, CT, SXT, CTX, ENR, FFC, DO, NA, CN, SH	13	9	0.9
14C	CE, SXT, CTX, FFC, DO, NA, CN, SH	8	7	0.6
15C	CE, TOB, SXT, CTX, FFC, NA, CN, SH	8	6	0.6
16C	AMC, CE, CT, SXT, CTX, FFC, NA, SH	8	7	0.6
17C	CE, DFX, TOB, CT, CTX, ENR, DO, NA, CN, SH	10	6	0.7
18C	AMC, CE, CT, SXT, CTX, FFC, DO, NA, SH.	9	8	0.6
19C	AMC, CE, CT, SXT, CTX, FFC, DO, NA, SH.	9	8	0.6
20C	AMC, CE, TOB, CT, SXT, CTX, FFC, DO, NA, SH.	10	9	0.7
21C	AMC, CE, DFX, TOB, CT, SXT, CTX, FFC, DO, NA, SH	12	9	0.9
22C	AMC, CE, DFX, TOB, CT, SXT, CTX, ENR, FFC, DO, NA, SH.	11	8	0.8
23C	CE, DFX, TOB, CT, SXT, CTX, ENR, DO, NA, SH.	11	7	0.8
24C	AMC, CE, DFX, TOB, CT, SXT, CTX, FFC, NA, CN, SH	11	8	0.8
25C	AMC, CE, TOB, CT, SXT, CTX, FFC, DO, NA, CN, SH.	11	8	0.8
26C	CE, DFX, CT, SXT, CTX, FFC, DO, NA, SH.	9	7	0.6
27C	AMC, CE, CT, CTX, FFC, NA, SH.	7	6	0.5
28C	AMC, CE, CT, SXT, CTX, FFC, DO, NA, SH.	9	8	0.6
29C	AMC, CE, DFX, CT, SXT, CTX, ENR, FFC, DO, NA, CN, SH	13	10	0.9
30C	AMC, CE, DFX, TOB, CT, SXT, CTX, ENR, FFC, DO, NA, CN, SH	13	9	0.9
31C	AMC, CE, CT, SXT, CTX, NA, CN, SH.	8	7	0.6
32C	AMC, CE, SXT, CTX, FFC, DO, NA, CN, SH.	9	8	0.6

Table 5. *Antibiotic resistance among P. aeruginosa isolated from broiler chicks in accordance to their importance to human health.*

Antimicrobial group	Antibiotic	Antibiotic category	No. of Strain	Resistant no.	Resistant%
Aminoglycosides	Gentamicin (CN)	II	32	20	62.5
	Tobramycin (TOB)	II	32	18	56.25
Cephalosporins	Cefotaxime (CTX)	I	32	32	100
	Cephradine (CE)	II	32	32	100
Fluoroquinolones	Enrofloxacin (ENR)	I	32	13	40.63
	Danofloxacin (DFX)	I	32	17	53.13
	Levofloxacin (LEV)	I	32	6	18.75
	Nalidixic acid (NA)	I	32	32	100
Penicillins	Amoxycillin (AMC)	I	32	30	93.75
Phenicoles	Florfenicol (FFC)	II	32	29	90.63
Polymyxins	Colistin (CT)	I	32	22	68.75
Sulfonamid & Trimethoprim	Sulphamethoxazole/Trimethoprim (SXT)	II	32	31	96.88
Tetracyclines	Doxycycline (DO)	II	32	29	90.63
aminocyclitols	spectinomycin	III	32	32	100

4. Discussion

Pseudomonas is a good example of environment associated infection and may cause a serious problem in poultry farms.

Birds at any age may be infected; young birds are most susceptible. Severely stressed or immunodeficient birds and concurrent infections with viruses and other bacteria enhance susceptibility to Pseudomonas infection.

In this investigation, a high prevalence of pseudomonas positive farm (69.57%) with high incidence of pseudomonas isolation from which the examined chicks (39.78%) was detected which constitute a hazard for both poultry and public health

These records are much higher than that reported by Saif–Edin, 1983 [19] who isolated the same organism with an incidence of 21.6% at kena Governorate.

Mohamed, 1996 [20] found that 13 strains of *P. aeruginosa* isolated from 150 baby chicks were collected from twenty broiler and balady flocks from different localities at Sharkia province during two years of

investigation. One hundred were in diseased conditions while the remaining was freshly dead, the incidence was 8.7%. Mohamed, 2004 [21] and Hebat Allah, 2004, [22] isolated *P. aeruginosa* from baby chicks at Assiut governorate in percentage of 17.6% for both. Hassan, 2013 [23] examined 150 samples from Hubbard and Ross broiler chickens (130 samples from diseased chickens and 20 samples from apparently normal chickens) and 50 samples from one- day-old chicks (40 samples from diseased chicks and 10 from apparently normal chicks) and Indicated that 38 and 4 isolates of *P. aeruginosa* were isolated from samples of chickens and chicks, with an incidence of 25.3% and 10% respectively. The elevation in the incidence of isolation indicated increased environmental pollution and decreased in the biosecurity programs applied in poultry farms as most developing countries as Egypt suffering from the release of pharmaceutical waste containing active pharmaceutical compounds from antibiotic manufacturing plants, into the rivers or the environment, which constituted a focus of infection with resistant organisms posing a significant threat to public health [24, 25].

On the other hands, Sensitivity testing of the isolated *P. aeruginosa* revealed that most isolates were mostly sensitive to 3rd generation quinolones (levofloxacin, Enrofloxacin and Danofloxacin) in incidences (81.25%) to (59.375%), (46.875%) respectively while complete resistant to 1st generation quinolones (Nalidixic acid). Also, the sensitivity to Aminoglycosides (Gentamycin and Tobramycin) ranged from 37.5% to 43.75% while Polymyxins showed 34.375%. The least sensitivity was towards Phenicoles (Florfenicol) and Tetracyclines (Doxycycline), 9.375% for each

Antibiotic sensitivity of the isolated *P. aeruginosa* was also detected by many authors. Kurkure *et al*., 2001 [26] stated that *P. aeruginosa* isolated from broiler were sensitive to gentamycin and ciprofloxacin in a percentage 88.57% and 62.85% respectively. Abd El- Tawab *et al.,* 2014 [27] reported that *P. aeruginosa* isolates were sensitive to colistin sulphate and norfloxacin. Also, Tawakol et al., 2018 [28] reported that *P. aeruginosa* isolates were highly sensitive to ciprofloxacin, colistin sulphate, norfloxacin and gentamycin with percentages of 90%, 90%, 70% and 65% respectively while Doxycycline, penicillin, ceftazidime and streptomycin showed resistance with a percentages of 75%, 65%, 60% and 50% respectively. The most worrisome characteristic of *P. aeruginosa* is its low antibiotic susceptibility, which is attributable to low permeability of the bacterial cellular envelopes and action of multidrug efflux pumps. In addition to this intrinsic resistance, *P. aeruginosa* can get resistance by mutation either in chromosomally encoded genes or by the horizontal gene transfers of antibiotic resistance determinants [29, 30].

On studying of antibiotic resistant pattern of the isolated *P. aeruginosa* the results showed that all the isolates were multidrug resistant to at least 1 agent in at least 5 antimicrobial categories used recording a multidrug resistant index ranged of 0.4 to 0.9 (table 4).

Unfortunately, rates of antibiotic resistance in *P. aeruginosa* are increasing worldwide [29, 31]. However, in the majority of the published studies, multidrug resistance was defined as resistance to at least three drugs from a variety of antibiotic classes, mainly aminoglycosides, antipseudomonal penicillins, cephalosporins, carbapenems and fluoroquinolones [32]. In Egypt, there is a lack of information on the degree of antimicrobial usage in poultry, medications through growth promoters included in their feed and among curative or preventive medicines. Another important element that has been overlooked in developing countries is the release of environmental pollution through pharmaceutical waste containing active pharmaceutical compounds from antibiotic manufacturing plants, into the rivers or the environment, contributes to the selection of antibiotic resistant organisms posing a significant threat to public health [24, 25]. *Pseudomonas aeruginosa* is also an opportunistic pathogen in human [31]. Antimicrobials used in poultry production are employed for therapeutic and non-therapeutic purposes [33, 34]. Many of these antibiotics employed in poultry production also serve as essential medicines for use in humans in many countries [33].

On the other hand, Investigating the drug- resistance among *P. aeruginosa* isolated from broiler chicks in accordance to their importance to human health (table 5), revealed complete resistance towards 3rd generation Cephalosporins and 1st generation quinolones (Nalidixic acid) followed by amoxicillin, colistine sulphate and 3rd generation quinolones which all classified according to WHO as a critical Important antibiotic for human use (Category I). Also, complete resistance was recorded among 1st generation cephalosporines, (cefradine) which classified as highly important antibiotics (Category II). A high resistance rates among Sulfonamid & Trimethoprim, Tetracyclines Phenicoles, Aminoglycosides and Complete resistance was found in all isolates towards the aminocyclitols spectinomycin which included as an important drug for human medicine (Category III).

Many of these antibiotics that employed in animal production also serve as essential medicines for use in humans in many countries [33, 35]. The misuse of antibiotics in food-animal production is one of the most important factors contributing to the global surge and spread in antibiotic resistance. [36, 37, 38]. With the global increase in antibiotic resistance, there is the need for all countries to preserve the effectiveness of essential antibiotics, especially those that are of critical importance [12, 39]. Also, careful use of antibiotics and the establishment of scientific monitoring systems are the best way to limit the adverse effects of the misapplication of antibiotics and to ensure the safety of animal-derived food and environment [40]. On the other hand, more researches for the development of new efficient and safe antibiotic alternatives with studying the effects of combined use of antibiotics and their alternatives for maintaining a healthy agricultural economy and preservation of potent antibiotics for efficacious therapy in human.

References

[1] K. D. Mena and C. P. Gerba (2009): Risk assessment of Pseudomonas aeruginosa in water. Rev Environ Contam Toxicol. 201: 71-115.

[2] Fekadu Kebede, (2010): *Pseudomonas* infection in chickens. Journal of Veterinary Medicine and Animal Health Vol. 2 (4), pp. 55-58, November 2010 Available online at http://www.academicjournals.org/JVMAH©2010 Academic Journals.

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

[4] M. W. Silby, C. Winstanley, S. A. Godfrey, S. B. Levy and R. W. Jackson (2011): *Pseudomonas* genomes: diverse and adaptable. FEMS Microbiol Rev 35: 652-80.

[5] S. L. Gellatly and R. E. Hancock (2013) *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis 67: 159-173.

[6] J. L. Vincent (2003): Nosocomial infections in adult intensive-care units. Lancet 61: 2068-77.

[7] A. Oliver, A. Mena, M. D. Macià (2008): Evolution of *Pseudomonas aeruginosa* pathogenicity: from acute to chronic infections. Baquero, F., Nombela, C., G. H. Cassell, J. A. Gutiérrez, (Eds). Evolutionary biology of bacterial and fungal pathogens. ASM Press pp 433-444. ISBN 978-1-55581-414-4. org/10.1098/rstb.2013.0571.

[8] PHAC. CIPARS 2008 Annual Report [homepage on the Internet]. Public Health Agency of Canada [updated 2011 October 26]. Available from: http://www.phac-aspc.gc.ca/cipars-picra/2008/index-eng.php Last accessed May 21, 2012.

[9] US FDA. NARMS Retail Meat Annual Report (2009): (homepage on the Internet), United States Food and Drugs Administration [updated 2011 June 02]. Available from: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistance Monitoring

[10] M. Harisberger, S. Gobeli, R. Hoop, J. Dewulf, V. Perreten and G. Regula (2011): Antimicrobial resistance in Swiss laying hens, prevalence and risk factors. Zoonoses Public Health. 2011 Sep; 58 (6): 377-87. doi: 10.1111/j.1863-2378.2010.01376.x. Epub 2010 Oct 12.

[11] WHO (2010): WHO model list of essential medicines. WHO pp: 1-43.

[12] G. Tomson and I. Vlad (2014): The need to look at antibiotic resistance from a health systems perspective. Ups J Med Sci 119: 117-124.

[13] E. W. Koneman ; S. D. Allen ; W. M. Janda; P. C. Schreckenberger, and W. C. Winn, (1997): Diagnostic Microbiology. 5th Ed. Philadelphia. Newyork.

[14] P. Quinn, B. Markey, W. Donnelly, F. Leonard, S. Fanning and D. Maguire (2011): Veterinary Microbiology and Microbial Disease (2nd edition), Wiley-Blackwell. A John Wiley& Sons, Ltd., Publication.

[15] CLSI, (2017): (Clinical and Laboratory Standards Institute), Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI Supplement M100, Clinical and Laboratory Standards Institute, Wayne, PA, 2017.

[16] WHO. (2017): World Health Organization, Critically Important Antimicrobials for Human Medicine – 5th Rev, World Health Organization, Geneva, 2017.

[17] P. C. Collignon, M. C. John, A. Antoine, A. M. Scott and Awa Aidara– Kane (2016): World Health Organization Ranking Of Antimicrobials According To Their Importance In Human Medicine: A Critical Step For Developing Risk Management Strategies To Control Antimicrobial Resistance From Food Animal Production. Clinical infectious diseases, volum 63, issue 8. 15 october 2016, pages 1087– 1093. http://doi.org/10.1093/cid/ciw475

[18] A. Chandran; A. A. M. Hatha; S. Varghese and K. M. Sheeja (2008): Prevalence of multidrug resistant E. coli serotypes in a Tropical Estuary, India. Microbes Environ. 23 (2): 153-158.

[19] Saif–Edin, M. El – Bakry (1983): Some studies on Pseudomonas infection in chickens. M. V. Sc., Thesis Presented to Fac. of Vet. Med., Assiut Univ.

[20] E. A. A. Mohamed (1996): Study on some bacterial causes of the early chick mortalities in Sharkia province.

[21] H. A. Mohamed, (2004): Studies on Pseudomonas species in chicken embryos and broiler in Assiut Governorate. Ass. Univ. Bull. Environ., 7: 23-31.

[22] A. Hebat Allah Mohamed (2004): Some studies on Pseudomonas species in chicken embryos and broilers in Assiut Governorate. Ass. Univ. Bull. Environ. Res., Vol. 7, No. 1.

[23] H. M. Hassan, (2013): Characterization of *Pseudomonas aeruginosa* isolated from different pathological lesions in chickens. M. V. Sc. Thesis (Microbiology), Fac. Vet. Med., Beni-Suef Univ.

[24] D. G. Joakim Larsson (2013): Pollution from drug manufacturing: review and perspectives, Phil. Trans. Roy. Soc. Lond. B Biol. Sci. 369 (1656) (2014) 20130571, https://doi. org/10.1098/rstb.2013.0571.

[25] P. Grenni, V. Ancona and A. B. Caracciolo (2018): Ecological effects of antibiotics on natural ecosystems: a review, Microchem. J. 136 (2018) 25–39 https://doi.org/10.1016/j. microc.2017.02.006.

[26] N. V. Kurkure ; D. R. Kalorey,; W. Shubhangi,; P. S. Sakhare, and A. G. Bhandarkar, (2001): Mortality in young broiler due to *Pseudomonas aeruginosa*. Ind. J. Vet. Res., 10 (1): 55-57.

[27] A. A. Abd El-Tawab; F. I. El-Hofy; D. F. Khater, and M. M. Al-Adl, (2014): PCR detection and gene sequence of Pseudomonas aeruginosa isolated from broiler chickens, Benha Veterinary Medical Journal, 27, 2: 449-455.

[28] M. M. Tawakol ; N. M. Nabil and R. M. Reda (2018): Molecular studies on some virulence factors of *pseudomonas aeruginosa* isolated from chickens as a biofilm forming bacteria. Assiut Veterinary Medical Journal Assiut Vet. Med. J. Vol. 64 No. 159 pp: 43-51.

[29] T. Strateva and D. Yordanov (2009): *Pseudomonas aeruginosa* a phenomenon of bacteria resistance. J Med Microb; 58: 1133-48. System/ucm257561.htm Last accessed October 9, 2012.

[30] R. A. Bonomo and D. Szabo (2006): Mechanisms of multidrug resistance in Acinetobacter species and *Pseudomonas aeruginosa*. Clin Infect Dis 2006; 43 (Suppl 2): S49-56.

[31] F. C. Tenover (2006). Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control; 34 (5): S3-10, discussion S64–73.

[32] E. B. Hirsch and V. H. Tam (2010): Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res 2010; 10 (4): 441-51.

[33] A. Annan-Prah, E. Agbemafle, P. T. Asare and S. Y. Akorli (2012) Antibiotic use, abuse and their public health implication: The contributory role of management flaws in the poultry industry in two Agro-ecological zones in Ghana. J Vet Adv 2: 199-208.

[34] N. Gilbert (2012): Rules tighten on use of antibiotics on farms. Nature 481: 125.

[35] S. McEwen, P. J. Fedorka-Cray (2002) Antimicrobial use and resistance in animals. Clin Infect Dis 34: S93-S106.

[36] M. Kohanski, D. J. Dwyer, J. J. Collins (2010): How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 8: 423-435.

[37] J. Davies, D. Davies (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74: 417-433.

[38] D. F. Maron, T. J. S. Smith, K. E. Nachman (2013): Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey Global Health 9: 48.

[39] WHO (2014): Antimicrobial resistance global report on surveillance. World Heal Organ 256.

[40] Guyue Cheng, Haihong Hao, Shuyu Xie, Xu Wang, Menghong Dai Lingli Huang and Zonghui Yuan (2014): Antibiotic alternatives: the substitution of antibiotics in animal husbandry. Frontier in microbiology, May 2014, Volume 5, Article 217, 1-15.