Bulgarian Journal of Veterinary Medicine, 2022 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2022-0040

Original article

EMERGENCY OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING *PSEUDOMONAS AERUGINOSA* ISOLATED FROM BROILER CHICKENS IN EGYPT

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Summary

Ahmed, A. S., S. A. Nasef & M. I. El Enbaawy, 2022. Emergency of extended-spectrum beta-lactamase-producing *Pseudomonas aeruginosa* isolated from broiler chickens in Egypt. *Bulg. J. Vet. Med.* (online first).

Multidrug-resistant (MDR) bacteria, such as extended-spectrum beta-lactamase (ESBL) *Enterobacteriaceae,* pose a challenge to human and animal health care systems. Antibiotic resistance in non-lactose fermenting pathogens such as *Pseudomonas aeruginosa* is increasing, making these clinical pathogens more difficult to treat. The most predominant *Pseudomonas* species that cause mortality among birds, especially chickens, is *P. aeruginosa*. The present study detected multi-drug resistance and ESBL-production among *P. aeruginosa* isolated from broiler chickens in different poultry farms. A total of 450 broiler chickens from 150 private farms were examined for the presence of *P. aeruginosa* using bacteriological and molecular methods. Samples were collected from the internal organs of the birds and subjected to bacteriological examination and identification. The bacteriological examination revealed that 29 farms (19.33%) were positive for *P. aeruginosa*. On the other hand, by PCR, only 25 farms (16.66%) were positive. All *P. aeruginosa* isolates were subjected to *in vitro* antimicrobial susceptibility testing against 15 antimicrobial agents by the disc diffusion method. *P. aeruginosa* isolates showed resistance to a wide range of antimicrobial agents, including trimethoprim/sulfamethoxazole (80%), followed by streptomycin (56%), colistin sulfate (48%), ceftazidime (32%), gentamicin and ciprofloxacin (28% each). In contrast, resistance levels to cefotaxime, oxytetracycline, and norfloxacin were 24%, 20%, and 16%, respectively. The lowest resistance was against apramycin (12%), fosfomycin, imipenem, and ertapenem (8%). All *P. aeruginosa* isolates were sensitive to amikacin and meropenem. The antibiotic-resistant pattern of the isolated *P. aeruginosa* revealed that the multi-drug resistance (MDR) level was 14/25 (56%). Only 16 isolates (64%) were ESBL producing. From the ESBL isolates, 9/16 (56.25%) were MDR. Molecular detection for antibiotic resistance gene revealed that bla_{TEM} was detected in 20/25 (80%) of *P. aeruginosa* isolates. From the bla_{TEM} gene-positive *P. aeruginosa,* 16/20 isolates (80%) were ESBL producers, and 11/20 (55%) were MDR. The results indicated that MDR and ESBL producing *P. aeruginosa* frequency reached an alarming level in poultry isolates in Egypt. In conclusion, 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 the food chain within established safe levels must be done.

Key words: bla_{TEM}, broiler, ESBL, MDR bacteria, *P. aeruginosa*

INTRODUCTION

P. aeruginosa infects birds of any age and young birds are the most susceptible (Stipkovits *et al.*,1993). It affects newly hatched chickens drastically, causing high mortality and mass embryonic deaths (Acquah *et al*., 2013). Birds can be infected with *P. aeruginosa* by a mechanical route through skin injury or use of contaminated needles during the vaccination process. Several factors, including the bird's immune status and presence of other concomitant infections, may enhance the susceptibility to *P. aeruginosa* infection (Gong *et al.,* 2018). The infection can also spread from infected to susceptible flocks. Infection with *P. aeruginosa*) is mainly associated with water, soil, and humid environments (Dinev *et al.,* 2013). Also, Fekadu (2010) found that the main reason of high mortalities in baby chicks was due to infection by *P. aeruginosa* invading shells of eggs or from the environment during hatching.

In processing poultry plants*, Pseudomonas* spp. have been the predominant organisms on chicken carcasses (Chen *et al*., 2020). Retail chicken products are incriminated as a primary source of *Pseudomonas* spp. for humans. Infection with *P. aeruginosa* induces severe pulmonary infection (Azam & Khan, 2019) and cystic lung fibrosis (Rybtke *et al.,* 2015), especially in immunosuppressed persons. Infection with *P. aeruginosa* in birds is associated with respiratory signs and septicaemia. Infection causes dyspnea, cheesy deposits on the serous surfaces lining the air sacs and peritoneal cavity, congestion of the internal organs, perihepatitis, and pericarditis (Shukla & Mishra, 2015).

The World Health Organization defined any infection that is transmitted naturally from animals to humans or from humans to animals as zoonosis (WHO, 2020). Approximately 2.4 billion diseased cases and 2.7 million deaths in humans annually, along with the negative impact on livestock production, are related to these zoonotic infections, especially in low-income countries (Grace *et al.,* 2012). Most infectious and fatal diseases that infect humans are of animal or animal product origin (Thompson & Kutz, 2019). There is a wide variety of bacterial zoonoses in poultry; among them, *P. aeruginosa* infection. It is also regarded as one of the most important pathogens that causes human opportunistic infection (Gellatly &Hancock, 2013). Many studies indicated infection of humans with *Pseudomonas* spp. through occupational contact with poultry carcasses or related products (Morales *et al.,* 2016).

P. aeruginosa is an opportunistic pathogen because of its innate resistance to disinfectants and antibiotics (Devanand & Saxena, 2013). *Pseudomonas* infection is a direct public health hazard (Abd El-Ghany, 2021). One of the main challenges with *P. aeruginosa* is its minor susceptibility to a lot of types of antimicrobials, making it a very hard pathogen to eliminate (Khattab *et al.,* 2015). It is defined as MDR by several mechanisms, including overexpression of the efflux pump and acquisition of ESBL (Chaudhary & Payasi, 2013).

ESBLs are enzymes effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime, and oxyimino-monobactam. A variety of these drugs are currently used in veterinary medicine, resulting in ESBL-producing Gram-negative bacteria (Bush *et al*., 1995) isolated from healthy and diseased poultry (Bortoloaia *et al*., 2011). ESBL enzymes, according to Ambler classification, are A and D classes. The most fa-

mous enzymes in A-class include bla_{TEM}, bla_{CTX-M}, and bla_{SHV}; they have been described in *P. aeruginosa* (Laudy *etal*., 2017). Globally, ESBLs are considered problematic and there is an increasing frequency of ESBL in different parts of the world (Ghafourian *et al*., 2015). When ESBLs are not identified on time, appropriate antimicrobial therapy is frequently delayed, resulting in poor clinical outcomes (Castanheira *et al*., 2021).

The emergence and dissemination of antibiotic-resistant bacteria threaten antibacterial therapy at a global scale (WHO, 2014). In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antimicrobial resistance from animals as a main cause of antibiotic resistance has emerged (Marshall & Levy, 2011). The extent of usage is expected to increase markedly over next years due to intensification of farming practices in most of the developing countries (Van Boeckel *et al.,* 2015). Within the African region, the knowledge on the true extent of antibiotic resistance is limited.

The chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year (FAO, 2017). A lot of antimicrobials are used to raise poultry in most countries (Boamah *et al.,* 2016). Also, many of these antimicrobials are considered essential in human medicine (WHO, 2017). The misuse of essential antimicrobials in animal production is likely to accelerate the development of antibiotic resistance in pathogens, as well as in commensal organisms. This may result in treatment failures, economic losses and could act as source of gene pool for transmission to humans. In addition, there are also human health concerns about the presence of antimicrobial residues in meat (Aalipour *et al.,* 2013), eggs (Goetting *et al.,* 2011) and other animal products (Mehdizadeh *et al.,* 2010).

With the global increase in antibiotic resistance, it is essential to monitor the resistance to antibiotics. The present work investigated the multi-drug resistance and ESBL-production among *P. aeruginosa* isolated from chickens and characterised ESBL-related *bla* genes, including bla_{TEM}.

MATERIALS AND METHODS

Samples

A total of 450 chickens were collected from 150 different broiler farms in Egypt. Three (1–4 days old) apparently healthy, clinically diseased, and recently dead chicks from each farm were collected in sterile plastic containers, kept in ice box and transported as soon as possible to the bacteriology unit of poultry diseases department-Animal Health Research Institute Dokki, Giza. Cervical dislocation and decapitation were used to euthanise chicks in a humane manner (Jaksch, 1981). The carcasses were promptly necropsied according to the standard procedures described by Lowenstine (1986). Samples were subjected to bacteriological and molecular examination. All samples were handled aseptically to prevent cross-contamination using sterile sampling materials.

During necropsy, pooled visceral organ samples (liver, heart, lung, yolk, and intestine) were collected from each bird, under complete aseptic conditions, inoculated into nutrient broth, then a loopful from the previously incubated broth was streaked onto the surface of nutrient agar, *Pseudomonas* cetrimide agar and MacConkey agar (HiMedia, Pvt. Ltd., India), and then incubated at 37 °C for 24 hours. The suspected colonies were identified biochemically (Quinn *et al*., 2011). Then

all *P. aeruginosa* positive by culture were screened for *16SrDNA* gene.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was done against 15 antimicrobial agents by agar disc diffusion method using Muller Hinton agar plates on confirmed *P. aeruginosa* isolates. The interpretation was as sensitive, intermediate and resistant according to the recommendations of The Clinical and Laboratory Standard Institute (CLSI, 2017). Strains that showed resistance to three or more antimicrobial classes were considered multidrug resistant (MDR) (Kiratisin *et al.,* 2008).

Determination of multi-drug resistance index (MDRI)

Multi-drug resistance 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 (Chandran *et al.,* 2008). Isolates with MDRI values of more than 0.2 were considered highly resistant.

Phenotypical detection of ESBL producing P. aeruginosa

All *P. aeruginosa* were screened for ESBLs production with the CLSI confirmatory test using both cefotaxime CTX (30 mg) and ceftazidime CAZ (30 mg) disks alone and in combination with clavulanate CA (10 mg). The test was considered positive when an increase in the growth-inhibitory zone around either the CTX/CA (2:1) and/or CAZ/CA (2:1) was 5 mm or greater of the diameter around the disk containing CTX or CAZ alone (CLSI, 2017).

Molecular identification

Extraction of DNA was done according to instructions for use of QIAamp DNA mini kit (Catalogue no.513). Briefly, 200 µL of the sample was added to 20 µL of proteinase K and 200 µL of AL lysis buffer and incubated at 56 ^{o}C for 10 min in a Biometra Tsc thermal block cycler. After incubation, 200 µL of 100% ethanol was added to the lysate and vortexed. The sample was then washed twice and centrifuged according to the manufacturer‘s instructions. DNA was eluted with 100 µL of elution buffer supplied in the kit. DNA concentration and integrity was checked using 2000C Nanodrop spectrophotometer (Thermo Fisher).

Oligonucleotide primers used in PCR were supplied from Metabion (Germany) (Table 1) amplifying 956 bp of the *P. aeruginosa16S rDNA* gene (Spilker *et al.,* 2004).

For PCR, primers were utilised in a 25 µL reaction containing 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µL of each primer of 20 pmol concentration, 4.5 µL of water, and 6 µL of DNA template. The reaction was performed in an Applied biosystem 2720

Table 1. Oligonucleotide primers sequences targeting *P. aeruginosa* genes

Gene	Sequence	Amplified product	Reference
16S rDNA	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	956 bp	Spilker *et al*. (2004)
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al*. (2003)

thermal cycler. PCR assay consisted of a primary denaturation step at 95 ^{o}C for 5 min, followed by 35 cycles (95 ^{o}C for 1 min., 52 ^{o}C for 1 min., 72 ^{o}C for 1 min.), and a final extension step at 72 ^{o}C for 10 min.

The PCR products were separated by electrophoresis according to Sambrook *et al*. (1989) with modification. Fifteen microliters of each PCR product were loaded in each gel lane of 1.5% agarose gel (Applichem). Electrophoresis grade agarose (1.5g) was prepared in 100 mL TBE buffer in a sterile flask, heated in microwave to dissolve all granules with agitation, and allowed to cool at 70 ^{o}C, then 0.5 µg/mL ethedium bromide was added and mixed thoroughly. Electrophoresis was done in 1× Tris Boric acid EDTA (TBE) buffer using 5V/cm gradients. A 100 bp DNA ladder (Fermentas) was used to determine the fragment sizes. The PCR photos were photographed and analysed by using a gel documentation system (Alpha Innotech, Biometra, Germany) through its computer software.

RESULTS

Out of 450 chickens collected from 150 private farms (3 samples per farm), 42/450 chickens (9.33%) and 35/450 chickens (7.77%) were positive for *P. aeruginosa* by culture and PCR methods, respectively. Meanwhile, 29 farms (19.33%) were found positive for *P. aeruginosa*. On the other hand, by PCR only 25 farms (16.66%) were positive for *P. aeruginosa* as shown in Table 2. Phenotypic identification revealed that the organism was a Gram-negative motile, haemolytic rod that produced colonies with bluish-green colour on the culture plate. It did not grow at 4 °C but grew well at 42 °C.

Percentages of isolation and *16SrDNA* gene detected from clinically diseased, recently dead, and healthy chickens are presented in Table 3, Fig 1 and 2.

The results of antimicrobial susceptibility testing for the isolated *P. aeruginosa* (Tables 4 and 5) revealed that *P. aeruginosa* PCR positive strains showed resistance to a wide range of antimicrobial agents, including trimethoprim/sulfamethoxazole (80%), followed by streptomycin (56%), colistin sulfate (48%), ceftazidime (32%), gentamicin and ciprofloxacin (28% each). In contrast, the resistance levels to cefotaxime, oxytetracycline, and norfloxacin were 24%, 20%, and 16%, respectively. On the other hand, the highest sensitivity was observed against apramycin (12%), fosfomycin, imipenem, and ertapenem (8%). All isolates were sensitive to amikacin and meropenem. The overall multi-drug resistance (MDR) level was 14/25 (56.00%).

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

Number of farms	Number of samples/ farm	Total number of chickens		Positive farms		Positive chickens	
				Number	%	Number	%
150	3	450	Bacterial isolation	29	19.3	42	9.34
			PCR	25	16.6	35	7.77

Table 3. Prevalence of *P. aeruginosa* according to the bird status from different farms.

	Number of tested samples			Culture-positive		PCR-positive	
	1-day old	2–4 days old	Total	Number	%	Number	%
Diseased	75	75	150	22	14.67	18	12.00
Recently Dead	75	75	150	15	10.00	14	9.33
Apparently healthy	75	75	150	5	3.34	3	2.00
Total	225	225	450	42	9.33	35	7.77

*The percentage was calculated according to the total number of examined samples.

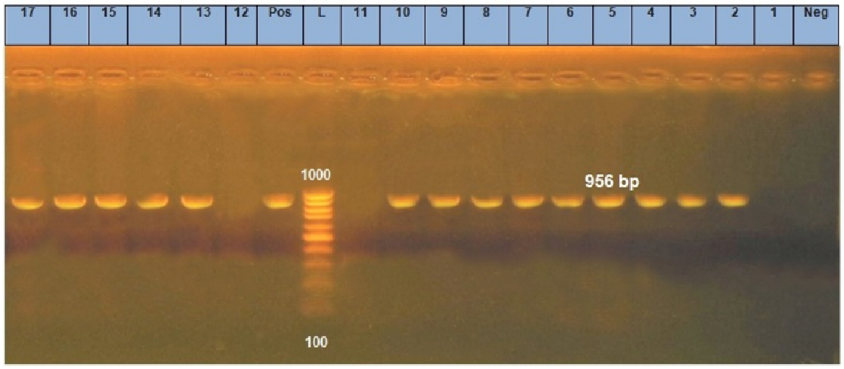

Fig. 1. Agarose gel electrophoresis of PCR for detection of *16S rDNA* gene in *P.aeruginosa* showing amplification of 956 bp in 17 examined farm samples. All samples were positive except 3 negative (lanes 1, 11 & 12). L (Ladder): DNA ladder (100–1000 bp).

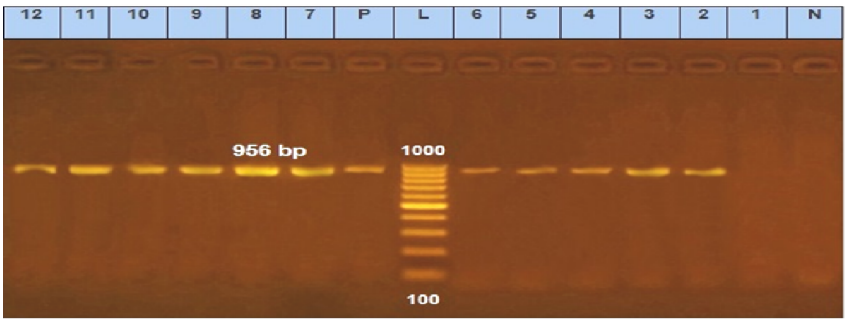

Fig. 2. Agarose gel electrophoresis of PCR for detection of *16S rDNA* gene in *P.aeruginosa* showing amplification of 956 bp in 12 examined farm samples. All samples were positive except one negative (lane 1). L (Ladder): DNA ladder (100–1000 bp); P: positive control; N: negative control.

Table 4. Antimicrobial resistance of *P. aeruginosa* strains to antibiotics

Agent	Symbol	Concentration	Resistant strains	
			Number	%
1.Aminoglycosides				
Streptomycin	S	10 µg	14	56
Gentamicin	CN	10 µg	7	28
Apramycin	APR	10 µg	3	12
Fosfomycin	FO	200 µg	2	8
2. Third-generation cephalosporins				
Ceftazidime	CAZ	30 µg	8	32
Cefotaxime	CTX	30 µg	6	24
3.Carbapenems				
Imipenem	IPM	10 µg	2	8
Ertapenem	ETP	10 µg	2	8
Meropenem	MEM	10 µg	0	0
4. Sulfonamides				
Trimethoprim/sulfamethoxazole	STX	1.25/23.75 µg	20	80
5.Polymyxins				
Colistinsulphate	CT	10 µg	12	48
6.Quinolones				
Ciprofloxacin	CIP	5 µg	7	28
Norfloxacin	NOR	10 µg	4	16
7. Tetracyclines				
Oxytetracycline	OT	30 µg	5	20

As shown in Table 5, phenotypically 16/25 (64%) of *P.aeruginosa* were ESBL producing strains. In addition, 9/16 (56.25%) of the ESBL-producing strains expressed an MDR phenotype. The bla_{TEM} gene was detected in 20 out of 25 *P. aeruginosa* (80%) (Fig 3). Of bla_{TEM} gene-positive *P. aeruginosa*, 16/20 (80%) were ESBL producers, and11/20 (55%) were MDR (Table 5).

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. *P. aeruginosa* is responsible for mortality in chickens and clinical signs including respiratory signs and septicaemia. Mortality begins from hatching and continues for 10–14 days or more (Bapat *et al.* 1985).

The World Health Organisation has categorised *P. aeruginosa* as a critical priority pathogen of zoonotic nature which was given a serious threat level due to MDR demonstrated against many antibio-

Table 5. Drug resistance patterns of the 25 strains of PCR positive *P. aeruginosa* isolated from chicks

Iso-late	Antibiotic	MDR index	MDR	ESBL phenotype	bla_{TEM} gene
1.	APR,ATP,SXT	0.2	–	–	+
2.	S,SXT,CT	0.2	+	+	+
3.	S,CN,FO,SXT,CT,CIP	0.4	+	–	_
4.	CAZ,SXT	0.1	-	+	+
5.	SXT,CT	0.1	–	+	+
6.	APR,CAZ,CTX, SXT	0.3	+	+	+
7.	S,CN,IPM,SXT, CT,CIP,OT	0.5	+	+	+
8.	S,APR,SXT,CT, CIP,OT	0.4	+	+	+
9.	CAZ,SXT,CT	0.2	+	+	+
10.	S,CTX,SXT	0.2	+	–	+
11.	S,CN,SXT,CT,CIP,NOR,OT	0.5	+	+	+
12.	CT	0.07	–	+	+
13.	S,CN,SXT,OT	0.3	+	+	+
14.	CAZ	0.07	-	+	+
15.	S,CN,CTX,SXT, CT,CIP,NOR,OT	0.53	+	+	+
16.	S,CN,FO,SXT,CIP,NOR,OT	0.5	+	–	_
17.	S,CN,CTX,IPM, SXT,CT,CIP,NOR,OT	0.6	+	+	+
18.	CAZ,SXT	0.1	–	–	+
19.	CAZ,CTX	0.1	–	+	+
20.	S,SXT	0.1	–	–	_
21.	S,ETP,SXT	0.2	+	–	+
22.	CAZ,OT	0.1	–	+	+
23.	CAZ,CTX,SXT	0.2	–	+	+
24.	CT	0.07	–	–	_
25.	S,SXT,CIP,OT	0.4	+	–	_
			14/25	16/25	20/25

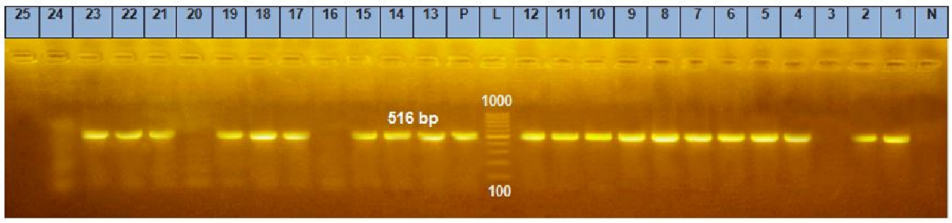

Fig. 3. Agarose gel electrophoresis of PCR for detection of bla_{TEM} gene in *P. aeruginosa* showing amplification of 516 bp in 25 examined samples. L (Ladder): DNA ladder (100–1000 bp); P: positive control; N: negative control. All samples were positive except 5 negative (Lanes 3, 16, 20, 24 & 25).

tics (Tacconelli *et al*., 2017; Grenni *et al*., 2018). Commercial poultry is possibly infected with *P. aeruginosa* at any age, especially young, with many losses, including embryo death, septicaemia, respiratory and enteric infections, with high mortality (Abd El-Ghany, 2021).

Extended-spectrum β-lactamases can be difficult to detect because they have different levels of *in vitro* activity against various cephalosporins. In clinical practice, susceptibility to cephamycins (cefoxitin, cefotetan) but resistance to third-generation cephalosporins (e.g., cefpodoxime, cefotaxime, ceftriaxone, and ceftazidime) or aztreonam should focus attentionon the possibility of ESBL production (Leekha *et al.,* 2011).

The present study detected multidrug resistance and ESBL production among *P. aeruginosa* isolated from chickens. Isolation and identification were performed by culture methods using specific media and characteristic biochemical tests.

The prevalence rate of *P. aeruginosa* positive farms was 19.3%, these results agreed with those of Shukla & Mishra (2015) having isolated *P. aeruginosa* at a rate of 20% and Elsayed *et al.* (2016) reported total isolation of *P. aeruginosa* of 38 out of 150 farms (25.3%). But disagreed with Badr *et al.* (2020), who recorded a high prevalence of *Pseudomonas* positive farms (69.57%). On the other hand the incidence of positive examined chickens was 9.34% in this study. This finding was nearly coordinated with Tawakol *et al.* (2012) who isolated *P. aeruginosa* from chickens with a percentage of (10.66%). Meanwhile, results in this study varied to some extent from Badr *et al.* (2020), who recorded high incidence of positive examined chickens (39.78%). There were variations in the distribution according to geographic regions.

In the current study, isolation rates from clinically diseased, recently dead, and healthy birds were 14.67%, 10%, and 3.34%, respectively. These records were consistent with data of Younes *et al*. (1990), who isolated *P. aeruginosa* from 20 dead chickens (4.9%). Chakrabarty *et al*. (1980) isolated *P. aeruginosa* with an incidence of 8% from 100 chickens suffering from respiratory symptoms, but our results disagreed with those of Shukla & Mishra (2015), who isolated *P. aeruginosa* from a clinically diseased and healthy birds at 30% and 12% respectively. These percentages differed from the current results, which may be attributed to the sample type.

The elevation in the incidence of isolation indicated decrease in the biosecurity programmes applied in poultry farms because most developing countries such as Egypt suffer from the release of pharmaceutical waste containing active pharmaceutical compounds from antibiotic manufacturing plants into the rivers or environment, which revealed that infection with resistant organisms is a significant threat to public health (Grenni *et al*., 2018).

Molecular techniques such as PCR were used to detect *P. aeruginosa 16Sr DNA* using specific primers to give an amplification product of 956 bp (Spilker *et al*., 2004) to confirm the existence of *P. aeruginosa* DNA in 35/450 chickens (7.77%). The same method was applied in the study of Shahat *et al*. (2019), who found *P. aeruginosa* DNA in 7 isolates out of 200 samples and added that *P. aeruginosa* could be detected by the PCR method to overcome the culture problems like false negative results.

In a study on antimicrobial sensitivity of isolated *P. aeruginosa*, high resistance to sulfamethoxazole-trimethoprim, strep-

tomycin, colistin sulfate and ceftazidime-wasdetected with a percentage of 80%, 56%, 48% and 32% respectively. On the other hand, low resistance to gentamicin, ciprofloxacin, cefotaxime, oxytetracycline, and norfloxacin was present with percentages of 28%, 28%, 24%, 20%, and 16%, respectively. The least resistance was towards apramycin (12%), fosfomycin, imipenem, and ertapenem (8%). None of the isolates were resistant to amikacin and meropenem (0%).

Several studies have detected extremely variable sensitivity of *P. aeruginosa* strains to different antibiotic classes. Beier *et al.* (2015) reported the highest resistance to sulfonamides (93.5%), while streptomycin showed resistance of 50%. These findings were consistent with the present high resistance against trimethoprim/sulfamethoxazole (80%), followed by streptomycin (56%). On the other hand, Egyptian chicken strains of *P. aeruginosa* showed 100% resistance to sulfamethazine (Shahat *et al.,* 2019).

A high (48%) resistance rate to colistin sulfate was found in the current study, which was dissimilar to that reported by Angadi *et al*. (2012), Nasreen *et al.* (2015), and Azimi *et al*. (2016), who reported resistance rates to colistin of 15.2%, 12.5%, and 2.5%, respectively.

Ceftazidime is the prescribed anti-pseudomonal third generation cephalosporin. Ceftazidime resistance rate was 32%, which was below that obtained by Ameen (2014) (42%) but higher than that obtained by Ansari *et al.* (2015) – 22.03%.

Of examined *P. aeruginosa* isolates, 28% were resistant to gentamicin (Elhariri *et al.,* 2017). Elevated rates of resistance (77.3%, 63%, and 93.7%) were found by Rashid *et al.* (2007), Rajat *et al*. (2012), and Nasreen *et al*. (2015), respectively. In a study performed by Hassuna *et al.* (2015), a 20% gentamicin resistance against *P. aeruginosa* was recorded. Moreover, Eraky *et al*. (2020) demonstrated that all isolated *P. aeruginosa* were sensitive to gentamicin and ciprofloxacin. Lower sensitivity to ciprofloxacin (62.85%) was observed by Kurkure *et al.* (2001). In the present work, *P. aeruginosa* resistance was 28%, being lower than those published by Elhariri *et al.* (2017), Rajat and co-workers (2012), Mohanasoundaram (2011), and Tam *et al.* (2010): 33.3%, 49%, 63.1% and 100%, respectively. Egyptian chicken strains of *P. aeruginosa* showed 100% susceptibility to ciprofloxacin and norfloxacin (Shahat *et al*., 2019).

In this study, the level of resistance to cefotaxime was 24%, disagreeing with rates found by Sedighi *et al.* (2015) and Norouzi *et al.* (2010), while Jabalameli *et al.* (2012) mentioned that more than 90% of the *P. aeruginosa* isolates were cefotaxime-resistant. Also, Ameen (2014) and Corona-Nakamura *et al*. (2001) found a high resistance against cefotaxime (80%).

Abd El-Tawab *et al*. (2014) and Tawakol *et al*. (2018) added that *P. aeruginosa* isolates were sensitive to norfloxacin. However, a low resistance rate of *P. aeruginosa* against imipenem and norfloxacin (8% and 20%) was reported. These results were consistent with other studies carried out in Egypt (Badr *et al.,* 2008; Osman *et al.,* 2012; Hassuna *et al.,*2015), which found low resistance to imipenem which seemed to be a promising therapy for *Pseudomonas* infection (Aniokette *et al.,* 2016). On the other hand, three other studies carried out by Diab *et al.* (2013), Zafer *et al.* (2014), and Hosu *et al.* (2021) found a higher frequency of imipenem resistance (72%, 39.34%, and 46.6%, respectively).

None of the *P. aeruginosa* isolates were resistant to amikacin and meropenem. These results were consistent with Ansari *et al.* (2015), who found no resistance to meropenem. On the contrary, Elhariri *et al*. (2017) found 14.2% resistance to meropenem. According to the China Antimicrobial Surveillance Network (CHINET) of 2020, the resistance rates of *P. aeruginosa* to imipenem and meropenem were 23.2% and 19.3%, respectively (Hu *et al.,*2021).

P. aeruginosa is defined as MDR when it resists one antimicrobial agent in three or more antipseudomonal antimicrobial classes (Magiorakos *et al.,* 2012). In general, bacteria use several mechanisms to resist antimicrobials, including modification, altering the drug target, implementing an alternative metabolic pathway (McDermott *et al*., 2003), and overexpression of at least two efflux systems (Llanes *et al*., 2004).

In the present study, *P. aeruginosa* expressed a multidrug-resistant phenotype level of 14/25 (56.00%). That finding agreed with Abd El Hamid (2014), Yousef (2014), and Hassuna *et al*. (2015), who found high percentages (75.8%, 57%, and 56%, respectively) of MDR *P. aeruginosa.* In contrast, Gill *et al.* (2011) and Ansari *et al.* (2015) obtained frequencies of MDR *P. aeruginosa* of 22.7% and 32.6%. Recently, Hosu *et al.* (2021) reported a total of 75 (36.8%) MDR *P. aeruginosa*.

It is important to detect ESBL producers to know the ESBL prevalence in animal-associated bacteria and limit the spread of these MDR organisms in veterinary settings (Liu *et al*., 2007). ESBLs are a cluster of β-lactamases that inactivates β-lactams, especially oxymino-β-lactams and monobactams, and are repressed by β-lactamase inhibitors, such as clavulanic acid (Laudy *et al*., 2017). The genes encoding ESBLs are often found on plasmids and harboured within transposons or insertion sequences, which enabled their spread from one organism to another (Castanheira *et al*., 2021).

The present results showed that 16/25 isolates of *P. aeruginosa* were phenotypically positive for ESBL production (64%). In addition, some of the ESBL-producing strains 9/16 (56.25%) expressed an MDR phenotype. Enzymes that are products of these genes were phenotypically detected in the isolates. Even though ESBLs enzymes were prevalent in 80% of the isolates, this rate was very close to that of Newman *et al.* (2015), who detected high ESBL production (90 to 98%) in Gram-negative isolates.

The high incidence of bla_{TEM} detection in the present study, with a percentage of 80%, was previously recorded. Igbinosa *et al.* (2012) detected bla_{TEM} antibiotic resistance gene in 100% *P. aeruginosa* and 40% in other *Pseudomonas* species. Hosu *et al.* (2021) applied real-time PCR and found a bla_{TEM} rate (79.3%), similar to ours. The bla_{TEM} derived extended-spectrum b-lactamases (ESBLs) have only been described in members of the family *Enterobacteriaceae* (Jacoby, 1994).

CONCLUSION

Most of the isolated MDR *P. aeruginosa* strains carried the bla_{TEM} gene. The problem of ESBL producing *P. aeruginosa* with MDR phenotype is anticipated to become more challenging. Therefore, monitoring antimicrobial resistance and early characterization of MDR and ESBL strains is the best defense against their spread.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Nesma Mohamed, Ahmed Erfan and the staff of microbiology unit at reference laboratory of poultry production (RLQP) for their kind support.

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Paper received 19.07.2022; accepted for publication 01.09.2022

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