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Phenotypic and genotypic characterization of *Pseudomonas aeruginosa* associated with airsacculitis in broiler chickens

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ABSTRACT

Airsacculitis is considered one of the respiratory signs caused by many viral and bacterial pathogens. This study aimed to investigate prevalence of airsacculitis syndrome among broiler chickens at Beni-Suef, El-Fayoum and El-Menia Governorates, Egypt and phenotypic as well as genotypic characterization of *P. aeruginosa* associated it. Therefore, 239 diseased or freshly dead broiler chickens were randomly collected from the different farms at these Governorates and were subjected to clinical and bacteriological examinations. *P. aeruginosa* isolates were investigated for antimicrobial susceptibility, ESβLs production, biofilm formation, haemolytic activity and pyocyanin production. Furthermore, 6 multidrug resistant *P. aeruginosa* isolates were investigated by PCR for presence of some antimicrobial resistance genes included *aad*B, *amp*C, bla_{CTX}, bla_{TEM} and *sul*1 genes as well as some virulence genes included *exo*S, *hly*A, *las*B, *las*I, *phz*M and *tox*A genes. Airsacculitis was recorded in 43.9% of the examined broiler chickens and *P. aeruginosa* was recovered from 53.3% of these cases. Antimicrobial susceptibility test revealed that *P. aeruginosa* isolates were highly resistant to sulfamethoxazole/trimethoprim, β-lactams and gentamicin while were highly sensitive to fluoroquinolones and phenicols. Furthermore, combined disc diffusion and cefinase disc tests revealed that 39.3% and 42.9% of *P. aeruginosa* isolates were ESβLs-producer, respectively. Biofilm formation, haemolytic activity and pyocyanin production were detected in all *P. aeruginosa* isolates. PCR revealed that all the investigated isolates have *amp*C, bla_{CTX} and bla_{TEM} genes while only 66.7% of them have *aad*B and *sul*1 genes. Also, it revealed that all the investigated isolates have *exo*S, *las*B, *las*I and *phz*M genes while only 83.3% and 66.7% of them have *tox*A and *hly*A genes, respectively.

Introduction

Without any doubt poultry are one of the most proper sources for animal protein for humans worldwide because of its short life cycle and the efficiency cost of production. In Egypt, a huge interest was paid to poultry sector to confront the incremental demands for animal protein. Infectious diseases of broilers are very important due to the high mortalities and growth retardation in addition to the high costs of antimicrobials used in their prevention or treatment (Abed *et al*., 2022).

Poultry are susceptible to various respiratory diseases which cause serious effect on poultry industry as a result of decreasing the produced protein and transmission of zoonotic pathogens (Contreras *et al*., 2007). Control of these diseases is considered one of the main problems in poultry industry.

Airsacculitis syndrome is a worldwide economically significant problem affecting broiler chickens and result in high morbidity and mortality (Abed *et al*., 2026). Furthermore, according to Food Standard Agency, airsacculitis is one of seven PM lesions having a public health concern (MLCSL, 2013).

Airsacculitis is caused by many viral and bacterial pathogens. Bacterial respiratory pathogens mostly colonize respiratory tract after a primary viral infection as avian influenza, infectious bronchitis, and Newcastle disease, other pathogenic bacteria as Mycoplasma gallisepticum, and Mycoplasma synoviae or presence of environmental factors as high ammonia levels (Abdelrahman, 2022).

Pseudomonas aeruginosa (*P. aeruginosa*) can cause respiratory infections in all birds kinds (Bakheet and Torra, 2020) causing high economic losses in poultry industry due to decrease egg production and the great mortalities especially in embryos and young chicks which may reach up to 100% during the first week of age (Abed, 2007). On the other hand, it is one of the main causes of zoonotic (Yaseen *et al*., 2020) and nosocomial infections in human (Mohamed *et al*., 2022).

Antimicrobial resistance (AMR) and emergence of multi-drug resistant (MDR) bacteria is considered one of the most significant problems facing bacterial diseases treatment. Food-producing animals as well as their environments are reservoirs for antimicrobial resistant bacterial pathogens and AMR genes which may be transmitted to human directly by the contact or indirectly through food production chain (WHO, 2011). AMR of *P. aeruginosa* is widely common and of public health concern (Radwan *et al*., 2016). There is a broad diversity of MDR *P. aeruginosa* isolates harboring resistance genes (Wiehlmann *et al*., 2007).

Extended-Spectrum β-Lactamases (ESβLs) and plasmid-mediated *amp*C β-lactamases perform a big risk in treatment of the bacterial diseases because of their important role in AMR induction (Carmo *et al*., 2014). ESβLs can hydrolyze the various β-lactam antibiotics, the 3rd and 4th generations of cephalosporins as well as monobactams and can be inhibited by the clavulanic acid (CA) (Poulou *et al*., 2014). Furthermore, ESβLs-coding plasmids may carry AMR genes of other antimicrobials classes leading to limitation of ESβLs-producers therapy and enhancing dissemination of ESβLs between the different bacteria (Zahar *et al*., 2009).

ESβLs-producers were reported worldwide (Zahar *et al*., 2009) and β-lactam resistant *P. aeruginosa* development became a huge risky problem particularly the incremental resistance against the 3rd and 4th generations of cephalosporins as well as carbapenems that are of particular concern (Abd El Tawab *et al*., 2016). There are many groups of ESβLs but TEM, CTX-M and SHV were the most detected types among the clinical isolates of Gram-negative (Bush and Fisher, 2011). Therefore, ESβLs-producers detection is essential for the epidemiological purposes and AMR limitation. CLSI, (2015) recommended combined disc diffusion (CDD) test for the phenotypic confirmation of ESβLs production.

P. aeruginosa is capable of production several virulence factors including enterotoxins, exotoxins, proteolytic enzymes, pigments, leuko-

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cidin, phospholipase and slime together are concerned with the high pathogenicity of *P. aeruginosa* (Wolska *et al*., 2002). Formation of biofilm by bacteria is considered a virulence and resistance mechanism (Cepas *et al*., 2019 and Ahmed *et al*., 2022). MDR *P. aeruginosa*, virulence genes existence and expression of certain virulence factors; mostly protease, elastase and siderophore, correlated with the pigment producing strains rather than the non-pigmented strains (Finlayson and Brown, 2011). *P. aeruginosa* has variable virulence genes on the chromosomes or plasmids as *tox*A, *las*B and *las*I (Morita *et al*., 2015).

Considering the above facts, this study aimed to investigate prevalence of airsacculitis syndrome among broiler chickens at Beni-Suef, El-Fayoum and El-Menia Governorates, Egypt as well as determination of the phenotypic and genotypic characters of *P. aeruginosa* associated it.

Materials and methods

Ethical approval

Institutional Animal Care and Use Committee of Beni-Suef University, Egypt approved protocol of the current work with ethical approval number BSU- IACUC-022-254.

Broiler chickens samples

During the period from January to October 2023, 239 diseased or freshly dead broiler chickens (2-5 weeks age) were randomly collected from the different farms at Beni-Suef, El-Fayoum and El-Menia Governorates and were subjected to clinical and post-mortem (PM) examination for airsacculitis detection.

Collected samples

A total of 105 airsacculitis samples were aseptically collected from the diseased and freshly dead broiler chickens. The collected samples were given serial numbers with detailed information, and were rapidly transferred under chilled conditions to the laboratory in Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

Isolation and identification of P. aeruginosa

Airsacculitis samples were aseptically cut into small pieces then inoculated into tryptone soya broth (TSB) (Oxoid, UK) and incubated under aerobic condition at 37˚C for 24 hrs. After that, a loopful from each inoculated broth was streaked onto tryptone soya agar (TSA) (Oxoid, UK) as well as MacConkey's agar (Oxoid, UK) and incubated under aerobic condition at 37˚C for 24-48 hrs.

Later, small and smooth colonies having a characteristic bluish-green pigment on TSA in addition to have serrated edges and non-lactose fermenter on MacConkey's agar were inoculated in/on cetrimide broth and agar media (HiMedia, India) and considered presumptively *P. aeruginosa*. They were morphologically and biochemically confirmed by using the standard tests described by Collee *et al*. (1996) and Quinn *et al*. (2011). Further confirmation was performed by using API 20 NE system (bioMerieux, France) according to instructions of the manufacture.

Antimicrobial susceptibility testing of P. aeruginosa isolates

Susceptibility of *P. aeruginosa* isolates were tested for 14 different antimicrobials belonging to 7 antimicrobial classes on Muller Hinton agar by using disc diffusion method as described by Clinical and Laboratory Standards Institute (2021). The antimicrobials included penicillin (P, 10µg), amoxycillin-clavulanic acid (AMC, 30µg), cefuroxime (CXM, 30µg), cefotaxime (CTX, 30µg), ceftazidime (CAZ, 30µg), cefepime (FEP, 30µg), amikacin (AK, 30µg), gentamicin (CN, 10µg), kanamycin (K, 30µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), florophenicol (FFC, 30µg), doxycycline (DO, 30µg) and sulphamethoxazole-trimethoprim (STX, 25µg) (Oxoid, UK).

Detection of ESβLs-producing P. aeruginosa isolates

Detection of ESβLs-producing *P. aeruginosa* isolates by combined disc diffusion test

ESβLs-producing *P. aeruginosa* isolates were detected by CDD test using CAZ, CTX, CXM and FEP discs alone and alongside AMC disc (Oxoid, UK). CAZ, CTX, CXM and FEP discs were placed on the inoculated plate around AMC disc with 2 cm center to center then incubated under aerobic conditions at 37˚C overnight. The tested isolate was interpreted as ESβLs producer on increasing the growth-inhibition zone diameter around one or more of the discs alongside AMC disc to at least 5 mm against the agent's zone diameter when tested alone (CLSI, 2021).

Detection of ESβLs-producing *P. aeruginosa* isolates by Cefinase disc test

ESβLs-producing *P. aeruginosa* isolates were detected by Cefinase disc test using CefinaseTM discs (bioMerieux, France) according to manufacturer's instructions.

Detection of biofilm-forming P. aeruginosa isolates

Biofilm-forming *P. aeruginosa* isolates were determined by using microtiter plate (MTP) assay as described by Osman *et al*. (2019). Overnight *P. aeruginosa* isolates cultures were diluted in 5 ml of TSB containing 0.5% w/v NaCl then incubated at 37˚C with shaking to optical density (OD) of 0.8 at A 620 nm. After that, the cultures were diluted with fresh TSB/NaCl in ratio of 1:40 and 150 µl of each dilution was inoculated into a well of a microtiter plate. The plate was sealed by para-film and was incubated at 37˚C for 2 days. Active HDPs was used for treatment in TSB for 1 day and distilled water (DW) was used as the positive control. Later, the wells were washed three times with DW and filled with crystal violet (CV) (Oxford, India) for 15 min. which then removed followed by washing the wells three times with DW then addition of 30% v/v acetic acid (EL Naser, Egypt).

ODs of adhering biofilms were measured by using ELISA reader (Labsystems, Multiskan, China) at A 620 nm. Each isolate was tested in triplicate and the assay redone three times. Isolates with values exceed 0.2 were classified as high biofilm-producers while those had values less than 0.081 were classified as non- or low-biofilm producers. Biofilm production magnitude was ascertained through formula; BF=AB/CW, where BF denotes biofilm formation, AB denotes OD at 620 nm for the stained attached bacteria whereas CW denotes OD at 620 nm for the stained control wells free from bacteria.

Detection of pyocyanin-producing P. aeruginosa isolates

Each isolate of *P. aeruginosa* was inoculated in glycerol alanine minimal broth medium and incubated at 37˚C aerobically for 24 hrs. Later, the tube was centrifuged and 5 ml of the supernatant was mixed with 3 ml of chloroform for extraction of pyocyanin. After that, pyocyanin was re-extracted into 1 ml of acidified water (0.2 M HCL) and was spectrophotometrically quantified by measuring the absorbance at 520 nm (Dénervaud *et al*., 2004).

Polymerase chain reaction for P. aeruginosa isolates

Six multidrug resistant *P. aeruginosa* isolates were investigated by PCR for presence of 5 AMR genes included resistance genes against aminoglycoside (*aad*B), β-lactams (*amp*C, bla_{CTX} and bla_{TEM}) and sulfonamide

Table 1. Primers sequences and amplified products for the different targeted genes for *P. aeruginosa* isolates.

Primers		Primer Sequence 5′-3′	Amplified product	Reference
Resistance genes	*aad*B	F GAGCGAAATCTGCCGCTCTGG R CTGTTACAACGGACTGGCCGC	319 bp	Frana *et al*. (2001)
	*amp*C	F TTCTATCAAMACTGGCARCC R CCYTTTTATGTACCCAYGA	550 bp	Srinivasan *et al*. (2005)
	bla_{CTX}	F ATGTGCAGYACCAGTAARGTKATGGC R TGGGTRAARTARGTSACCAGAAYCAGCGG	593 bp	Archambault *et al*. (2006)
	bla_{TEM}	F ATCAGCAATAAACCAGC R CCCCGAAGAACGTTTTC	516 bp	Colom *et al*. (2003)
	*sul*1	F CGGCGTGGGCTACCTGAACG R GCCGATCGCGTGAAGTTCCG	433 bp	Ibekwe *et al*. (2011)
Virulence genes	*exo*S	F GCGAGGTCAGCAGAGTATCG R TTCGGCGTCACTGTGGATGC	118 bp	Winstanley *et al*. (2005)
	*hly*A	F AACAAGGATAAGCACTGTTCTGGCT R ACCATATAAGCGGTCATTCCCGTCA	1177 bp	Piva *et al*. (2003)
	*las*B	F ACAGGTAGAACGCACGGTTG R GATCGACGTGTCCAAACTCC	1220 bp	Finnan *et al*. (2004)
	*las*I	F ATGATCGTACAAATTGGTCGGC R GTCATGAAACCGCCAGTCG	606 bp	Bratu *et al*. (2006)
	*phz*M	F ATGGAGAGCGGGATCGACAG R ATGCGGGTTTCCATCGGCAG	875 bp	Finnan *et al*. (2004)
	*tox*A	F GACAACGCCCTCAGCATCACCAGC R CGCTGGCCCATTCGCTCCAGCGCT	396 bp	Matar *et al*. (2002)

(*sul*1), as well as 6 virulence genes included genes encoding for α-haemolysin (*hly*A), exotoxin A (*tox*A), exotoxin S (*exo*S), pyocyanin (*phz*M) and quorum sensing (*las*B and *las*I). Sequences of the used primers (Metabion, Germany) and size of the amplified products of the target genes were summarized in Table 1.

Results

Prevalence of airsacculitis among the investigated broiler chickens

PM examination revealed presence of airsacculitis in 105 broiler chickens from the examined samples with a prevalence of 43.9%.

Prevalence of P. aeruginosa among the investigated broiler chickens

P. aeruginosa isolates (n.= 56) were recovered from the airsacculitis cases found in the investigated broiler chickens (n=105) with a prevalence of 53.3%.

Antimicrobial susceptibility of P. aeruginosa isolates

P. aeruginosa isolates showed highest resistance against STX (100%) followed by CTX (96.4%) and then P (87.5%). After that, the resistances against CN, CAZ, AMC, FEP and CXM were 76.8, 73.2, 73.2, 69.6 and 62.5%, respectively. On the contrary, they showed highest sensitivity to LEV (91.1%) followed by CIP (87.5%), FFC (53.6%) and AK (50%) (Table 2).

ESβLs production by P. aeruginosa isolates

Phenotypic detection of ESβLs production by using CDD and cefinase tests revealed that 39.3% (n=22) and 42.9% (n=24) of *P. aeruginosa* isolates were ESβLs-producers, respectively.

Table 2. Results of antimicrobial susceptibility testing of *P. aeruginosa* isolates.

Class	Antimicrobial agent	Disc content (µg)	*P. aeruginosa* tested isolates (*n=56*)					
			R		I		S	
			No.	%	No.	%	No.	%
Penicillins	Penicillin	10	49	87.5	3	5.4	4	7.1
	Amoxycillin-Clavulanic Acid	30	41	73.2	7	12.5	8	14.3
Cephalosporins	Cefuroxime	30	35	62.5	5	8.9	16	28.6
	Cefepime	30	39	69.6	5	8.9	12	21.4
	Ceftazidime	30	41	73.2	4	7.1	11	19.6
	Cefotaxime Sodium	30	54	96.4	-	-	2	3.6
Aminoglycosides	Gentamicin	10	43	76.8	2	3.6	11	19.6
	Kanamycin	10	21	37.5	11	19.6	24	42.9
	Amikacin	30	19	33.9	9	16.1	28	50
Fluoroquinolones	Ciprofloxacin	5	5	8.9	2	3.6	49	87.5
	Levofloxacin	5	3	5.4	2	3.6	51	91.1
Tetracyclines	Doxycycline HCl	30	27	48.2	3	5.4	26	46.4
Phenicols	Florophenicol	30	15	26.8	11	19.6	30	53.6
Pot. Sulphonamides	Sulfamethoxazole/trimethoprim	25	56	100	-	-	-	-

%: was calculated according to the number of tested *E. coli* isolates (n=56).

Virulence factors of P. aeruginosa isolates

Haemolytic activity of *P. aeruginosa* isolates

All *P. aeruginosa* isolates were haemolytic. Most of the isolates (52/56; 92.9%) showed β-haemolysis while only few isolates (4/56; 7.1%) showed α-haemolysis.

Biofilm formation by *P. aeruginosa* isolates

MTP assay revealed that all *P. aeruginosa* isolates were high biofilm-formers where they had OD values exceeding 0.2 with average value of 0.460±0.1334.

Pyocyanin production by *P. aeruginosa* isolates

Spectrophotometric quantification of pyocyanin revealed that all *P. aeruginosa* isolates were pyocyanin-producers with an average value of 4.359±1.870 µg/ml.

Prevalence of antimicrobial resistance and virulence genes among the investigated P. aeruginosa isolates

Regarding to AMR genes, PCR revealed that all the investigated *P. aeruginosa* isolates (n=6) harboured *amp*C, bla_{CTX} and bla_{TEM} genes meanwhile both *aad*B and *sul*1 genes were found in only 66.7% (n=4) of the investigated isolates (Table 3 and Figures 1-3).

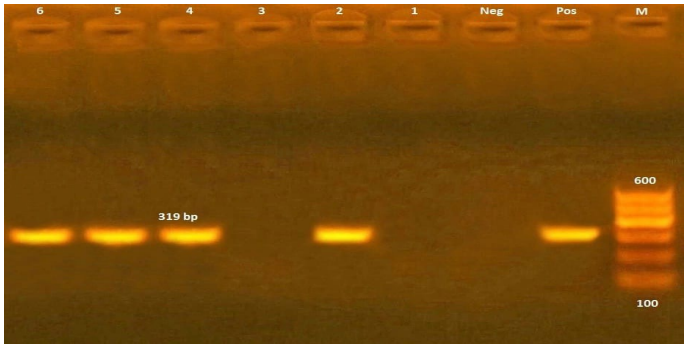

Fig. 1. Agarose gel electrophoresis for PCR products which targeted *aad*B gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 319-bp in all the investigated isolates except isolates number 1 and 3.

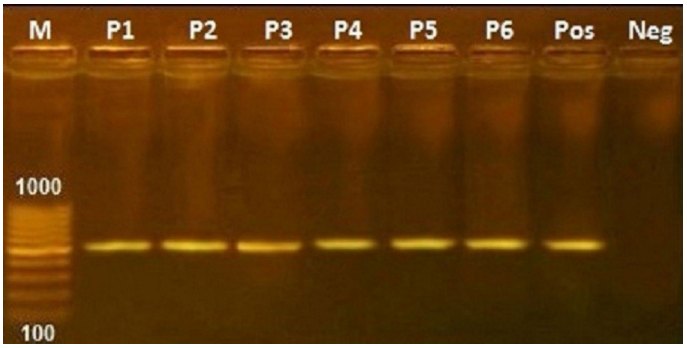

Fig. 2. Agarose gel electrophoresis for PCR products which targeted ampC gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 550-bp in all the investigated isolates.

While regarding virulence genes, PCR revealed that all the investigated *P. aeruginosa* isolates harboured *exo*S, *las*B, *las*I and *phz*M genes followed by *tox*A and *hly*A genes which were found in 83.3% (n=5) and 66.7% (n=4) of the investigated isolates, respectively (Table 3 and Figures 4-8).

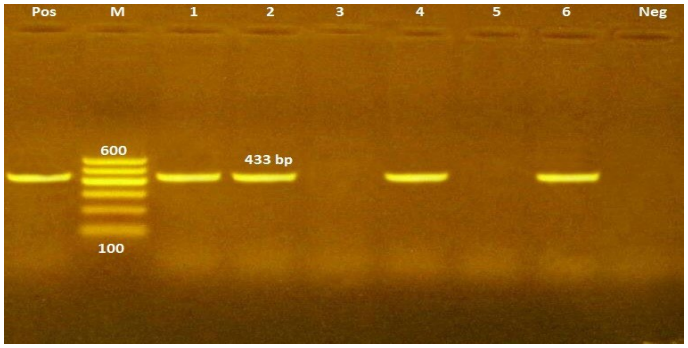

Fig. 3. Agarose gel electrophoresis for PCR products which targeted *sul*1 gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 433-bp in all the investigated isolates except isolates number 3 and 5.

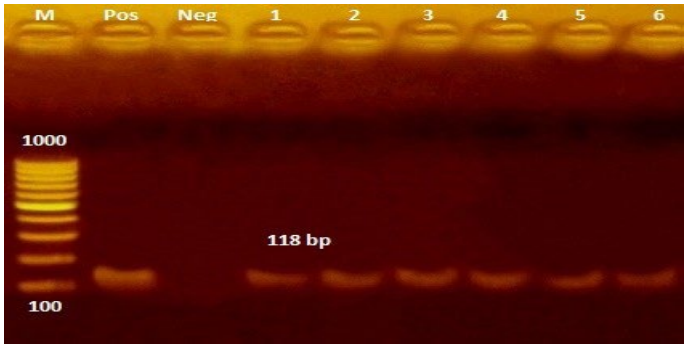

Fig. 4. Agarose gel electrophoresis for PCR products which targeted *exo*S gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 118-bp in all the investigated isolates.

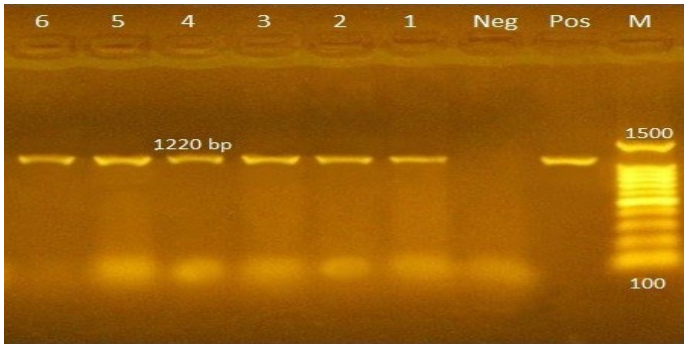

Fig. 5. Agarose gel electrophoresis for PCR products which targeted *las*B gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 1220-bp in all the investigated isolates.

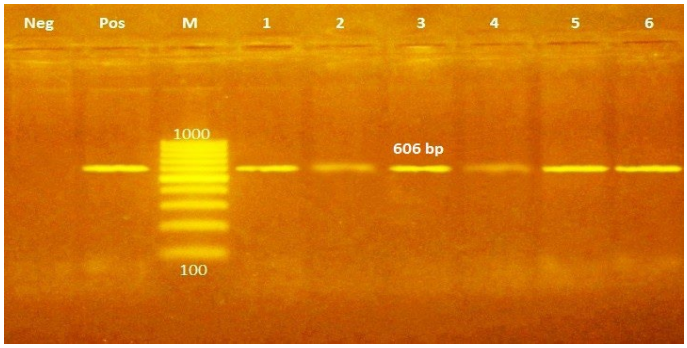

Fig. 6. Agarose gel electrophoresis for PCR products which targeted *las*I gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 606-bp in all the investigated isolates.

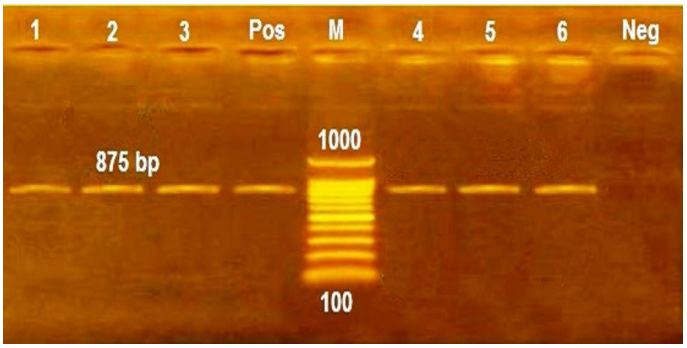

Fig. 7. Agarose gel electrophoresis for PCR products which targeted *phz*M gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 875-bp in all the investigated isolates.

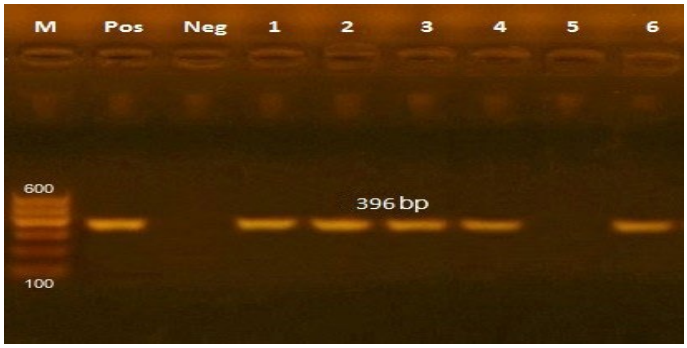

Fig. 8. Agarose gel electrophoresis for PCR products which targeted *tox*A gene in *P. aeruginosa* isolates. Lane M: DNA ladder (100 bp), lane Pos.: Positive control, lane Neg.: Negative control and lanes 1-6: DNA extracted from *P. aeruginosa* isolates showing positive bands at 396-bp in all the investigated isolates except isolate number 5.

Table 3. Prevalence of different tested resistance and virulence genes in the examined *P. aeruginosa* isolates (n.= 6).

The tested Gene		Positive	
		No.	%
Resistance genes	*aad*B	4	66.7
	*amp*C	6	100
	bla_{CTX}	6	100
	bla_{TEM}	6	100
	*Sul*1	4	66.7
Virulence genes	*exo*S	6	100
	*hly*A	4	66.7
	*las*B	6	100
	*las*I	6	100
	*phz*M	6	100
	*tox*A	5	83.3

%: was calculated according to the number (No.) of the tested isolates (n=6).

Discussion

Respiratory tract diseases of poultry have a big economic significance worldwide where they cause high mortalities and variable morbidity in poultry and subsequently large economic losses in poultry sectors. They are complex syndromes that may be caused by one microorganism or more in addition to the stress factors that exaggerate the disease (Abed, 2007). Airsacculitis is considered one of the respiratory signs which caused by many viral and bacterial pathogens (Abdelrahman, 2022).

In this study, PM examination revealed that prevalence of airsacculitis among the investigated broiler chickens was 43.9%. This result was supported by Barnes *et al*. (2008) and Syuhada *et al*. (2014) who recorded that airsacculitis was one of the major PM lesions in the chronic respiratory diseases of broiler chickens while slightly lower prevalence of airsacculitis (32.3%) and (34.6%) were recorded by Abd (2007) and Gomis *et al*. (2001), respectively.

Regarding to prevalence of *P. aeruginosa* among the investigated airsacculitis samples, it was recovered from 53.3% of them while Hassan (2013), Satish and Priti (2015) and Wolska *et al*. (2002) reported that prevalence of *P. aeruginosa* among airsacculitis samples were 25.3%, 30% and 26.8%, respectively. Also, much lower prevalence was recorded by Radwan *et al*. (2022); 8.3%.

Antimicrobials are the most important tools for controlling the bacterial diseases, to reduce their prevalence and mortalities. Furthermore, they can be used as growth promoters to maintain birds' productivity and health limiting the great economic losses that occur in poultry sectors globally (Radwan *et al*. 2021b). Therefore, antimicrobial susceptibility testing is valuable to choice the suitable antimicrobial for therapy and prophylaxis. Also, it is very important for MDR bacteria detection which has a public health concern.

Antimicrobial susceptibility testing results of our *P. aeruginosa* isolates run parallel to those recorded by Radwan *et al*. (2022) who found that *P. aeruginosa* isolates had high resistance against β-lactams and SXT in addition to high sensitivity to fluoroquinolones as well as those reported by Hassan *et al*. (2018) who found that *P. aeruginosa* isolates had complete or high resistance to AMC, DO, STX and AK as well as high susceptibility to CIP and K. Also, Emam (2006) and Kamel (2011) recorded nearly similar results where they found that *P. aeruginosa* isolates had high resistance against β-lactams and CN as well as high sensitivity to fluoroquinolones. Furthermore, Radwan *et al*. (2021b) reported that *P. aeruginosa* isolates had high resistance against β-lactams, CN and SXT as found in this study while they reported that the isolates had high resistance against fluoroquinolones, phenicols and tetracyclines in contrast to our results.

EsβLs-producing bacteria are defined as those which develop resistance against β-lactams through β-lactamases production that hydrolyze or inactivate antimicrobials having an oxyimino side chain including 3rd and 4th generations of cephalosporins as well as monobactams meanwhile unable to inactivate carbapenems and cephamycins (Abd El Tawab *et al*., 2016). They can be prohibited by β-lactamase inhibitors as CA and tazobactams (Bush and Fisher, 2011 and Ghodousi *et al*., 2015).

EsβLs are considered the major cause of Gram-negative bacteria resistance against β-lactams (Rawat and Nair, 2010) and their production may be attributed to the widespread use of β-lactams (Chaudhary and Aggarwal, 2004). β-lactamases genes are mainly carried on the plasmids (Abd El Tawab *et al*., 2016) in addition to the other MGEs (Ghodousi *et al*., 2015) and so they could be disseminated among the different bacteria (Ghodousi *et al*., 2015). ESβLs and carbapenemases-producing bacteria are MDR representing a great public health concern (Rahman *et al*., 2018). Gram-negative bacteria frequently produce β-lactamases and carbapenemases (Meletis, 2016).

In this study, most *P. aeruginosa* isolates were resistant to the tested β-lactams, therefore, they were investigated by CDD and cefinase tests for detection of ESβLs production which confirmed that 39.3% and 42.9% of *P. aeruginosa* isolates were EsβLs-producers, respectively. Our results agreed with those recorded by Begum *et al*. (2013) and Radwan *et al*. (2018) who found that 35.4% and 40% of *P. aeruginosa* isolates were EsβLs-producers, respectively. On the contrary, lower prevalences of EsβLs-produces were reported among *P. aeruginosa* isolates by Aggarwal *et al*. (2008); 20.3%, Rafiee *et al*. (2014); 6.5%, Shaikh *et al*., (2015); 25.1% and Umadevi *et al*. (2011); 19.4%. Also, Radwan *et al*. (2021b) reported higher prevalence of EsβLs-produces among *P. aeruginosa* isolates; 66.7%.

P. aeruginosa can produce variable extracellular components increasing their virulence such as fluorescein, pyocyanin, haemolysins, phospholipases and biofilm (Kebede, 2010). Moreover, Parija (2012) enumerated four exopigments produced by *P. aeruginosa* strains including pyocyanin (bluish-green color and easily identified on the agar medium), fluorescin (yellowish-green color), pyorubin (red color) and pyomelanin (brown color). In this study, *P. aeruginosa* isolates were phenotypically investigated

for some virulence factors included biofilm formation, haemolytic activity and pyocyanin production.

The haemolysins are cytolytic exotoxins invade the host cells, degrade RBCs membrane and facilitate the bacterial iron uptake from hemoglobin (Moraveji *et al*., 2014). The haemolysins expression is considered the main mechanism deployed by bacteria to facilitate the bacterial infection and prohibit the host defense (Almeida *et al*., 2013) targeting the host phagocytic cells (Radwan *et al*., 2021a). Expression of haemolysins must be considered a favoring for development of extra-intestinal infections (Kassé *et al*., 2016). In the current study, all *P. aeruginosa* isolates were haemolytic and mostly β-haemolytic as reported by Abd El Halim (2022).

Production of slime and surfaces' attachment capability to enhance formation of biofilm is a fundamental factor in bacterial pathogenicity (El-Seedy *et al*., 2019). Biofilms play a vital role in emergence and dissemination of AMR in microbial population (Morente *et al*., 2013). They can impair antimicrobial therapy by raising the AMR level up to 1000 times and therefore huge antimicrobial concentrations are essential for achieving bacteriostatic or bactericidal effects within a biofilm (Hall and Mah, 2017). Such resistance may be ascribed to the insufficient antimicrobial concentrations reaching the biofilms parts and metabolic inactivity, in addition to active antimicrobial degradation mechanisms which prevent accumulation of antimicrobials to the effective concentrations on the biofilm-forming bacteria (Ahmadi *et al*., 2017). The biofilms formation could be enhanced by the sub-inhibitory concentrations of antimicrobials (Abd El Halim, 2022). Furthermore, biofilm production could be injurious to the host tissues through enhancing release of the phagocytic lysosomal enzymes (Hermeyer *et al*., 2011). Moreover, biofilm-forming bacteria become more resistant to the opsono-phagocytosis (Srednik *et al*., 2017).

In the present study, MTP assay revealed that all *P. aeruginosa* isolates were high biofilm-formers in agreement with results of Tras *et al*. (2019) who found that all *P. aeruginosa* isolates can form biofilm. While such result was higher than those recorded among *P. aeruginosa* isolates by Abd El Halim (2022); 87.5%, Radwan *et al*. (2021b); 63.6% and Radwan *et al*. (2022); 40%.

Pyocyanin is a blue active pigment produced by *P. aeruginosa* (Kanthakumar *et al*., 1993). It is considered one of the most significant virulence factors of *P. aeruginosa* have a great concern (Fuse *et al*., 2013). Pyocyanin was the most studied phenazine with redox abilities causing damage of the host cells (Lau *et al*., 2004) and enhances *P. aeruginosa* virulence through prohibiting many host cellular functions as gene expression, innate immune mechanisms, cellular respiration, energy metabolism, and electron transport (Rada and Leto, 2013). Also, it can induce neutrophils apoptosis limiting the local inflammatory reactions and bacterial clearance (Allen *et al*., 2005). Furthermore, pyocyanin has great antimicrobial effects against different Gram-positive and Gram-negative bacteria (El-Gohary, 2004). In this study, all *P. aeruginosa* isolates were pyocyanin-producers with a mean value of 4.359±1.870 µg/ml.

The incremental AMR is receiving great attention globally and in Egypt (Radwan *et al*., 2016). Emergence and dispersal of MDR bacteria is a huge and incremental problem in both medical and veterinary fields (Radwan *et al*., 2021a). Poultry and their environments are major reservoirs for resistant bacteria and AMR genes which could be transmitted to human directly or indirectly (WHO, 2011).

P. aeruginosa genome is highly changeable due to insertion of the various MGEs (Wiehlmann *et al*., 2007) and acquires defensive mechanisms permitting maintenance of AMR genes in addition to many degradative enzymes and secretion systems affecting human infection (Frank, 2012). Many AMR and virulence genes were reported on *P. aeruginosa* plasmids (Kelly *et al*., 2009) that are the primary vector for dissemination of AMR between bacteria (Radwan *et al*., 2016). Therefore, in the present study 6 multidrug resistant *P. aeruginosa* isolates were examined by PCR for presence of 5 AMR genes included *aad*B, *amp*C, bla_{CTX}, bla_{TEM} and *sul*1.

P. aeruginosa follow variable molecular mechanisms for developing its AMR against β-lactam antibiotics like a) ESβL generation, b) ESβL encoding genes acquisition from the environmental bacteria, c) expression level increasing of chromosome-encoding β-lactamase genes (*bla*), d) *bla* genes mobilization by the combination with the integrons and horizontal transmission to the other bacteria, e) plasmid-mediated carbapenemases dispersal; like metallo-β- lactamases, and f) porin genes expression stoppage and efflux pump-based AMR (Radwan *et al*., 2021b). ESβLs are the main causes of β-lactams resistance in Gram-negative bacteria (Rawat and Nair, 2010). The most common ESβLs enzymes in the clinical isolates were CTX-M (Pitout *et al*., 2005), TEM-1 and 2 (Abd El Tawab *et al*., 2016; Bush and Fisher, 2011; Ghodousi *et al*., 2015) and SHV-1 (Abd El Tawab *et al*., 2016; Ghodousi *et al*., 2015).

In the present study, *amp*C, bla_{CTX} and bla_{TEM} genes were found in all the tested *P. aeruginosa* isolates in agreement with results of Abd El Tawab *et al*. (2016), Bora *et al*., 2014, Ghodousi *et al*. (2015) and Pitout *et al*. (2005) who suggested that TEM, and CTX-M were the main genetic groups of ESβLs in the clinical isolates of Gram-negative bacteria.

The *aad*B gene is encoding for aminoglycoside adenyltransferase (ANT2-Ia) enzyme production (Poole, 2011) which is concerned with aminoglycoside resistance, including CN, K and tobramycin (Radwan *et al*., 2021b). In this study, *aad*B gene was found in 66.7% of the investigated *P. aeruginosa* isolates. This result agreed nearly with that reported by Radwan *et al*. (2021b) who found *aad*B gene in 50% of the investigated *P. aeruginosa* isolates.

P. aeruginosa is regarded as one of the most virulent pathogens that causes ravaged diseases leading to great mortality in poultry farms (Kebede 2010). *P. aeruginosa* can produce many virulence factors especially exotoxin A encoding by *tox*A gene, pyocyanin regulated by *phz*M gene that cause host cell damage, elastase enzyme which degrades host defense and damages the epithelial cells, type III secretion system that can inject the eukaryotic cells cytoplasm with toxic effector proteins (Abd El-Halim, 2020). The most universal primers used in PCR for *P. aeruginosa* isolates is mainly relying on detection of 5 significant enterotoxin genes including *tox*A, *phz*M, *exo*S, *las*B and *exo*U (Shi *et al*., 2012).

In the present study, PCR was used for examination of 6 MDR *P. aeruginosa* isolates for presence of 6 virulence factors genes included *exo*S, *hly*A, *las*B, *las*I, *phz*M and *tox*A. PCR revealed that all the investigated *P. aeruginosa* isolates harbored these genes except *tox*A and *hly*A genes which were found in only 83.3% and 66.7% of the examined isolates, respectively.

*Tox*A gene is encoding for exotoxin A which is an important ADP-ribosyl transferase toxin (Liu, 2009). It was regarded as a significant virulence factor in the clinical isolates of *P. aeruginosa* from the different affections in chicken especially respiratory diseases (Tartor and El-Naenaeey, 2016). The *tox*A granted ability of *P. aeruginosa* for induction multiple clinical lesions in the different organs through its inhibitory effect on production of cytokines resulting in minimizing the host potential for infection clearance (Abd El-Halim, 2020). Therefore, the transmission potential of highly virulent *P. aeruginosa* strains from the broiler chickens to humans has public health concern. Furthermore, *P. aeruginosa* is considered one of the top five bacterial pathogens causing health care-associated infections respecting to virulence factors genes (Elmaraghy *et al*., 2019).

Respecting to the reported prevalence of *tox*A gene (83.3%), this result was reinforced by El-Gohary (2004) and Bahaa El-Din *et al*. (2008) who found *tox*A gene in 80% and 89.4% in the tested *P. aeruginosa* isolates, respectively. On contrary, Radwan *et al*. (2021b) didn't detect *tox*A gene in all the tested *P. aeruginosa* isolates and many previous studies reported presence of *tox*A gene in all the investigated *P. aeruginosa* isolates (Abd El-Halim, 2020, Hasssan *et al*., 2020, Hosni, 2016, Morales-Espinosa *et al*., 2017, Sabharwal *et al*., 2014 as well as Tartor and El-Naenaeey, 2016). Also, higher prevalences of *tox*A gene were reported by Elmaraghy *et al*. (2019); 95.7%, Lavenir *et al*. (2007); 95%, Taee *et al*. (2014); 97.2% and Wolska and Szweda (2009); 91.8%.

Quorum sensing is an essential bacterial method for their communication and talking through specific chemical molecules which used for

regulation of their action against the host by regulating production of their AMR and virulence factors determinants (Sabharwal *et al*., 2014). Quorum sensing gene (*las*I) is an auto-inducer which was positively linked to virulence traits secretion by *P. aeruginosa* as elastase enzyme and pyocyanin (Bratu *et al*., 2006). *Las*B gene is another quorum sensing gene encoding also for elastase enzyme that is an extracellular endo-peptidase (proteolytic enzymes) and was implicated as a very important virulence factor of *P. aeruginosa* (Wolz *et al*., 1991). It was regarded as an immune-regulatory protein affecting host immunity and damaging the epithelial cells (Dulon *et al*., 2004).

In this study, PCR revealed that all the investigated *P. aeruginosa* isolates had *las*I gene as previously recorded by Abd El Halim (2020) and Hosni (2016) while Hassan *et al*. (2020), Kadhim and Ali (2014), Radwan *et al*. (2018), Sabharwal *et al*. (2014) and Senturk *et al*. (2012) found that 80%, 78.3%, 30%, 75% and 83.3% of the investigated *P. aeruginosa* isolates by harbored *las*I gene. Concerning the recorded prevalence of *las*B gene, our result was similar to that reported by Morales-Espinosa *et al*. (2017) who found *las*B gene in all the investigated *P. aeruginosa* isolates while lower prevalences were reported by Benie *et al*. (2017); 89%, Hassan *et al*. (2020); 80% and Tartor and El-Naenaeey (2016); 85.7%

Pyocyanin is resulted from phenazine conversion by *phz*M gene (Mavrodi *et al*., 2001). The *phz* operon encoding proteins required for phenazine conversion were identified by Finnan *et al*. (2004). PCR revealed that all the investigated *P. aeruginosa* isolates had *phz*M gene in accordance with results of Abd El Halim (2022), Hosni (2016) and Morales-Espinosa *et al*. (2017) while lower prevalence of *phz*M gene were found among *P. aeruginosa* isolates by Finnan *et al*. (2004); 94.1%, and Jamunadevi *et al*. (2012); 85.7%. Furthermore, Radwan *et al*. (2021b) as well as Tartor and El-Naenaeey (2016) didn't detect *phz*M gene among the investigated *P. aeruginosa* isolates.

Exoenzyme S (*exo*S) is an important virulence factor of *P. aeruginosa* where it has an antiphagocytic property permitting the host defense overcoming and resulting establishment of the infection and damage the tissues (Rocha *et al*., 2003). There are 4 effector proteins described in *P. aeruginosa* (*exo*S, *exo*U, *exo*Y and *exo*T). They share in tolerance of innate immune responses and possess enzymatic activity resulting in host cells physiology disturbances and bacterial clearance inhibition (Sato *et al*., 2006). It was reported that *exo*S and *exo*U had the major effects on virulence meanwhile *exo*Y and *exo*T had minor effects but had great role in pathogenesis (Jia *et al*., 2006). Most the clinical *P. aeruginosa* isolates harbored *exo*S or *exo*Y genes while *exo*U gene was less frequent (Feltman *et al*., 2001). The reported prevalence of *exo*S gene in this study (100%) agreed with results of El-Sayed *et al*. (2016) and Radwan *et al*. (2021b) who found that all the investigated *P. aeruginosa* isolates harbored *exo*S gene while Zhao *et al*. (2012), Tartor and El-Naenaeey (2016) and Benie *et al*. (2017) found that 58%, 79% and 89% of the investigated *P. aeruginosa* isolates harbored *exo*S gene, respectively.

Conclusion

This study demonstrated that *P. aeruginosa* is one of the main causes of airsacculitis among broiler chickens at Beni-Suef, El-Fayoum and El-Menia Governorates, Egypt. *P. aeruginosa* isolates showed high resistance against most of the tested AMs and ESβLs production was confirmed in many of them. All *P. aeruginosa* isolates were haemolytic and producers for both biofilm and pyocyanin. Furthermore, all the investigated MDR *P. aeruginosa* isolates harbored several AMR and virulence genes posing a serious risk to broiler chickens and humans.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abd El Halim, S.A., 2022. Phenotypic and genotypic characterization of some bacterial pathogens associated with subclinical mastitis in sheep and goats. Ph.D. Thesis (Microbiology), Fac. Vet. Med., Beni-Suef Univ., Egypt.

Abd El Tawab, A.A., Mahmoud, H.B., El-Hofy, F.I., El-khayat, M.E., 2016. Prevalence of bla_{TEM} and bla_{SHV} genes in genomic and plasmid DNA of ESβL producing *Escherichia coli* clinical isolates from chicken. BVMJ 31, 167-177.

Abd El-Halim, M.W., 2020. Genotypic characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens. M. V. Sc. Thesis (Microbiology), Fac. Vet. Med., Beni-Suef Univ., Egypt.

Abdelrahman, A.A., 2022. Investigation on the causes of respiratory affections in broilers associated with high mortalities. M. V. Sc. Thesis (Poultry Diseases), Fac. Vet. Med., Beni-Suef Univ., Egypt.

Abed, A.H., 2007. A contribution towards the bacterial pathogens associated with respiratory problems in broiler chickens. M. V. Sc. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.

Abed, A.H., Orabi, A.H., Al-Safty, M.M., Hashish, H.A.A., Meabed, A.M.H. 2026. Phenotypic and genotypic characterization of avian pathogenic *E. coli* in airsacculitis of broiler chickens. J. Adv. Vet. Res. 16, 251-258.

Abed, A.H., Orabi, A., Hassan, W., Gaber, G., 2022. Resistance and virulence genes constellation associated with biofilm forming avian pathogenic *E. coli* recovered from broiler chickens. J. Egyt. Vet. Med. Assoc 82, 189-208.

Aggarwal, R., Chaudhary, U., Bala, K., 2008. Detection of extended spectrum β-lactamase in *P. aeruginosa*. Indian. J. Pathol. Microbiol. 51, 222-224.

Ahmadi, M.R., Derakhshandeh, A., Shirian, S., Daneshbod, Y., Ansari-Lari, M., Nazifi, S., 2017. Detection of bacterial biofilm in uterine of repeat breeder dairy cows. Asian Pac J. Reprod 6, 136-139.

Ahmed, O.A., Afifi, M.M., Salem, W.M., 2022. Efficacy of aqueous, ethanolic and ethyl acetate extracts of Curcuma longa in the treatment of multidrug-resistant *Pseudomonas aeruginosa* clinical isolates associated with urinary tract infections. JES 27, 1-9.

Allen, L., Dockrell, D.H., Pattery, T., Lee, D.G., Cornelis, P., Hellewell, P.G., Whyte, M.K.B., 2005. Pyocyanin production by *Pseudomonas aeruginosa* induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in-vivo. J Immunol. 174, 3643-3649.

Almeida, L.M.D., Almeida, M.Z.P., Mendonça, C.L.D., Mamizuka, E., 2013. Comparative analysis of agr groups and virulence genes among subclinical and clinical mastitis *Staphylococcus aureus* isolates from sheep flocks of the northeast of Brazil. Braz.J.Microbiol. 44, 493-498.

Archambault, M., Petrov, P., Hendriksen, R.S., Asseva, G., Bangtrakulnonth, A., Hasman, H., Aarestrup, F.M., 2006. Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among *Salmonella enterica* serovar Corvallis from Thailand, Bulgaria, and Denmark. Microb. Drug Resist. 12, 192-198.

Bahaa El-Din, A., El-Nagdy, M., Badr, R., El-Sabagh, A., 2008. *Pseudomonas aeruginosa* exotoxin A: Its role in burn wound infection and wound healing. Egypt, J. Plast. Reconstr. Surg. 32, 59-65.

Bakheet, A.A., Torra, D.E., 2020. Detection of *Pseudomonas aeruginosa* in dead chicken embryo with reference to pathological changes and virulence genes. AJVS 65, 81-89.

Barnes, H.J., Nolan, L.K., Vaillancourt, J.P., 2008. Colibacillosis, In Diseases of poultry, Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E., editors,12th ed, Blackwell Publishing, Ames, IA. pp. 691-732.

Begum, S., Salam, M.A., Alam, K.F., Begum, N., Hassan, P., Haq, J.A., 2013. Detection of extended spectrum B-lactamase in *Pseudomonas* species isolated from two tertiary care hospitals in Bangladesh. BMC res. Notes 6, 7.

Benie, C.K.D., Dadie, A., Guessennd, N., Kouame, N., N'gbesso, D., Kouadio, N.A., 2017. Molecular identification and virulence factors of *Pseudomonas aeruginosa* strains isolated from animal products. J. Bacteriol. Mycol. 4, 94.

Bratu, S., Gupta, J., Quale, J., 2006. Expression of the *las* and rhl quorum-sensing systems in clinical isolates of *Pseudomonas aeruginosa* does not correlate with efflux pump expression or antimicrobial resistance. J. Antimicrob. Chemother. 58, 1250-1253.

Bora, A., Hazarika, N.K., Shukla, S.K., Prasad, K.N., Sarma, J.B., Ahmed, G., 2014. Prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian J. Pathol. Microbiol. 57, 249-254.

Bush, K., Fisher, J.F., 2011. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-negative bacteria. Annu Rev Microbiol. 65, 455-478.

Carmo, L.P., Neilsen, L.R., da Costa, P.M., Alban, L., 2014. Exposure assessment of extended- spectrum beta-lactamases *amp*C beta-lactamases producing *E. coli* in meat in Denmark. Infect. Ecol. Epidmiol. 5, 4.

Cepas, V., López, Y., Muñoz, E., Rolo, D., Ardanuy, C., Martí, S., Xercavins, M., Horcajada, J.P., Bosch, J., Soto, S.M., 2019. Relationship between biofilm formation and antimicrobial resistance in Gram-Negative bacteria. Microb. Drug Resist. 25, 72-79.

Chaudhary, U., Aggarwal, R., 2004. Extended spectrum β-lactamases-An emerging threat to clinical therapeutics. Indian J. Med. Microbiol. 22, 75-80.

Clinical and Laboratory Standards Institute (CLSI), 2015. Performance Standards for Antimicrobial Susceptibility Testing. 25th informational supplement update. CLSI supplement M 100-S25, Wayne, PA.

Clinical and Laboratory Standards Institute (CLSI), 2021. Performance Standards for Antimicrobial Susceptibility Testing. 31st Ed. CLSI supplement M100. Wayne, PA.

Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A., 1996. Practical Medical Microbiology 14th Ed.

Colom, K., Pèrez, J., Alonso, R., Fernández-Aranguiz, A., Lariño, E., Cisterna, R., 2003. Simple and reliable multiplex PCR assay for detection of bla_{TEM}, bla_{SHV} and $bla_{OXA.1}$ genes in *Enterobacteriaceae*. FEMS Microbiol. Lett. 223, 147-151.

Contreras, A., Sierra, D., Sanchez, A., Corrales, J.C., Marcoc, P.C., Paape, M.J., Gonzalo, C., 2007. Mastitis in small ruminants. Small Rumin Res 68, 145-153.

Dénervaud, V., TuQuoc, P., Blanc, D. *et al*. 2004. Characterization of cell-to cell signaling-deficient *Pseudomonas aeruginosa* strains colonizing in tubated patients. J. Clin. Microbiol. 42, 554-562.

Dulon, S., Dubouix, A., Ollier, M., Marty, N., 2004. Longitudinal survey of virulence factors: *Exo*U, *Exo*S and PLC produced by *Pseudomonas aeruginosa* cystic fibrosis isolates. Int J Antimicrob Agents 24, S210-S210.

El-Gohary, A.H.A., 2004. Further investigation of *Pseudomonas aeruginosa* with reference to products and genetic profile. Ph. D. Thesis (Microbiology), Fac. Vet. Med., Cairo Univ., Egypt.

Elmaraghy, N., Abbadi, S., Elhadidi, G., Hashem, A., Yousef, A., 2019. Virulence genes in *Pseudomonas aeruginosa* strains isolated at Suez Canal University hospitals with respect to the site of infection and antimicrobial resistance. Int. J. Clin. Microbiol. Biochem. Technol. 2, 8-19.

El-Sayed, M.S.A, Ammar, A.M., Al Shehri, Z.S., Abd-El Rahman, H., Abd-ElRahman, N.A., 2016. Virulence repertoire of *Pseudomonas aeruginosa* from some poultry farms with detection of resistance to various antimicrobials and plant extracts. Cell Mol. Biol. 62, 124.

El-Seedy, F.R., Abed, A.H., Wafaa, M.M.H., Bosila, A.S., Mwafy, A., 2019. Antimicrobial resistance and molecular characterization of pathogenic *E. coli* isolated from chickens. J. Vet. Med. Res. 26, 280-292.

Emam, H.E.F., 2006. Bacteriological and histopathological studies on *P. aeruginosa* infection in chickens M.V.Sc Thesis (Bacteriology, Immunolgy and Mycology), Fac. Vet. Med., Suez Canal Univ., Egypt.

Feltman, H., Schulert, G., Khan, S. , Jain, M. , Peterson, L., Hauser, A.R., 2001. Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. Microbiol. 147, 2659-2669.

Finlayson, E.A., Brown, P.D., 2011. Comparison of antibiotic resistance and virulence factors in pigmented and non-pigmented *Pseudomonas aeruginosa*. West Indian Med. J. 60, 24-32.

Finnan, S., Morrissey, J.P., Gara, F.O'., Boyd, E.F., 2004. Genome diversity of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients and the hospital environment. J. Clin. Microbiol. 42, 5783-5792.

Frana, T.S., Carlson, S. A., Griffith, R.W., 2001. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. Appl. Environ. Microbiol. 67, 445-448.

Frank, D.W. 2012. Research topic on *Pseudomonas aeruginosa*, biology, and genetics host pathogen interactions. Front. Microbiol. 3, 20.

Fuse, K., Fujimura, S., Kikuchi, T., Gomi, K., Iida, Y., Nukiwa, T., Watanabe, A., 2013. Reduction of virulence factor pyocyanin production in multidrug-resistant *Pseudomonas aeruginosa*. J. infect. Chemother. 19, 82-88.

Ghodousi, A., Bonura, C., Dinoto, A.M., Mammina, C., 2015. Extended-spectrum ß-lactamase, *Amp*C-Producing, and fluoroquinolone-resistant *Escherichia coli* in retail broiler chicken meat, Italy. Foodborne Pathog. Dis. 12, 619-625.

Gomis, S.M., Riddell, C., Potter, A.A., Allan, B.J., 2001. Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with simultaneous occurrence of cellulitis and other colibacillosis lesions. Can. J. Vet. Res. 65, 1-6.

Hall, C.W., Mah, T., 2017. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol. Rev. 41, 276-301.

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

Hassan, W.H., Abed, A.H., Abd Al-Wanis, S.A., Al-Sayed, M.A.Y., 2018. Phenotypic and genotypic characterization of oxidase positive Gram negative bacilli isolated from broiler chickens. J. Vet. Med. Res. 25, 30-40.

Hassan, W.H., Kassim, A.M.K., Shany, S.A.S., Salam, H.S.H., 2020. Virulence and resistance determinants in *Pseudomonas aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt. J. Adv. Vet. Anim. Res. 7, 452-463.

Hermeyer, K., Jacobsen, B., Spergser, J., Hewicker-Trautwein, M., 2011. Detection of Mycoplasma bovis by in-situ hybridization and expression of inducible nitric oxide synthase, nitrotyrosine and manganese superoxide dismutase in the lungs of experimentally infected calves. J. Comp. Pathol. 145, 240-250.

Hosni, A.R.H., 2016. Studies on bacterial pathogens associated with proventriculitis in chickens. M. V. Sc. Thesis (Microbiology), Fac. Vet. Med., Beni-Suef Univ., Egypt.

Ibekwe, A.M., Murinda, S.E., Graves, A.K., 2011. Genetic diversity and antimicrobial resistance of *Escherichia coli* from human and animal sources uncovers multiple resistances from human sources. PLoS ONE, 6, e20819.

Jamunadevi, S., Balashanmugam, P., Muralitharan, G., Kalaichelvan, P.T., 2012. Molecular characterization of pathogenic and non-pathogenic *Pseudomonas aeruginosa* with special reference to phenazine gene. J. Modern. Biotechnol. 1, 70-74.

Jia, J., Wang, Y., Zhou, L., Jin, S., 2006. Expression of *Pseudomonas aeruginosa* toxin *Exo*S effectively induces apoptosis in host cells. Infect. Immun. 74, 6557-6570.

Kadhim, D., Ali, M.R., 2014. Prevalence study of quorum sensing groups among clinical isolates of *Pseudomonas aeruginosa*. Int. J. Curr. Microbiol. App. Sci. 3, 204-215.

Kamel, M.F., 2011. A contribution towards bacteriological and immunological studies on bacterial causes of enteritis in broilers M. V. Sc. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.

Kanthakumar, K., Taylor, G., Tsang, K.W., Cundell, D.R., Rutman, A., Smith, S., Wilson, R., 1993. Mechanisms of action of *Pseudomonas aeruginosa* pyocyanin on human ciliary beat in-vitro. Infect. Immun. 61, 2848-2853.

Kassé, F.N., Fairbrother, J.M., Dubuc, J., 2016. Relationship between *Escherichia coli* virulence factors and postpartum metritis in dairy cows. J. Dairy Sci. 99, 4656–4667.

Kebede, F., 2010. *Pseudomonas* infection in chicken. J. Vet. Med. Anim. Health. 2, 55-58.

Kelly, B.G., Vespermann, A., Bolton, D.J. 2009. The role of horizontal gene transfer in the evolution of selected foodborne bacterial pathogens. Food Chem. Toxicol. 47, 951-968.

Lau, G.W., Ran, H., Kong, F., Hassett, D.J., 2004. The role of pyocyanin in *Pseudomonas aeruginosa* infection. Trends Mol. Med. 10, 599-606.

Lavenir, R., Jocktane, D., Laurent, F., Nazaret, S., Cournoyer, B., 2007. Improved reliability of *Pseudomonas aeruginosa* PCR detection by the use of the species-specific ecfX gene target. J Microbiol Methods 70, 20-29.

Liu, D., 2009. Molecular detection of foodborne pathogens. 1st Ed., Taylor & Francis CRC Press, Boca Raton, Florida, USA.

Matar, G.M., Ramlawi, F., Hijazi, N., Khneisser, I., Abdelnoor, A.M., 2002. Transcription levels of *Pseudomonas aeruginosa* exotoxin A gene and severity of symptoms in patients with otitis externa. Curr. Microbiol. 45, 350-354.

Mavrodi, D.V., Bonsall, R.F., Delaney, S.M., Soule, M.J., Phillips, G., Thomashow, L.S., 2001. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAOI. J. Bacteriol. 183, 6454-6465.

Meat and Livestock Commercial Services Ltd (MLCSL), 2013. An Evaluation of Food Chain Information (FCI) and Collection and Communication of Inspection Results (CCIR). FSA project FS145002 report.

Meletis, G., 2016. Carbapenem resistance: Overview of the problem and future perspectives. Ther. Adv. Infec. Dis. 3, 15-21.

Mohamed, M.A., Mohamed, H.A., Afifi, M.M., 2022. Prevalence of MDR *Pseudomonas aeruginosa* in intensive care units and burned patients. JES 27, 10-15.

Morales-Espinosa, R., Delgado, G., Espinosa, L.F., Isselo, D., Mendez, J.L., Rodriguez, C. *et al.* 2017. Fingerprint analysis and identification of strains ST309 as a potential high risk clone in a *Pseudomonas aeruginosa* population isolated from children with bacteremia in Mexico City. Front. Microbiol. 8, 313.

Moraveji, Z., Tabatabaei, M., Shirzad Aski, H., Khoshbakht, R., 2014. Characterization of hemolysins of Staphylococcus strains isolated from human and bovine, southern Iran. Iran. J. Vet. Res. 15, 326-330.

Morente, E.O., Fernández-Fuentes, M.A., Burgos, M.J.G., Abriouel, H., Pulido, R.P., Gálvez, A., 2013. Biocide tolerance in bacteria. Inter. J. Food Microbiol. 162, 13-25.

Morita, R.Y., Tomida, J., Kawamura, Y., 2015. Efflux-mediated fluoroquinolone resistance in the multidrug-resistant *Pseudomonas aeruginosa* clinical isolate PA7: identification of a novel MexS variant involved in upregulation of the mexEF-oprN multidrug efflux operon. Front. Microbiol. 6, 8.

Osman, K., Orabi, A., Elbehiry, A., Hanafy, M.H., Ali, A.M., 2019. *Pseudomonas* species isolated from camel meat: Quorum sensing-dependent virulence, biofilm formation and antibiotic resistance. Future Microbiol. 14, 609-622.

Parija, S.C., 2012. Textbook of Microbiology and Immunology (Ebook). 2nd Ed. Puducherry, India: Elsevier.

Pitout, J.D.D., Nordmann, P., Laupland, K.B., Poirel, L., 2005. Emergence of *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESβLs) in the community. J. Antimicrob. Chemother. 56, 52-59.

Piva, I.C., Pereira, A.L., Ferraz, L.R., Silva, R.S.N., Vieira, A.C., Blanco, J.E., Blanco, M., Blanco, J., Giugliano, L.G., 2003. Virulence markers of enteroaggregative *Escherichia coli* isolated from children and adults with diarrhea in Brasília, Brazil. J. Clin. Microbiol. 41, 1827-1832.

Poole, K. 2011. *Pseudomonas aeruginosa*: Resistance to the max. Front. Microbiol. 2, 65.

Poulou, A., Grivakou, E., Vrioni, G., Koumaki, V., Pittaras, T., Pournaras, S., Tsakris, A., 2014. Modified CLSI Extended spectrum β-Lactamase (ESβL) confirmatory test for phenotypic detection of ESBLs among *Enterobacteriaceae* producing various β. Lactamases. J. Clinc. Microbio. 52, 1483-1489.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., Fitzpatrick, E., 2011. Veterinary Microbiology and Microbial Disease, 2nd Ed., John Wiley & Sons, Hoboken, NJ, USA, , p. 893.

Rada, B., Leto, T.L., 2013. Pyocyanin effect on respiratory epithelium: Relevance in *Pseudomonas aeruginosa* airway infection. Trends Microbiol. 21, 73-81.

Radwan, I.A., Abd El-Halim, M.W., Abed, A.H., 2021a. Molecular characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens. J. Vet. Med. Res. 27, 128-142.

Radwan, I. A., Abed, A.H., Abd Al-Wanis, S.A., Abd El-Aziz, G.G., El-Shemy, A., 2016. Antibacterial effect of cinnamon and oreganium oils on multidrug resistant *Escherichia coli* and Salmonellae isolated from broiler chickens. J. Egypt. Vet. Med, Assoc. 76, 169-186.

Radwan, I.A., Abed, A.H., Abd Allah, M.M., Abd El-Latif, M.A.A., 2018. Bacterial pathogens associated with cellulitis in chickens. J. Vet. Med. Res. 25, 68-79.

Radwan, I.A., Moustafa, M.M.M., Abdel-Wahab, S.H., Ali, A., Abed, A.H., 2022. Effect of essential oils on biological criteria of Gram-negative bacterial pathogens isolated from diseased broiler chickens. Inter. J. Vet. Sci. 11, 59-67.

Radwan, I.A., Shany, S.A.S., Amin, S.S.E., Abed, A.H., 2021b. Inhibitory effects of carvacrol on bla_{TEM} and *exo*S genes expression in ESβL producing *Pseudomonas aeruginosa* isolated from kidney lesions of broiler chickens. Adv. Anim. Vet. Sci. 9, 1408-1415.

Rafiee, R., Eftekhar, F., Tabatabaei, S.A., MinaeeTehrani, D., 2014. Prevalence of extended-spectrum and metallo β-lactamase production in *Amp*C β -lactamase producing *Pseudomonas aeruginosa* isolates from burns. Jandishapur J. Microbiol. 7, e16436.

Rahman, S.U., Ali, T., Ali, I., Khan, N.A., Han, B., Gao, J., 2018. The growing genetic and functional diversity of extended spectrum Beta-Lactamases. Biomed. Res. Int. 26, 9519718.

Rawat, D., Nair, D., 2010. Extended-spectrum beta-lactamases in Gram negative bacteria. J. Glob. Infect. Dis. 2, 263-274.

Rocha, C.L., Coburn, J., Rucks, E.A., Olson, J.C., 2003. Characterization of *Pseudomonas aeruginosa* exoenzyme S as a bifunctional enzyme in J774A.1 macrophages. Infect. Immun. 71, 5296-5305.

Sabharwal, N., Dhall, S., Chhibber, S., Harjai, K., 2014. Molecular detection of virulence genes as markers in *Pseudomonas aeruginosa* isolated from urinary tract infections. Int. J. Mol. Epidemiol. Genet. 5, 125-134.

Satish, S., Priti, M., 2015. *Pseudomonas aeruginosa* infection in broiler chicks in Jabalpur. Inter. J. Extensive Res. 6, 37-39.

Senturk, S., Ulusoy, S., Bosgelmez-Tinaz, G., Yagci, A., 2012. Quorum sensing and virulence of *Pseudomonas aeruginosa* during urinary tract infections. J. Infect. Dev. Ctries 6, 501-507.

Shaikh, S., Fatima, J., Shakil, S., 2015. Prevalence of multidrug resistant and extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* in atertiary care hospital. Saudi J. Biol. Sci. 22, 62-64.

Shi, H., Trinh, Q., Xu, W., Zhai, B., Luo, Y., Huang, K., 2012. A universal primer multiplex PCR method for typing of toxinogenic *Pseudomonas aeruginosa*. Appl. Microbiol. Biotechnol. 95, 1579-1587.

Srednik, M.E., Tremblay, Y.D.N., Labrie, J., Archambault, M., Jacques, M., Alicia, F.C., Gentilini, E.R., 2017. Biofilm formation and antimicrobial resistance genes of coagulase-negative staphylococci isolated from cows with mastitis in Argentina. FEMS Microbiol. Lett. 364, 1.

Srinivasan, V., Nam, H.M., Nguyen, L.T., Tamilselvam, B., Murinda, S.E., Oliver, S.P., 2005. Prevalence of antimicrobial resistance genes in Listeria monocytogenes isolated from dairy farms. Foodborne Pathog. Dis. 2, 201-211.

Sato, H., Feix, J.B., frank, D.W., 2006. Identifecation of superoxide dismutase as a cofactors for the *Pseudomonas* type toxin, *Exo* U. biochem. J. 45, 10368-103750.

Syuhada, N.Z.A., Hair-Bejo, M., Zakaria, Z., Omar, A.R., Khairani-Bejo, S., 2014. Isolation of *Escherichia coli* from various organs of broiler chickens with complicated chronic respiratory disease. In WPSA and WVPA conference At Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor, 2013

Taee, S.R., Khansarinejad, B., Abtahi, H., Najafimosleh, M., Ghaznavi-Rad, E., 2014. Detection of algD, oprL and *exo*A genes by new specific primers as an efficient, rapid and accurate procedure for direct diagnosis of *Pseudomonas aeruginosa* strains in clinical samples. Jundishapur J. Microbiol. 7, e13583.

Tartor, Y., El-Naenaeey, H., 2016. RT-PCR detection of exogenes expression in multi-drug resistant *P. aeruginosa*. Cell Mol. Biol. (Noisy-le-grand). 22, 62, 56-62.

Tras, B., Dinc, A. D., Uney, K., 2019. The effect of N-acetylcysteine on the treatment of clinical endometritis and pregnancy rate in dairy cows. Eurasian J. Vet. Sci. 30, 133-137.

Umadevi, S., Joseph, N.M., Kumari, K., Easow, J.M., Kumar, S., Stephen, S., Srirangaraj, S., Raj, S., 2011. Detection of extended spectrum B-lactamases, *amp*C beta lactamases and metallo-betalactamases in clinical isolates of ceftazidime resistant *Pseudomonas aeruginosa*. Braz. J. Microbiol. 42, 1284-1288.

Wiehlmann, L., Wagner, G., Cramer, N., Siebert, B., Gudowius, P., Morales, G., Köhle, T., van Delden, C., Weinel, C., Slickers, P., Tümmler, B., 2007. Population structure of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U.S.A. 104, 8101–8106.

Winstanley, C., Kaye, S.B., Neal, T.J., Chilton, H.J., Miksch, S., Hart, C.A., the Microbiology Ophthalmic Group 2005.: Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis. J. Med. Microbiol. 54, 519-526.

Wolska, K., Jakubaczak, A., Bukowski, K., 2002. Pathogenicity of *P. aeruginosa*. Adv. Agric. Sci. 8, 27-38.

Wolska, K., Szweda, P., 2009. Genetic features of clinical *Pseudomonas aeruginosa* strains. Pol. J. Microbiol. 58, 255-260.

Wolz, C., Hellstern, E., Haug, M., Galloway, D. R., Vasii, M.L., Doring, G., 1991. *Pseudomonas aeruginosa Las*B mutant constructed by insertional mutagenesis reveais elastolytic activity due to alkaline proteinase and the *Las*A fragment. Molec. Microbiol. 5, 2125-2131.

World Health Organization (WHO), 2011. Tackling antibiotic resistance from a food safety perspective in Europe. http://www.euro.who.int/data/assets/pdf_file/0005/136454/e94889.pdf.

Yaseen, M., Abdelaziz, M., Abdel-moneam, D., Abd-Elhay, E., Wassif, I., Fawzy, M., Moustafa, M. 2020. Phenotypic and genotypic characterization of the pathogenic *Pseudomonas aeruginosa* isolated from cultured Pangasianodon hypophthalmus in Egypt. EJABF 24, 453-467.

Zahar, J.R., Lortholary, O., Martin, C., Potel, G., Plesiat, P., Nordmann, P., 2009. Addressing the challenge of extended-spectrum β-lactamases. Curr. Opin. Investig. Drugs 10, 172-180.

Zhao, R.Z., Zheng, Y.J., Chen, Q., 2012. Carriage of the *Pseudomonas aeruginosa* virulence factors and prognosis after infection. Zhonghua Er Ke Za Zhi. 50, 672-677.