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RESEARCH ARTICLE

Molecular Characterization of Extensively Drug-Resistant *Pasteurella multocida* Isolated From Apparently Healthy and Diseased Chickens in Egypt

Radwa A Elalamy[1], Yasmine H Tartor[2*], Ahmed M Ammar[2], Ibrahim E Eldesouky[3,4] and Abo Elkheir I Esawy[1]

[1]Departmentof Bacteriology, Animal Health Research Institute, Dokki, Giza, Egypt; [2]Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, 44511, Zagazig, Egypt; [3]Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt; [4]Veterinary Diagnostic Laboratory, Ministry of Environment, Water and Agriculture, King Abdul Aziz Road, Al Bahah 65528, Saudi Arabia
*Corresponding author: jasmen21@yahoo.com; yasminehtartor@zu.edu.eg

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ABSTRACT

Pasteurella multocida is a significant pathogen causing fowl cholera, a highly contagious disease of worldwide economic importance. Indiscriminate use of antimicrobials accelerates the emergence of resistance that represents a serious challenge for controlling *P. multocida* infection. In this study, the prevalence of *P. multocida* in apparently healthy and diseased chickens, capsular genotyping, antimicrobial resistance patterns, and some resistance genes were determined. Lung, trachea, bone marrow, and spleen samples were collected from 200 diseased and 100 apparently healthy chickens from ten layers and broilers commercial flocks for isolation of *P. multocida*. Confirmatory identification was done using *P. multocida* specific Polymerase chain reaction (PM-PCR) and multiplex PCR for capsular genotyping. *Pasteurella multocida* isolates were additionally tested for pathogenicity in mice. Antimicrobial resistance patterns towards 18 antimicrobials and detection of *tet*H, $Bla_{ROB1,}$ *aphA-1*, and *erm*X genes were determined. *Pasteurella multocida* isolates were recovered from 10% of the diseased chickens and 4% of apparently healthy layers. All isolates were capsular type A, and susceptible to only one or two antimicrobial classes. Extensively drug resistance was found to gentamicin, ampicillin, erythromycin, trimethoprim/ sulphamethoxazole, tobramycin, colisitin, penicillin, cefotaxime, chloramphenicol, and doxycycline. Low resistance level was observed to ofloxacin (12.5%) and neomycin (41.67%). All isolates harbored *tet*H, followed by *aphA-1* (70.83%) and Bla_{ROB1} (8.3%). The obtained findings warrant attention to the emergence of extensively drug-resistant *P. multocida* from apparently healthy and diseased chickens. Consequently, prudent use of antimicrobials to treat infected birds efficiently, changing the utilization of antimicrobials in chicken feed both for prophylaxis and growth promotion is mandatory.

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INTRODUCTION

Pasteurella multocida (*P. multocida*) is a Gram-negative encapsulated bacterium that colonizes the respiratory tract of healthy animals and birds, and occasionally causes pasteurellosis under stress conditions. *Pasteurella multocida* causes fowl cholera, a highly contagious peracute/ acute or chronic disease resulting in high mortality and devastating economic losses in commercials and backyard poultry production (Xiao *et al*., 2015; Christensen and Brandford, 2016). Actually, *P. multocida* capsular serotypes A, B, D, E, and F and 16 somatic serovars have been identified (Townsend *et al*., 2001). A diversity of laboratory diagnostic methods has been used for identification of *P. multocida* with variable results. Morphological and biochemical typing for phenotypic characterization are strenuous and tedious. Therefore, molecular assays are most important as they surpass the hinders of phenotyping and furthermore gives data regarding the capsular type of *P. multocida* (Rajeev *et al*., 2011).

Regardless of using antibiotics as an effective tool for controlling *P. multocida* infection, a direful increase in multidrug-resistant (MDR) *P. multocida* strains due to excessive utilization of antimicrobials that impose extensive selective pressure on antibiotic resistance genes represents a serious challenge for antibiotic use in disease treatment (Khamesipour *et al*., 2014; Oh *et al*., 2018). MDR *P. multocida* isolates have been reported from avian in Brazil (Furian *et al*., 2016), Pekin duck in China (Zhu *et al*., 2019), pig in Korea (Oh *et al*., 2018), ovines in Spain (Cid *et al*., 2019), and cattle in Iran (Khamesipour *et al*., 2014). Hence, antibiotics sensitivity testing of *P. multocida* is of utmost significance to detect the suitable antimicrobial agent to be used (Cid *et al*., 2019). In various occasions, distinctive genotypes can be at the origin of similar antimicrobial resistance patterns. Therefore, the diversity and distribution of antibiotic resistance genes ought to be resolved (Roberts *et al*., 2013). The prevalence and resistance phenotypes of *P. multocida* serotypes can change significantly as per geographical distribution and overtime in a certain region.

To date, scant literatures are available on extensively drug-resistant (XDR) avian *P. multocida* isolates as well as its prevalence among poultry flocks in Egypt. Along these lines, the present study determines the occurrence of *P. multocida* in apparently healthy and diseased chickens, capsular typing, antimicrobial resistance patterns, and some antimicrobial resistance genes in the recovered isolates.

MATERIALS AND METHODS

Samples: Lung, trachea, bone marrow, and spleen samples (300 of each) were collected from 200 diseased and recently dead chickens with a history of respiratory distress (150 layers and 50 broilers) and 100 apparently healthy chickens (70 layers and 30 broilers) during June 2017 to May 2018 from ten commercial flocks in Dakahlia Governorate, Egypt. Samples were packaged in polyethylene bags, labeled and transferred to the laboratory for bacteriological examination.

Isolation and Identification of *P. multocida:* Swabs from trachea and bone marrow and loop full from lung and spleen were inoculated on 7% sheep blood agar and incubated for 24-48 h at 37°C. *Pasteurella multocida* colonies were identified through the colonial morphology, characteristic bipolarity, and biochemical tests (Glisson *et al*., 2008). MacConkey agar media (Oxoid; Cambridge, United Kingdom) was used for differentiation of *P. multocida* from *Pasteurellaceae* members. Isolates were maintained in Brain Hear Infusion (BHI) broth (BD Bioscience, USA) with 10% glycerol at -80°C for further analysis.

***P. multocida* specific PCR (PM-PCR) assay:** QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used for DNA extraction following the manufacturer's instructions. *Pasteurella multocida kmt*1 gene was amplified in Mastercycler® nexus thermal cycler (Eppendorf, Germany) using KMT1T7 and KMT1SP6 primers (Townsend *et al*., 1998) (Table 1).

Capsular typing of *P. multocida* isolates: Multiplex PCR assay using five sets of oligonucleotide primers (Table 1) was employed (Townsend *et al*., 1998). The amplification reaction consisting of 25 µl EmeraldAmp GT PCR master mix (Takara Bio USA, Inc.), 1µl (20 pmol) of each primer pair (Invitrogen; Carlsbad, USA), 6 µl extracted DNA, and 9 µl nuclease free water.

Molecular detection of antimicrobial resistance genes: Several PCR protocols were conducted for *tet*H, *Bla*$_{ROB1}$, *erm*X, and *aphA*-1 genes detection (Table 1). A positive control (*P. multocida* ATCC® 15742™) and negative control were included in each run.

Amplicons along with 100 bp DNA ladder(Thermo Fisher Scientific, Bedford, MA, USA) were electrophoresed using 1-2% agarose gel (Invitrogen UltrapureTM Agarose®, California, USA) containing ethidium bromide (0.5 µg/ml), visualized and photographed using ultraviolet transilluminator and gel documentation system (Alpha Innotech, Germany).

***In-vivo* testing of *P. multocida* pathogenicity:** Experimental infection complied with relevant professional and institutional animal welfare policies established by the Ethical Committee of Kafrelsheikh University, Egypt. SPF-Swiss albino mice weighting about 16-20 gm were used (3 mice for each isolate and control group). The mice were accommodated for one week, kept in separate cages, and provided with clean water and solid feed. Each isolate was grown in BHI broth for 18 h, 0.2 ml of inoculum (10^7 CFU/ml) were inoculated intraperitoneally to mice, and observed for 72 h. Non-infected mice were inoculated with phosphate buffered saline (Varte *et al.,* 2014). During postmortem examination, heart blood, lung, spleen, and liver were collected, inoculated on blood agar and recovered colonies were identified. Impression smears were prepared from heart blood and stained with Giemsa stain.

Antimicrobial sensitivity testing: *P. multocida* isolates were tested for their susceptibility to 18 antimicrobial agents (Oxoid®, Basingstoke, Hampshire, UK) (Table2) following the M31–A3 CLSI guidelines (CLSI 2018). MDR isolates display resistance to one antimicrobial agent in three or more antimicrobial classes and those susceptible only to ≤ 2 antibiotic classes were XDR (Magiorakos *et al*., 2012).

Data analysis: The statistical package program SPSS (IBM SPSS Statistics for Windows, Version 23 Armonk, NY, IBM Corp.) was used for data analysis. Kruskal-Wallis test was employed to compare between the susceptibility of isolates to antimicrobial classes and antimicrobial agents. Data were considered significant at a P value < 0.05.

RESULTS

Isolation and identification of *P. multocida* from apparently healthy and diseased chickens: Dew drop-like mucoid non-hemolytic colonies were subjected for further biochemical identification.

The isolates fail to grow on MacConkey agar and were Gram-negative bipolar coccobacilli upon microscopical examination. The recovered isolates were positive for catalase and oxidase, indole production, nitrate reduction, D-glucose, D-mannitol, galactose, fructose, and sucrose fermentation. *Pasteurella multocida* isolates were negative to methyl red, voges–proskauer, urease, citrate utilization, gelatin liquefaction tests and failed to ferment arabinose, rhamnose, inositol, raffinose, and salicin.

Table 1: Target genes and oligonucleotide primers used in the study

Target gene	Primer	Sequence (5' -3')	Amplified product (bp)	References
*kmt*1	KMT1T7	ATCCGCTATTTACCCAGTGG	460	Townsend *et al*., 1998
	KMT1SP6	GCTGTAAACGAACTCGCCAC		
*hya*D-*hya*C	CAPA-F	TGCCAAAATCGCAGTCAG	1044	Townsend *et al*., 2001
	CAPA-R	TTGCCATCATTGTCAGTG		
*bcb*D	CAPB-F	CATTTATCCAAGCTCCACC	760	
	CAPB-R	GCCCGAGAGTTTCAATCC		
*dcb*F	CAPD-F	TTACAAAAGAAAGACTAGGAGCCC	657	
	CAPD-R	CATCTACCCACTCAACCATATCAG		
*ecb*J	CAPE-F	TCCGCAGAAAATTATTGACTC	511	
	CAPE-R	GCTTGCTGCTTGATTTTGTC		
*fcb*D	CAPF-F	AATCGGAGAACGCAGAAATCAG	851	
	CAPF-R	TTCCGCCGTCAATTACTCTG		
*tet*H	F	ATACTGCTGATCACCGT	1076	Wang *et al*., 2017
	R	TCCCAATAAGCGACGCT		
Bla_{ROB1}	F	AATAACCCTTGCCCCAATTC	685	
	R	TCGCTTATCAGGTGTGCTTG		
*erm*X	F	GAGATCGGRCCAGGAAGC	488	
	R	GTGTGCACCATCGCCTGA		
aphA-1	F	TTATGCCTCTTCCGACCATC	489	
	R	GAGAAAACTCACCGAGGCAG		

Table 2: Antimicrobial resistance phenotype and antimicrobial resistance genes of extensively drug-resistant *P. multocida* isolates

No. of isolates	Isolation source	Resistance phenotype	Resistance to antimicrobial classes	*AMR genes
1	Bone marrow of diseased layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, APR, CIP, AMC, VAN	nine classes	*Tet H, blaROB-1, Aph-1*
3	Lung of diseased layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, NEO, APR, TET, VAN, NIT	nine classes	*Tet* H
4	Lung of diseased layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, NEO, APR, CIP, AMC, TET, NIT	nine classes	*Tet* H, *aphA-1*
3	Trachea of diseased layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, CIP, AMC, TET, VAN, NIT	ten classes but remain susceptible to OFX, NEO, APR	*Tet* H, *aphA-1*
1	Spleen of diseased layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, OFX, NEO, APR, CIP, TET	eight classes	*Tet* H, bla_{ROB-1}, *aphA-1*
3	Lung of diseased broiler	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, APR, CIP, AMC, TET, VAN	nine classes	*tet*H, *aphA-1*
2	Lung of diseased broiler	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, NEO, APR, CIP, TET, VAN, NIT	ten classes but remain susceptible to OFX, AMC	*tet*H, *aphA-1*
1	Trachea of diseased broiler	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, AMC, TET, NIT	eight classes	*tet*H, *aphA-1*
2	Trachea of diseased broiler	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, APR, CIP, AMC, VAN, NIT	ten classes but remain susceptible to TET, OFX, NEO	*tet*H
2	Lung of apparently healthy layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, APR, CIP, AMC, NIT	nine classes	*tet*H, *aphA-1*
2	Spleen of apparently healthy layer	AMP, PEN, ERY, CHL, DOX, TOB, CTX, GEN, CST, SXT, OFX, APR, CIP	eight classes	*tet*H

*AMR: antimicrobial resistance genes. Beta-lactams: PEN, penicillin10 IU; AMP, ampicillin 10 µg/mL; CTX, cefotaxime 30 µg/mL; AMC, amoxicillin/clavulanic acid 30 µg /mL;tetracyclines: TET, tetracycline 30 µg /mL; DOX, doxycycline 30 µg /mL; macrolides: ERY, erythromycin 10 µg /mL; folate pathway inhibitors: SXT, trimethoprim/sulfamethoxazole 30 µg /mL;aminoglycosides: GEN, gentamycin 10 µg /mL; APR, apramycin 50 µg /mL; NEO, neomycin 30 µg/mL; TOB, tobramycin10 µg/mL; phenicols: CHL, chloramphenicol 30 µg / mL; fluoroquinolones: CIP, ciprofloxacin 10 µg /mL; OFX, ofloxacin 5 µg /mL; VAN, vancomycin 2 µg /mL; Nitrofurans: NIT, nitrofurantoin 300 µg/mL; lipopeptides: CST, colistin 10 µg/mL.

Based on the phenotypic characterization, 24 *P. multocida* isolates (8%) were identified from the examined chickens. Among the isolates, 20 were obtained from diseased chickens (12 from layers and 8 isolates from broilers) at a prevalence rate of 10%. Interestingly, 4 isolates were detected from apparently healthy layers (5.7%) while no isolates were recovered from the apparently healthy broilers. All isolates yielded a 460 bp amplicon of *P. multocida kmt1* gene (Fig. 1).

Capsular genotyping of *P. multocida* isolates: Phenotypically, all *P. multocida* isolates had similar cultural, morphological and biochemical characteristics and couldn't be differentiated by conventional methods. Therefore, multiplex PCR was performed for capsular serogrouping of isolates. All *P. multocida* isolates gave an amplicon of 1044 bp for *hya*D-*hya*C gene that associated with capsular biosynthesis of serogroups A. whereas no amplicon was found to genes of the other capsular types (Fig. 2).

Antimicrobial resistance genes: Screening of the recovered isolates to the presence of antimicrobial resistance genes associated to tetracycline resistance (*tet*H), ampicillin and penicillin resistance (Bla_{ROB1}), tilmicosin (*erm*X) and neomycin (*aph*1) resistance was performed using uniplex PCR. The predominant gene detected in all isolates (100%) was *tet*H gene followed by *aph*1 gene (70.83%), while Bla_{ROB1} gene was detected in two isolates (8.3%) (Fig.3). However, *erm*X gene was not identified among the tested isolates.

***P. multocida* pathogenicity in mice:** All *P. multocida* isolates caused death of the challenged mice within 24 to 36 h post-inoculation while control mice survived. No significant variation was observed for mortality pattern. At necropsy, lungs were congested with areas of consolidation, red hepatization, and fibrin deposition. The liver was enlarged and congested. Serosanguinous exudate was observed in thoracic cavity.

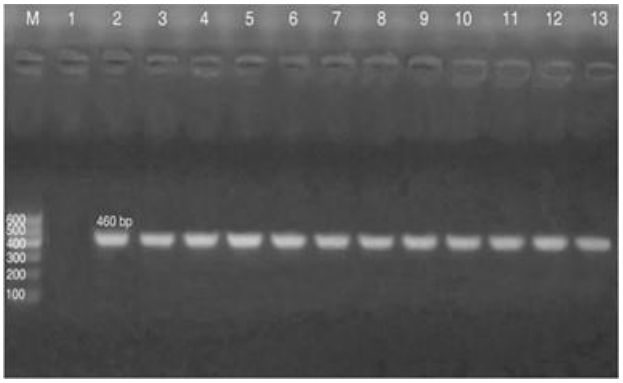

Fig. 1: Agarose gel electrophoresis for the amplified products of *kmt*1 gene specific for *P. multocida.* Lane M, 100 bp DNA ladder; lane 1, control negative; lane 2, control positive and lanes 3-13, positive *P. multocida* isolates at 460 bp.

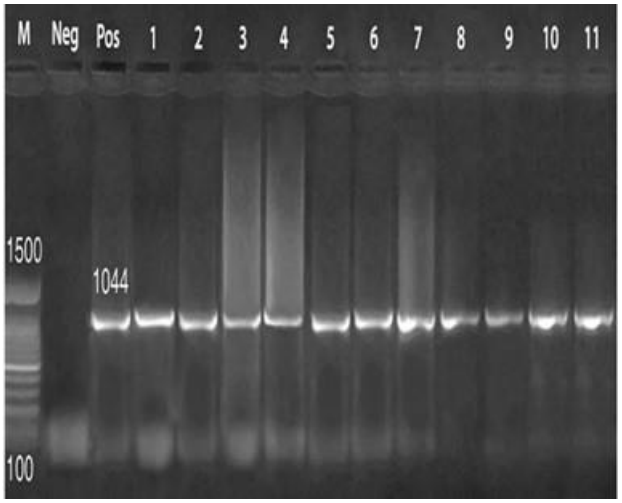

Fig. 2: Amplified products of *P. multocida* multiplex capsular PCR assay. Lane M, 100 bp DNA ladder; lane Pos, positive control; lane Neg, negative control; lanes 1-11, *P. multocida* isolates positive for the capsular antigen type A at 1044 bp.

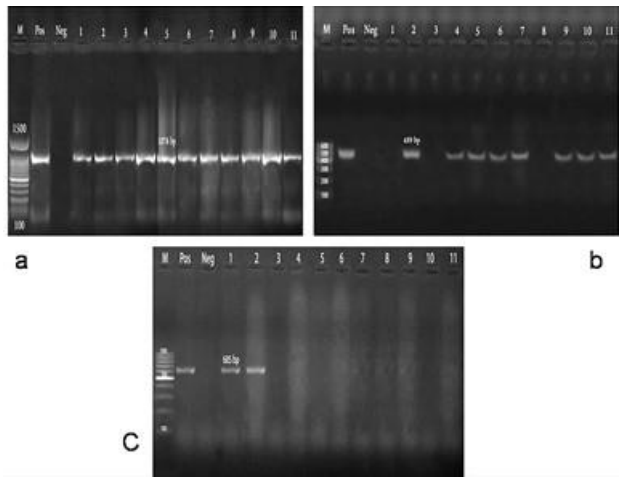

Fig. 3: Amplified products of *P. multocida* antimicrobial resistance genes. A: Amplicons of *tet*H gene. Lane M, 100 bp DNA ladder; lane Pos, positive control; lane Neg, negative control; lanes,1-11, *P. multocida* isolates positive for *tet*H gene at 1076bp. B: Amplicons of *aphA*-1 gene. Lane M, 100 bp ladder; lane Pos, positive control; lane Neg, negative control; lanes 2, 4-7, 9-10 and 11, *P. multocida* isolates positive for *aphA-1* gene at 489bp; lanes 3 and 8, negative isolates. C: Amplicons of bla_{ROB1} gene. Lane M,100 bp DNA ladder; lane Pos, positive control; lane Neg, negative control; lane 1&2, *P. multocida* positive isolates for bla_{ROB1} gene at 685bp; lanes 3-11, negative isolates.

The re-isolated colonies showed typical cultural characteristics on blood agar. Giemsa stained blood smears revealed bipolar *P. multocida*.

Antimicrobial susceptibility of *P. multocida* isolates: *Pasteurella multocida* phenotypic resistance patterns are depicted in Table 2. It exhibited extensively drug-resistance to the tested antimicrobial agents. No statistically significant differences were found between susceptibility of isolates to the tested antimicrobial classes (P=0.462) and antimicrobial agents (P=0.454). Six isolates were resistant to 16 antimicrobial agents from the different antimicrobial categories, 12 isolates possessed resistance to 15 antimicrobial agents and six isolates showed resistance to 14 and 13 antimicrobials (3 isolates for each). The highest level of resistance was found to penicillin, ampicillin, cefotaxime, erythromycin, trimethoprim / sulphamethoxazole, tobramycin, gentamicin, colistin, chloramphenicol, and doxycycline (100%); followed by apramycin and ciprofloxacin (83.33%, each), tetracycline, and amoxicillin/clavulanic acid (66.67% each), nitrofurantoin (63.6%), vancomycin (62.5 %), neomycin (41.67%), and ofloxacin (12.5%). *Pasteurella multocida* isolates were mostly sensitive to ofloxacin (87.5%) followed by neomycin (58.33%), nitrofurantoin (41.67%), vancomycin (37.5%), amoxicillin/clavulanic acid, and tetracycline (33.33%, for each).

DISCUSSION

In the current study, the total prevalence rate of *P. multocida* among the examined chickens was 8% whereas; *P. multocida* was recovered from 10% of chickens with a respiratory disease and 4% of apparently healthy layers. Similarly, an overall prevalence rate of 7.6% was reported in backyard chickens in Upper Egypt (Mohamed and Mageed, 2014). In Bangladesh, Panna *et al.* (2015) announced a prevalence rate of 11.42% and a 12.4% was reported in southwest Nigeria (Victor *et al*., 2016). Meanwhile, in Plateau state a much lower prevalence (1.2%) was reported (Kwage *et al*., 2013). In Brazil, Rigobelo *et al*. (2013) found *P. multocida* in the diseased (13.3%) and apparently healthy birds (3.3%). Hence, carrier birds might play a role in cholera transmission (Christensen and Bisgaard, 2000). The differences in the prevalence rates among studies from different countries may be attributed to breed and age of birds, environment and the bacterial strain. In consistent with Hasan *et al*. (2010) a higher rate of isolation was found among layer chickens.

As affirmed by various investigations, molecular methods have demonstrated its significance in surpassing the limitations of phenotypic methods. The capsular genotyping is an accurate and rapid assay for identification the capsular type of *P. multocida* (Townsend *et al*., 2001). Subsequently, further confirmation of the isolates was conducted on the basis of *kmt*1 gene detection. Our results showed that the phenotypically identified *P. multocida* isolates yielded the predicted amplicons of 460 bp which is specific for *P. multocida* (khamesipour *et al*., 2014; Mehmood *et al*., 2016).

Using multiplex PCR for *P. multocida* capsular genotyping revealed that all isolates were capsular type A. This finding was in accordance with the fact that capsular type A is the predominant group of *P. multocida* infecting poultry (Christensen and Bisgaard, 2000; Mohamed and Mageed, 2014). However, the present finding differs from earlier investigation that fowl cholera in avian are caused by capsular group D:3 and F:3 (Chawak *et al*., 2000).

In the present study, all *P. multocida* strains were virulent to mice. The obtained results are in accordance with previous findings (Mohamed*et al*., 2012). Therefore, this is vital in light of the fact that the tested isolates could be profoundly pathogenic causing significant economic losses in the poultry industry.

Excessive and unjustified utilization of antimicrobials has resulted in the development of MDR *P. multocida* strains (Oh *et al*., 2018). Tetracyclines are among the widely used drugs both for prophylaxis and as growth promoter in poultry industry (Babesta *et al*., 2012). This explains the development of antimicrobial resistance among isolates in the present study and previous literature (Babesta *et al*., 2012; Furian *et al*., 2016). Furthermore, the resistance rates for most antimicrobials among isolates were higher than those reported for avian isolates in Brazil (Furian *et al*., 2016), ovine isolates in Spain (Cid *et al*., 2019) or porcine isolates in Korea (Oh *et al*., 2018) due to the higher amount of antibiotics used in avian industry in Egypt. The high frequency of non-susceptibility to beta-lactam antibiotics, macrolides, quinolones, sulphonamides, phenicoles, aminoglycosides have been observed (Furian *et al*., 2016; Victor *et al*. 2016; Cid *et al*., 2019).

Most isolates were sensitive to ofloxacin (87.5%), neomycin (58.33%), nitrofurantoin (41.67%), vancomycin (37.5%) and amoxicillin/clavulanic acid, tetracycline (33.33%, for each). Relatively similar findings declared that nitrofurantoin was the best antimicrobial agent with 50% resistance (Victor *et al*., 2016). Otherwise, previous literatures declared that *P. multocida* exhibited a high sensitivity (97%) to gentamicin and amoxicillin (Furian *et al*., 2016), cephalosporins and tertracyclines (80%) (Dashe *et al*., 2013), ciprofloxacin, florfenicol, streptomycin, and sulfamethoxazine/trimethoprim (100%) (Mohamed *et al*., 2012). These findings indicate that performing antibiotic sensitivity test is essential to control fowl cholera due to the emerging drug-resistance in *P. multocida* (Panna *et al*., 2015).

Here, the *P. multocida* strains with perceived phenotypic resistance profiles were tested for antimicrobial resistance genes. We investigated the existence of resistance genes for β-lactams, macrolides and tetracyclines, that were classified by the World Organization for Animal Health as fundamentally essential antimicrobials in veterinary medicine. The results indicated that all *P. multocida* isolates carried *tet*H gene (100%), providing an explanation for the tetracycline resistance phenotype. San Millan *et al*. (2008) and Babetsa *et al*. (2012) reported nearly similar results 75 and 72.2%, respectively. bla_{ROB1} β-lactamase that confer penicillins and cephalosporins resistance is one of the Ambler Class A β-lactamases that can be highly inhibited by β-lactamase inhibitors (Bush and Brandford, 2016). The presence of bla_{ROB1} proposes that the resistance to ampicillin and penicillin is mostly actuated by the β-lactamase enzyme. Among penicillin and ampicillin resistant isolates, only 8.3% of isolates carried bla_{ROB1} gene. The obtained results differ remarkably from those of San Millan *et al*. (2008) who identified bla_{ROB1} gene in all examined *P. multocida* strains due to the existence of bla_{ROB1} gene as a part of small plasmids. *aphA-1* gene was detected in 70.83% of tested isolates. As confirmed previously, strains harboring *aphA-1* generally showed resistance to kanamycin, amikacin, and neomycin (Klima *et al*., 2014). This finding was in agreement with those described by Wang *et al*. (2017). Non-detection of genes that mediate the resistance to macrolide proposes that genes other than those screened for here may confer the resistance or that there is another drug-resistance mechanism, for example, mutations in the specific target region of the macrolide in the bacterial ribosome (Peric *et al*., 2003) or plasmid mediated resistance (Zhu *et al*., 2019).

In conclusion, PM-PCR and capsular PCR are efficient assays for rapid precise identification and serogrouping of *P. multocida* to reform fowl cholera diagnosis. To impede the emergence of XDR *P. multocida* strains, it is mandatory to test the antimicrobial efficacy to recognize the most efficient antimicrobial agents for treatment and changing the use of antimicrobials for prophylaxis and at subtherapeutic levels in chicken feed as growth promoters.

Authors contribution: AA, IE, and AE conceived and designed the study. RE, IE, and YT carried out the experiments and performed the data analysis. All authors interpreted the data. YT, IE, and RE wrote the manuscript. All authors revised the manuscript and approved the final version.

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