Isolation, Identification and Antimicrobial susceptibility patterns of *Pasteurella multocida* Isolated from Diseased Rabbits

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Abstract

Pasteurella multocida is a widespread pathogen associated with major animal diseases of considerable economic significance to livestock sector in Egypt. *Pasteurella* species (spp.) isolated from rabbits are poorly characterized. Therefore, this study was conducted to isolate and study the phenotypic characteristics and antimicrobial susceptibility profiles among *P. multocida* organisms recovered from diseased rabbits. Isolation of *P. multocida* was attempted from the collected nasal swabs and tissue samples of rabbits. A total of 16 *P. multocida* isolates (8%) were recovered from lung tissues and nasal swabs with incidence rates of 6.5 and 1.5%, respectively. The pure cultures were identified on the basis of their cultural, morphological and biochemical properties, pathogenicity studies in rabbits and molecular recognition by a species specific PCR assay. The *in vitro* antibiotic sensitivity results revealed the occurrence of antimicrobial resistance among all the isolates. The resistance was more frequent to doxycycline, tetracycline and colistin (100% each), followed by erythromycin, cefotaxime, chloramphenicol, ciprofloxacin and trimethoprim/sulfamethoxazole (93.8% each). Meanwhile, low levels of susceptibility were found; with the greatest level of sensitivity to nitrofurantoin (75%). All the examined strains demonstrated remarkably multi-drug resistant (MDR) patterns with resistance to at least 8 drugs. The results of this investigation provide useful information on the diagnosis and antimicrobial resistance patterns of *P. multocida* for selection of appropriate antimicrobial agents.

Key words: Isolation, characterization, *P. multocida*, rabbits, antibiogram.

Introduction

Pasteurellosis is a highly contagious disease of rabbits caused by *Pasteurella multocida* (*P. multocida*) ***(El Tayeb et al., 2004)***. The disease is characterized by various clinical signs including respiratory distress (snuffles) with purulent nasal discharge, pneumonia, abscesses and septicemia ***(Premalatha et al., 2009)***.

Accurate laboratory diagnosis of pasteurellosis is based mainly on the occurrence of typical clinical symptoms and pathological findings and isolation and identification of the suspected bacterial causative organism on the basis of its microscopic demonstration and cultural and biochemical characteristics ***(Kumar et al., 2012)***.

Antimicrobial therapy especially the choice of effective antibiotics is still an effective tool for treatment of pasteurellosis. However, excessive use of antibiotics in animal production accelerates the emergence of MDR strains of *P. multocida* to most of the widely used commercial antimicrobial agents being a controversial issue ***(Arora et al., 2005)*** with a large potential for transfer of this resistance from animals to humans through the use of antibiotics in animal production. The implication of this potential transfer is the increased treatment costs, prolonged illness due to treatment failure and sometimes death, thereby affecting the economy of Egypt ***(Kelly et al., 2004)***.

As a result, antibiotic resistance among pathogenic bacteria is recognized as a growing problem in both the veterinary and human medicine fields ***(White et al., 2002)***. This warrants the need for pre-testing of antibiotics sensitivity against pasteurella isolates to find out effective antimicrobial agents to be used by the veterinarians ***(Varte et al., 2014).***

Proper isolation and identification of *P. multocida* besides better understanding of their antimicrobial resistance profiles is prerequisite for adapting successful control measures of pasteurellosis in rabbits. Therefore, the present investigation was undertaken with the aim to isolate and characterize *P. multocida* isolates from diseased rabbits in various districts of Sharkia Governorate in addition to documentation of their antibiogram patterns.

Materials and methods

Clinical specimens

A total of 200 samples consisting of nasal swabs (53) and tissues from lung (72), liver (32), heart (30) and spleen (13) were collected from clinically sick rabbits with respiratory manifestations from 6 districts of Sharkia Governorate during the period of March 2015 to March 2017 for isolation of *P. multocida*. Following collection, the samples were conveyed to the bacteriology unit of the

Microbiology Laboratory, Faculty of Veterinary Medicine, Zagazig University in special ice-filled containers within 6 h of sampling for culture and microbiological examination.

Isolation and characterization of *Pasteurella* spp.

The collected samples were processed as per the procedures previously described ***(Cheesbrough, 2006)***. Until very recently, conventional methods for detection and diagnosis of infection with pasteurella relied on observation of the stained bacterium by microscopy and/or its isolation by *in vitro* culturing on selective media, followed by full phenotypic characterizations.

Preliminary isolation of pasteurella organisms was conducted adopting the techniques suggested earlier by other authors ***(Songer and Post, 2005)***. Briefly, each prepared sample was inoculated into brain heart infusion (BHI) broth (Oxoid, Basingstoke, Hampshire, England, UK) and incubated aerobically at 37°C for 24 h. Subsequently, a loopfull from the inoculated broth was streaked onto blood agar medium supplemented with 5% fresh sheep blood and clindamycin, trypticase soya agar supplemented with 5% defibrinated sheep blood (BTSA) and MacConkey`s agar media (Oxoid, Basingstoke, Hampshire, England, UK). After overnight incubation at 37°C under aerobic conditions, growth and colony characteristics on the various media were examined and the colonies suggestive of *Pasteurella* spp. were purified and cultured again on blood agar. Afterwards, the purified suspected colonies were subjected to Gram staining for demonstration of the cellular morphology and to Giemsa staining for characterization of the bacterial bipolar cells.

Initially, all the isolates that do not grow on MacConkey`s agar and show Gram negative coccobacilli characteristics were further confirmed as *Pasteurella* spp. using conventional biochemical tests including catalase, indole, oxidase and urease production according to the standard laboratory procedures ***(Mutters et al., 1989)***.

Presumptive isolates were confirmed as *P. multocida* by PCR amplification of the species specific *kmt*1 gene fragment using the protocol established preciously ***(Townsend et al., 1998)***

Pathogenicity test

Experimental infection collaborated with relevant professional and institutional animal welfare policies. All animal studies were performed in accordance with the guidelines and permission of the Committee on the Ethics of Animal Experiments of the Faculty of Veterinary Medicine, Zagazig University, Egypt and general good health was observed regularly.

The pure cultures of *P. multocida* isolates were subjected to pathogenicity studies. Two rabbits with an average body weight of 1.5-

2 kg were used for each isolate and all animals were kept under hygienic measures. Rabbits were infected intranasally (IN) with 1 ml of inoculum containing 2×10^5 colony forming units (CFU)/ml, while negative control rabbits were given 1 ml phosphate buffered saline ***(Katoch et al., 2015)***. All rabbits were observed for 48 h and mortality was recorded. The dead ones were subjected to post mortem examinations and heart blood, lungs, spleen, liver and kidneys were collected and subjected to re-isolation of *P. multocida* onto 5% sheep blood agar. Heart blood smears and impression smears prepared from liver, spleen and lung from the dead rabbits were stained with Giemsa's stain and examined microscopically.

Antimicrobial sensitivity assay

All the isolates were subjected to *in vitro* antibiotic susceptibility testing by standardized disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England, UK) plates supplemented with 5% defibrinated sheep blood ***(Bauer et al., 1966)***. The following 17 commercially available antimicrobial agents (Oxoid, Basingstoke, Hampshire, England, UK) were tested: erythromycin (15 µg), apramycin (15 µg), gentamicin (10 µg), neomycin (30 µg), tobramycin (10 µg), chloramphenicol (30 µg), colistin (10 µg), tetracycline (30 µg), doxycycline (30 µg), nitrofurantoin (300 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amoxicillin (25 µg), amoxicillin/clavulanic acid (20/10 µg), ampicillin (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg) and penicillin G (10 IU). The diameters of the inhibition zones around each antibiotic disc were measured and subsequently matched with the respective standard inhibition zone diameters of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoint table version 7.1 2017 ***(EUCAST, 2017)*** to interpret the test culture as sensitive, intermediate or resistant. Isolates that were resistant to at least three different antibiotic classes were classified as MDR.

Results

Isolation of *P. multocida* from diseased rabbits in Sharkia Governorate

Sixteen pasteurella isolates were obtained from 200 collected samples of infected rabbits (8%) as proved by bacteriological examination and biochemical characterization. The contamination with *P. multocida* was observed only in lung tissues and nasal swabs with incidence rates of 6.5 and 1.5%, respectively. No *P. multocida* were recovered from liver, heart and spleen tissues. Among different localities in Sharkia Governorate, Hahia was more likely to have *P. multocida* (16.7%); meanwhile, Mashtool El Sook, showed the

lowest incidence rate of *P. multocida* (3.3%) (Table 1).

Characterization of *P. multocida*

The 16 clinical isolates were subjected to a full phenotypic characterization via standard bacteriological techniques to be identified as *Pasteurella* spp. The addition of clindamycin on 5% sheep blood agar medium rendered it the best selective medium for pasteurella isolation in pure cultures and with little contamination. Initial examination of the growth characteristics revealed that all pasteurella cultures exhibited luxuriant growth onto 5% sheep blood agar and BTSA, with typical cultural characteristics of small, smooth, glistening, translucent, convex and non haemolytic dew-drop like colonies; however, the isolates failed to grow on MacConkey`s agar after overnight incubation at 37º C.

Microscopical examination of Gram stained smears of suspected isolates revealed characteristic Gram negative, non-spore forming and coccobacillary organisms arranging singly, in pairs or in short chains. Moreover, typical bipolar staining rods were evident in Giemsa stained smears of pasteurella fresh cultures.

All strains presumed to belong to *Pasteurella* spp. were confirmed through a wide range of uniform biochemical activities. Biochemical profiles of the recovered isolates indicated that they produced positive reactions for oxidase, catalase and indole production tests; however, they were found to be negative for urease production test. The above tested criteria identified those bacterial isolates as *Pasteurella* spp.

All 16 isolates were confirmed as *P. multocida* based on the PCR amplifications of *kmt*1 species specific gene fragments that were electrophoresed to approximately 460 bp (Figure 1).

Experimental infections of *P. multocida*

All the rabbits belonging to the negative control group remained pasteurella free with no clinical or pathological signs of the disease observed among them.

Pathogenicity tests of field pasteurella isolates revealed that all rabbits showed signs of depression during the first 2-3 h of injection, fever, tremor, weakness, loss of appetite, rough hair and increased urination in addition to the appearance of symptoms of snuffle, subcutaneous abscess and death occurring 24-48 h post infection.

Post mortem examinations of experimental rabbits revealed the pathognomic characteristic lesions. Grossly, there were severe haemorrhages in subcutaneous blood vessels and capillaries, septicemia, sever congestion and haemorrhage of the internal organs, especially for trachea and lungs, fibrinopurulent pneumonia, and enlargement of spleen.

P. multocida was further re-isolated from the heart blood, lungs, liver, spleen, and kidneys of the dead

rabbits onto 5 % sheep blood agar giving their typical cultural characteristics.

On Giemsa staining, heart blood smears and tissue impression smears prepared from liver, spleen and lung revealed the presence of characteristic bipolar organisms suggestive of *P. multocida*.

Antimicrobial sensitivity assay

The results of *in vitro* susceptibilities of all *P. multocida* isolates to a panel of 17 antibiotics of different groups using disc diffusion test is depicted in table 2. In spite of the different sources from which the isolates were obtained, there was a great uniformity of response to the antibacterial agents tested. The range of resistance to different individual antimicrobial agents was more diverse among isolates from lung tissues than those from nasal swabs.

Antimicrobial susceptibility profiles of pasteurella isolates obtained in this study are displayed in table 3. Generally, pasteurella isolates were resistant to most of the widely used commercial antimicrobial agents. All clinical isolates exhibited absolute resistance to doxycycline, tetracycline and colistin (100% each), followed by erythromycin, cefotaxime, chloramphenicol, ciprofloxacin and trimethoprim/ sulfamethoxazole (93.8% each). Furthermore, the resistance was more frequent to apramycin, neomycin, amoxicillin/clavulanic acid and amoxicillin (75% each). On the other hand, there were notably little antibiotic susceptibilities in the isolates, with the greatest level of sensitivity to nitrofurantoin (75%), followed by tobramycin (43.8) and variable sensitivity frequencies to penicillin G, ampicillin and gentamicin.

All the examined strains demonstrated remarkably MDR patterns with resistance to at least 8 drugs. A considerable number of isolates (13/16, 81.25%) were resistant to thirteen or more antibiotics and one isolate possessed resistance to all tested drugs (6.25%) (Table4).

Table (1): *Incidence of P. multocida from nasal and lung samples of diseased rabbits in various localities of Sharkia Governorate*

Locality (No)	No. of *P. multocida* isolates (%)		Total (%)
	Nasal swabs (53)	Lung tissues (72)	
Minea El kamh (65)	1 (1.9)	3 (4.2)	4 (6.2)
San-El Hagar (50)	1 (1.9)	3 (4.2)	4 (8)
Mashtool El sook (30)	0.0	1 (1.4)	1 (3.3)
Hahia (30)	1 (1.9)	4 (5.6)	5 (16.7)
Fakous (10)	0.0	1 (1.4)	1 (10)
Zagazig (15)	0.0	1 (1.4)	1 (6.7)
Total (200)	3 (5.7)	13 (18.1)	16 (8)

Table (2): *Antibiogram patterns of all 16 P. multocida isolated from nasal and lung samples of diseased rabbits*

Isolate source	Code No	B-lactams				Aminoglycosides				Macrolides	Tetracyclines		Polymyxins	Cephalosporins	Phenicols	Quinolones	Sulfonamides	Nitrofurans
		AX	AM	AMC	P	CN	TOB	APR	N	E	DO	TE	CT	CTX	C	CIP	SXT	F
Nasal swabs	P13	S(20)	-	-	-	-	-	S(27)	-	-	-	-	-	-	-	-	-	-
	P14	S(18)	S(19)	-	-	-	S(17)	S(17)	-	-	-	-	-	-	-	-	-	-
	P15	-	S(17)	-	-	-	S(19)	-	S(18)	-	-	-	-	-	-	-	-	S(14)
Lung tissues	P1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S(15)
	P2	-	-	-	S(19)	-	-	-	-	-	-	-	-	-	-	-	-	S(15)
	P3	-	-	S(18)	S(20)	-	-	-	-	-	-	-	-	-	-	-	-	-
	P4	S(20)	-	-	S(22)	-	-	-	-	-	-	-	-	-	-	-	-	S(12)
	P5	S(17)	-	S(18)	-	S(17)	-	-	-	-	-	-	-	-	-	-	-	S(22)
	P6	-	S(20)	S(17)	S(34)	-	-	-	-	S(32)	-	-	-	S(26)	S(22)	S(31)	S(42)	S(18)
	P7	-	S(17)	-	S(20)	-	S(18)	S(17)	-	-	-	-	-	-	-	-	-	S(22)
	P8	-	S(18)	-	-	-	S(19)	-	S(18)	-	-	-	-	-	-	-	-	S(20)
	P9	-	-	-	-	S(17)	S(17)	-	S(18)	-	-	-	-	-	-	-	-	S(12)
	P10	-	-	-	-	S(20)	S(18)	-	S(19)	-	-	-	-	-	-	-	-	S(22)
	P11	-	-	S(18)	S(19)	S(19)	-	S(19)	-	-	-	-	-	-	-	-	-	S(21)
	P12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P16	-	S(18)	-	-	S(17)	S(17)	-	-	-	-	-	-	-	-	-	-	S(19)

Number between brackets shows the inhibition zone diameters in mm.

S: Sensitive -: Resistant

AX: Amoxicillin AM: Ampicillin AMC: Amoxicillin/clavulanic acid
P: Penicillin G CN: Gentamicin TOB: Tobramycin
APR: Apramycin N: Neomycin E: Erythromycin Do: Doxycycline
TE: Tetracycline CT: Colistin CTX: Cefotaxime
C: Chloramphenicol Cip: Ciprofloxacin SXT: Trimethoprim/sulfamethoxazole
F: Nitrofurantoin

Table (3): *Sensitivity profiles of 16 P. multocida against 17 antimicrobial agents*

Antibiotic	No (%) of sensitive isolates	No (%) of resistant isolates
Nitrofurantoin	12 (75)	4 (25)
Tobramycin	7 (43.8)	9 (56.3)
Penicillin G	6 (37.5)	10 (62.5)
Ampicillin	6 (37.5)	10 (62.5)
Gentamicin	5 (31.3)	11 (68.8)
Apramycin	4 (25)	12 (75)
Neomycin	4 (25)	12 (75)
Amoxicillin/clavulanic acid	4 (25)	12 (75)
Amoxicillin	4 (25)	12 (75)
Cefotaxime	1 (6.3)	15 (93.8)
Trimethoprim/sulfamethoxazole	1 (6.3)	15 (93.8)
Tetracycline	0.0	16 (100)
Chloramphenicol	1 (6.3)	15 (93.8)
Ciprofloxacin	1 (6.3)	15 (93.8)
Erythromycin	1 (6.3)	15 (93.8)
Doxycycline	0.0	16 (100)
Colistin	0.0	16 (100)

Table (4): *Multi drug resistance patterns of P. multocida isolated from nasal and lung samples of diseased rabbits*

No. of Antibiotics	No. of resistant isolates	
	Nasal swabs (3)	Lung tissues (13)
8	0.0	1 (7.7%)
12	0.0	2 (15.4 %)
13	2 (66.7 %)	5 (38.5 %)
14	0.0	1 (7.7%)
15	1 (33.3 %)	2 (15.4%)
16	0.0	1 (7.7%)
17	0.0	1 (7.7%)

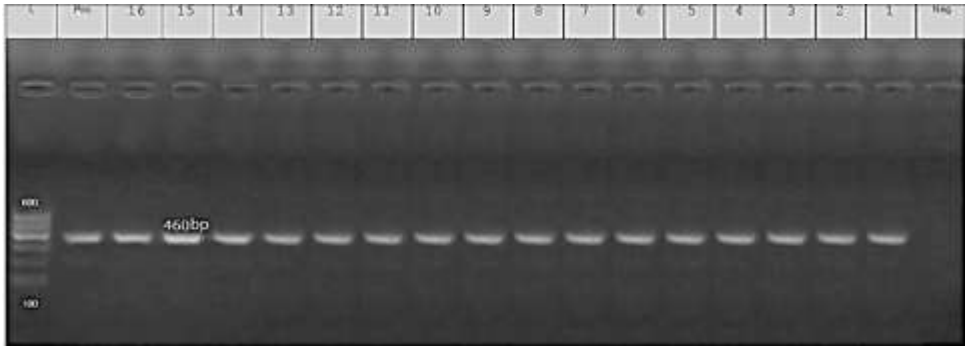

Figure 1: *Agarose gel electrophoresis showing typical amplification products for kmt1 species specific gene. Lane L: DNA molecular size marker (100-bp), lanes 1-16: Pasteurella multocida isolates from infected rabbits, lane Pos.: positive control, lane Neg.: negative control.*
PCR amplification products and their sizes in base pairs (bp) are indicated.

Discussion

Rabbit pasteurellosis "snuffles" continues to be a major concern for the rabbit industry with potential fatal consequences. In this study, we described isolation, identification and antibiotic susceptibility patterns of *P. multocida* isolated from diseased rabbits in different localities of Sharkia Governorate, Egypt.

As shown in table 1, out of 200 collected samples, 16 (8%) were positive for *P. multocida* isolates. This prevalence rate was consistent with other previous reports in Brazil with varying frequencies observed in some Brazilian rabbitries (7.4 - 10%) ***(Ferreira et al., 2012)*** and in Kafr El-Sheikh (9.4%) ***(Asran et al., 2016)***. On the other hand, the prevalence of *P. multocida* infection was low when compared with previous reports in various Governorates in Egypt; Kaliobeya (27%) ***(Mazed et al., 2013)*** and Sharkia (47.5%) ***(Suelam. and Abdel Samie, 2011)***.

Isolation percentages obtained in this study were rather low considering the total number of animals examined. This may be due to the fact the samples were not taken from flocks in the acute stage of infection and massive usage of antibiotics during rabbit breeding in Egypt.

Notably, the isolates were recovered only from lung tissues and nasal swabs with incidence rates of 6.5 and 1.5%, respectively. In comparison, other investigators also isolated *P. multocida* from nasal swabs and lung tissues (6 and 3.4%, respectively) and failed to isolate *P. multocida* from other collected samples **(Asran *et al.*, 2016)**.

It was suggested that the great variations in the distribution frequencies of *P. multocida* may be

attributed to individuality of health or immunological status of the sampled rabbits, sources of rabbit colonies, differences in geographic locations, environmental conditions, sanitation, ventilation, genetic resistance and vaccination.

The recovered isolates were subjected to a full phenotypic characterization depending on standard culture and biochemical methods. All the isolates showed typical cultural characteristics of smooth, dew drop and non haemolytic colonies onto 5% sheep blood agar and BTSA. No growth was observed in MacConkey`s agar. Gram staining of the prepared smears revealed characteristic Gram negative coccobacillary organisms. Giemsa stained smears exhibited typical microscopical features of bipolar staining. The isolates subjected to biochemical tests were positive for indole, oxidase and catalase production; however, they were negative for urease production test. These findings coincide with the descriptive features described recently ***(Premalatha et al., 2009; Verma et al., 2013 and Kour et al., 2016)***.

The above mentioned traditional methods are labor intensive and their results can be inconclusive creating difficulties in arriving at pivotal decisions. Many such variations have been described previously ***(Ekundayo et al., 2008)***. This means the necessity for use of additional assays to ensure a foolproof bacterial identity. Therefore, in recent years, genotypic identification methods have proved a great success in overcoming some limitations of traditional phenotypic procedures.

Molecular confirmation by PCR amplification of *kmt*1 gene proved to be quick, easy, specific and sensitive assay for the confirmation of *P. multocida* isolates. All the recovered *P. multocida* isolates in the current research were found to be positive for PCR amplifications of *kmt*1 gene yielding amplified products at the expected size of 460 bp. These findings confirmed the results obtained earlier ***(Townsend et al., 1998; Verma et al., 2013 and Kour et al., 2016)***.

All *P. multocida* field isolates subjected to rabbit inoculation tests killed the animals within 24 – 48 h. The main pathological findings were haemorrhage in subcutaneous blood vessels and capillaries, septicemia, sever congestion and haemorrhage of the internal organs, fibrinopurulent pneumonia, and enlargement of spleen. Giemsa staining of prepared smears revealed the presence of characteristic bipolar organisms. Similar observations were obtained in another study conducted in India ***(Katoch et al., 2015)*** indicating that *P. multocida* strains are highly pathogenic for rabbits.

Infections with *P. multocida* are commonly managed by broad spectrum antimicrobials. Monitoring the antimicrobial susceptibility trends of *P. multocida*

is an important aid to veterinarians for selecting the most efficacious therapeutic agents. The current antibiotic susceptibility findings as well as those reported in a previous study in Iran ***(Khamesipour et al., 2014)*** indicated that nitrofurantoin was the most active drug against the recovered *P. multocida* isolates (75%). Variable sensitivity frequencies recorded to penicillin G and ampicillin in our study were in conformity with data of previous literatures ***(Khamesipour et al., 2014 and Jabbari et al., 2015)***. The judicious use of these drugs by practitioners will go a long way in controlling cases of pasteurellosis in rabbits in the study area.

The inferior activities of antimicrobials in the last two decades may account for the shift in the antibiotic sensitivity spectrum of *P. multocida* with a pronounced clinical resistance against conventional antibiotics, which lead to problems in treating the animals suffering from pasteurellosis. Studies have therefore reported the occurrence of resistance to a large number of antimicrobial agents among *P. multocida* isolates.

A remarkable observation in this study is the resistance shown by a high proportion of isolates to doxycycline, tetracycline and colistin, followed by erythromycin, cefotaxime, chloramphenicol, ciprofloxacin, trimethoprim/ sulfamethoxazole, apramycin, neomycin, amoxicillin/clavulanic acid and amoxicillin. Our observations are generally in agreement with previous reports by different investigators ***(Balakrishnan et al., 2012; Jabbari et al., 2015 and Yakubu et al., 2015)***. The apparent inability of these conventional drugs to be effective against *P. multocida* isolates signifies a serious consequence to rabbit farmers and clinicians because this will severely undermine the effective control of pasteurellosis.

There is some confusion with regard to the susceptibility of *P. multocida* to aminoglycoside antibiotics. Un like other authors who reported a high activity of gentamicin against the tested isolates ***(Premalatha et al., 2009; Khamesipour et al., 2014; Varte et al., 2014; Yakubu et al., 2015*** the present study documented a relatively high resistance of *P. multocida* clinical isolates to gentamicin (68.8%).

All the examined strains demonstrated remarkably MDR patterns with resistance to at least 8 drugs. This phenomenon is presumably attributed to the extensive and pervasive use of antimicrobial agents by farmers and veterinary practitioners. Similar reports on the emergence of MDR *P. multocida* strains have also been made earlier ***(Espinosa et al., 2012 and Yakubu et al., 2015)***. Antimicrobial resistance in *P. multocida* has been linked to small plasmids. The coexistence and spread of these small plasmids has

resulted in the emergence of multiresistant *P. multocida* isolates.

References

Arora AK, Virmani S, Jand SK and Oberoi MS (2005): Isolation, characterization and antibiogram of *Pasteurella multocida* isolates from different animal species. The Indian Journal of Animal Sciences, 75 (7): 749-752.

Asran EA, Khalil SA and Hegazy AM (2016): Identification and molecular analysis of *Pasteurella multocida* isolated from rabbits. Alexandria J. Vet. Sci., 48 (1): 34-41.

Balakrishnan G, Roy P, Nagarajan K, Selvaraj J and Murali Manohar B (2012): Isolation, identification and antibiogram of *Pasteurella multocida* isolates of rabbits suffering from pasteurellosis. IJAVMS, 6 (1): 58-61**.**

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966): Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496.

Cheesbrough M (2006): District Laboratory Practice in Tropical Countries. 2nd edition. London English Language Book Society. pp: 100-194

Ekundayo SO, Odugbo MO, Olabode AO and Okewole PA (2008): Phenotypic variability among strains of *Pasteurella multocida* isolated from avian, bovine, caprine, leporine and ovine origin. African Journal of Biotechnology, 7 (9): 1347-1350.

El Tayeb AB, Morishita TY and Angrick EJ (2004): Evaluation of *Pasteurella multocida* isolated from rabbits by capsular typing, somatic serotyping, and restriction endonuclease analysis J. Vet. Diagn. Invest., 16 (2): 121-125.

Espinosa I, Báez M, Vichi J and Martínez S (2012): Antimicrobial resistance and genes associated to the host-microbe interaction of *Pasteurella multocida* isolates from swine in Western Cuba. Rev. Salud Anim., 34 (3): 151-158.

European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2017): Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. Available: http://www.eucast.org/. Accessed 10 March 2017. pp. 76-77.

Ferreira TSP, Felizardo MR, de Gobbi DDS, Gomes CR, Filsner PHLN, Moreno M, Paixão R, Pereira JJ and Moreno AM (2012): Virulence genes and antimicrobial resistance profiles of *Pasteurella multocida* strains isolated from rabbits in Brazil. The Scientific World Journal, 2012, Article ID 685028, 6 pages.

Jabbari AR, Rezaei E and Esmaelizad M (2015): Distribution of virulence adhesin associated genes and antimicrobial susceptibility in *Pasteurella multocida* from ovine pasteurellosis in Iran. International Journal of Molecular and Clinical Microbiology, 5 (2): 556-563.

Katoch S, Verma L, Sharma M, Asrani RK, Kumar S, Chahota R, and Verma S. (2015): Experimental study of the pathogenicity of *Pasteurella multocida* capsular type B in rabbits. J. Comp. Pathol., 153 (2-3): 160-166.

Kelly L, Smith DL, Snary EL, Johnson JA, Harris AD, Wooldridge M and Morris JG Jr (2004): Animal growth promoters: to ban or not to ban? A risk assessment approach. Int. J. Antimicrob. Agents, 24 (3): 205-212.

Khamesipour F, Momtaz H and Mamoreh MA (2014): Occurrence of virulence factors and antimicrobial resistance in *Pasteurella multocida* strains isolated from slaughter cattle in Iran. Front. Microbiol., 5: 536.

Kour A, Verma S, Singh G, Katoch S, Sharma M and Singh G (2016): Characterization and different antigenic preparations of *P. multocida* along with their quantitative and qualitative analysis. Journal of Animal Research, 6: 733-741.

Kumar JK, Reddy PH, Devi MC (2012): Isolation and partial characterization of *Pasteurella multocida* from poultry farms around Tirupati. Journal of Microbiology and Biotechnology Research, 2 (3): 393-395.

Mazed IM, Sharaf EM, Zakary EM and Atwa EI (2013): Comparison between traditional methods and real time PCR for diagnosis of *Pasteurella multocida* from diseased rabbits. Benha veterinary medical journal, 24 (1): 12-18.

Mutters R, Mannheim W and Bisgaard M. (1989): Taxonomy of the group. In: Adlam C, Rutter JM, editors, pasteurella and pasteurellosis. Academic Press, London, United Kingdom. pp 3–34.

Premalatha N, Senthil Kumar K, Purushothaman V, Ravikumar G and Muraalimanohar B (2009): Incidence of pasteureloosis (Snuffles) in a rabbit farm. Tamilnadu J. Veterinary and Anim. Sci. 5 (6): 269-271.

Songer JG and Post KW (2005): Veterinary Microbiology: Bacterial and Fungal Agents of Animal Disease. 1st edition, St. Louis, MO: Elsevier Saunders.

Suelam IIA. and Abdel Samie LK (2011): Molecular diversity of *Pasteurella multocida* isolated from different rabbit outbreaks at Zagazig suburbs, Egypt. Global Vet., 6 (2): 208-212.

Townsend, K.M., Frost, A.J., Lee, C. W., Papadimitriou, J.M. and Dawkins, H.J.S., 1998. Development of PCR assays for species and type specific identification of *Pasteurella multocida* isolates. Journal of Clinical Microbiology, 36: 1096-1100.

Varte Z, Dutta TK, Roychoudhury P, Begum J and Chandra R (2014): Isolation, identification, characterization and antibiogram of *Pasteurella*

multocida isolated from pigs in Mizoram with special reference to progressive atrophic rhinitis. Veterinary World, 7 (2): 95-99.

Verma S, Sharma M, Katoch S, Verma L, Kumar S, Dogra V, Chahota R, Dhar and Singh G. (2013): Profiling of virulence associated genes of *Pasteurella multocida* isolated from cattle. Vet. Res. Commun., 37 (1): 83-89.

White DG, Zhao S, Simjee S, Wagner DD and McDermott PF (2002): Antimicrobial resistance of foodborne pathogens. Microbes Infect., 4 (4): 405-412.

Yakubu D, Moshood R, Paul A, Blessing O and LolabO (2015): Phenotypic characteristics of *Pasteurella multocida* isolated from commercial chickens affected by fowl cholera in Jos, Nigeria. J. World's Poult. Res., 5 (3): 59-63.

الملخص العربى

تعد الباستريلا مالتوسيدا مسبب مرضى واسع الانتشار ومرتبط بأمراض حيوانية كبيرة ذات أهمية اقتصادية هامة لقطاع الثروة الحيوانية في مصر. ولان أنواع الباستريلا المعزولة من الأرانب لم توصف بصورة واضحة, لذلك تم اجراء هذه الدراسة لعزل ودراسة الخصائص المظهرية وانماط الحساسية للمضادات الحيوية لميكروبات الباستريلا مالتوسيدا المعزولة من الارانب المريضة. وقد تم عزل الباستريلا مالتوسيدا من مسحات الأنف وعينات الأنسجة المجمعة من الأرانب و تم الحصول على 16 عزلة باستريلا مالتوسيدا (8٪) من انسجة الرئة ومسحات الأنف بمعدلات عزل 6.5 و 1.5٪، على التوالي. وقد نم تصنيف المستعمرات النقية على أساس اشكالها على اطباق الزرع، وخصائصها المورفولوجية والبيوكيميائية، ودراسات اختبار المرضية في الأرانب والتعرف الجزيئي باستخدام تفاعل انزيم البلمرة المتسلسل الخاص بانواعها. كشفت نتائج الحساسية للمضادات الحيوية في المعمل عن حدوث مقاومة لمضادات الميكروبات بين جميع العزلات. وكانت المقاومة أكثر حدوثا للدوكسيسيكلين والتتراسيكلين والكوليستين (100٪ لكل منهما) ويليهم الاريثروميسين، السيفوتاكسيم، الكلورامفينيكول، السيبروفلوكساسين و التريمثوبريم / سلفاميثوكسازول (93.8٪ لكل منهما). بينما تم الحصول على مستويات منخفضة من الحساسية للمضادات المستخدمة مع أكبر مستوى من الحساسية للنيتروفورانتوين (75٪). وقد أظهرت جميع السلالات التي تم فحصها أنماطا من المقاومة المتعددة للادوية بشكل ملحوظ مع مقاومة لما لا يقل عن 8 مضادات حيوية. لذا توفر نتائج هذه الدراسة معلومات مفيدة عن تشخيص وأنماط مقاومة الباستريلا مالتوسيدا لمضادات الميكروبات لاختيار العوامل المناسبة المضادة للميكروبات.