Plant Archives Vol. 20, Supplement 1, 2020 pp. 536-544 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GENOTYPIC AND PHYLOGENIC CHARACTERIZATION OF *PASTEURELLA MULTOCIDA* AND THEIR ANTIBIOGRAM ISOLATED FROM MOUTH SWABS OF PET CATS AND DOGS IN BAGHDAD, IRAQ

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Abstract

A century ago, the *Pasteurella multocida* was an important pathogen in veterinary medicine. Knowing the organism by the traditional method of diagnosis based on the isolation, microscopic examination and identification of *Pasteurella multocida* are not sufficient to determine the identity of the organism, but it is important to know the serotype of the organism. The aims of this study were to assess the presence of *Pasteurella multocida* in the oral cavity of Iraqi pet cats and dogs. One hundred tonsillar swab samples were taken from Aden veterinary hospital, Baghdad, Iraq from December 2018 to August 2019. The samples were placed in a cool box and carried to the laboratory. This is the first reported isolates of *Pasteurella multocida* from pet cats and dogs in Baghdad-Iraq. The diagnosis of *Pasteurella multocida* in these samples was detected by combining direct culture and isolation, Polymerase chain reaction (PCR) using 16SrRNA identified three isolates were *Pasteurella* multocida, which was substantiated by inoculation of VITEK® 2 compact. Two isolates were *Pasteurella multocida subsp. septica* and one were *Pasteurella multocida subsp. gallicida*. The groupings were stored in NCBI GenBank with the accession number of the same two isolate was NR_115138.1 and AF326324.1 with the identity of 99%, 100 and 99% respectively. All three isolated were sensitive against many antibiotics tested like (Ampicillin, Penicillin G, Trimethoprim-Sulfamethoxazole, Streptomycin, Ampicillin/Sulbactam, Tetracyclin, Amoxicillin- Clavulanic acid, Chloramphenicol, Erythromycin, Clarithromycin, Doxycycline, Cefixime, Ceftriaxone, Gentamicin, Trimethoprim and Enrofloxacin) using disc diffusion method except one isolate was resist to gentamicin.

***Keywords*:** *Pasteurella multocida*, dogs and cats, PCR, VITEK® 2 compact, sequencing, Antibiotics.

Introduction

P. multocidaa is a zoonotic bacteria that is Gram-negative, *Pasteurella* genus mainly colonizes the upper respiratory tract and oral pharyngeal system of animals, especially cats and dogs (Smith, 1955; Bisgaard, 1993; Król *et al.,* 2011). Pets serve valuable social roles in society (Anderson *et al*., 1992; Parslow and Jorm, 2003). People acquire pets for many reasons, including companionship, recreation, and protection (Smith, 2012). Despite these benefits, pets present zoonotic risks, especially for immunocompromised hosts (Trevejo *et al*., 2005).

P multocida was isolated from healthy and sick cat's mouths, as well as from healthy dogs where it was 54% in the tonsils and in healthy dog noses 10%. The isolate percent was more in cats than in than dogs (Tindall and Harrison, 1972) Besides this microorganism being pathogenic to dogs and cats, The species *Pasteurella* are among the most popular commensals and opportunistic bacteria present household and wild animals all over the world (Harper *et al*., 2006). It is absent in the normal flora of the human (Capriotti *et al*., 2009).*Pasteurella* species Infections in wild and domestic animals can cause different illness; the majority of diseases among human include cat or dog scratches, chewing, as well as scratching (Shirzad Aski and Tabatabaei, 2016).The preferred medium for the recovery of *Pasteurella* spp. is blood agar (Smith and Baskerville 1983, Garlinghouse *et al*., 1981). The bipolar coloration is often lost and the *Pasteurella* looks like small cocciform sticks by subsequent culturing. They do not form spores, but they have a capsule (Angen *et al.*, 1999).

Positive reactions happened with many biochemical test such as oxidase, catalase, etc. The vitek used for more adequet diagnosis in the recent study while th VITEK its specificity was (89%) of the *Pasteurella spp* (Zangenah *et al.*, 2013). Vitek database, and the requirement for an analysis affirmation incited us to perform 16S rRNA sequencing, although *Pasteurella multocida* as rapidly as possible, PCR methods play a crucial role in the diagnosis of clinical laboratories accurate screening of microorganisms It made notable progress When diagnosing bacterial substances, Particularly where presence of organisms is of importance (Relman and Persing, 1996).

Antimicrobial therapy is a very important method for control and prevention, infectious diseases (Brogden *et al*., 2007; Kehrenberg *et al*., 2001; Lion *et al*., 2006). The global public health problem is known as a widespread resistance to antibiotics in pathogenic bacteria from animals and environmental sources (Bronzwaer *et al*., 2002; White *et al*., 2002). In general, *Pasteurella multocida* isolates are sensitive to most widely used antibiotics, but the random use of these antibiotics helps the emergence of resistant strains.

Due to all the above information, this study designed with many aims such as isolation of *Pasteurella* spp from of dogs and cats, identification of the isolates using VITEK® 2 compact and molecular technique. and Perform antimicrobial susceptibility test of the isolates.

Material and Methods

Collection of Samples: One hundred and twenty-five tonsillar swabs were taken from Aden veterinary hospital, Baghdad, Iraq from December 2018 to August 2019. The samples were placed in a cool box then delivered to the laboratory.

Bacterial identification: The identification of the isolates was made according to (Quinn *et al*., 2004) by Gram stain when a single colony from blood agar was taken by a loop and spread on a clean slide and fixed with heat and staining with gram stain according to (Jawetz *et al*., 2007) and then

the bacterial cell were examined under oil immersion, after that a set of Biochemical tests was done such as Oxidase, catalase, Indole test and Urease test

Identification of isolates using VITEK®2 system: Each assay cards include 64 holes, each with a unique test base. Material tests different metabolic processes such as acidification, alkalinisation, hydrolysis of the enzymes, and Fermentable sugars. The visually transparent layer from both faces of the card enables sufficient oxygen delivery thus preserving a closed cavity.

Antimicrobial sensitivity test : the antimicrobial used were Ampecilin, Penicillin G, Trimethoprim-Sulfamethoxazole, Streptomycin, Ampicillin/Sulbactam, Tetracyclin, Amoxicillin-Clavulanic acid, Chloramphenicol, Erythromycin, Clarithromycin, Doxycycline, Cefixime, Ceftriaxone, Gentamicin, Trimethoprim and Enrofloxacin screened against *P. multocida* according to the Mueller-Hinton agar disk diffusion process

DNA Extraction : Genomic DNA was isolated from bacterial growth according to the protocol of ABIO pure Extraction.

Primers: The primers used in this study was 16srRNA gene The primers were amplified from plate-grown bacteria 16S1, 5_AGAGTTTGATCCTGGCTCAG, and 16S2, 3_TACGGTTACCTTGTTACGACTT primer sequence (5' to 3').

Gene sequence : The gene sequence 16S rRNA has been established and the sequence was compared with the NCBI GenBank with the accession number.

Results

1. Dog and Cat samples:

Over a six month period of study, the data of the animals samples summarized in the table (1).

Table 1 : Explaining the source, types, number of the samples of animal isolates:

Source of samples	Types of animals	Type of samples	No. of samples	No. of isolates	Percentage%
Aden veterinary hospital	Dog	Tonsillar swabs	60	0	0%
		Oral swabs	15	0	0%
		Nasal swabs	10	0	0%
	Cat	Tonsillar swabs	40	3	7.5%
Total			125	3	0.024%

2: Identification of the bacteria: The results showed the growth of non-heamolytic, small, rough, round, and greyish colonies on blood agar after incubation for 24 hrs at 37 °C as explained in Figure (1). *Pastuerella* apperars as small gram-negative coccobacilli, arranged in pairs or short chains as explained in Figure (2).

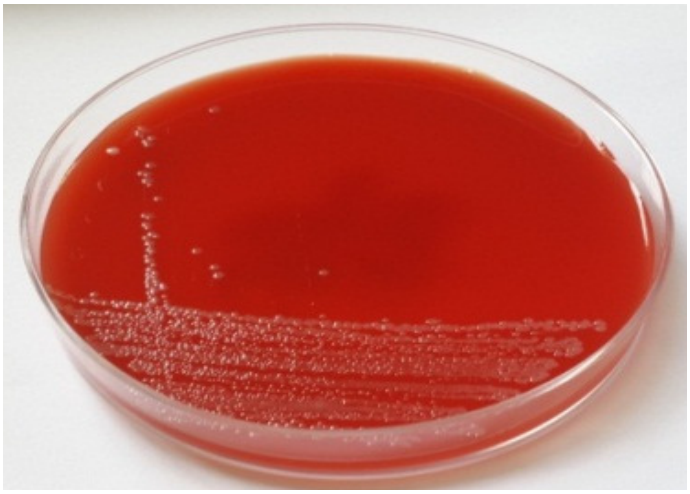

Fig. 1 : Growth of *Pasteurella multocida* on blood agar after incubation for 24 hrs at 37 °C show non-heamolytic, small, round and greyish colonies.

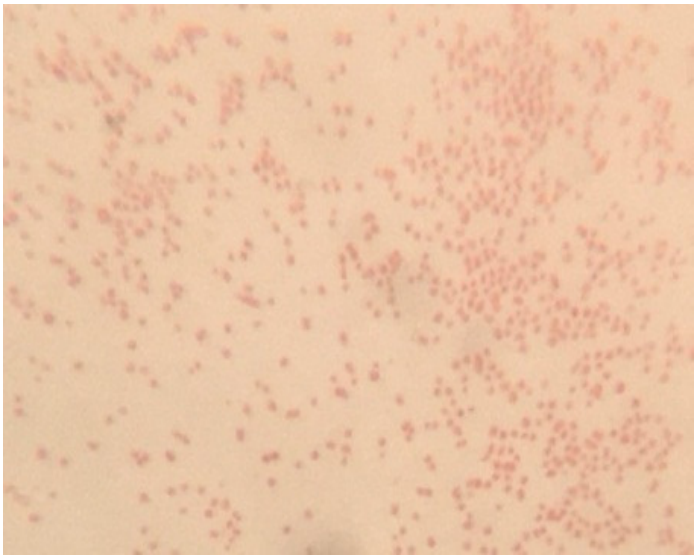

Fig. 2 : *Pasteurella multocida* gram staining showed gram negative coccobacilli

A set of biochemical test done like Oxidase, Catalase, Indole, Urease and Sulfide Indole Motility test (SIM).

3. VITEK® 2 microbial ID/AST testing ***system***

The result of tests by using ***VITEK®*** 2 compact system shown in figure (3-5).

bioMérieux Customer:
System #:

Laboratory Report

Printed Apr 27, 2019 08:38 CDT
Printed by: System

Patient Name: B1, .
Isolate: 1-1 (Approved)

Patient ID: gfjdjdf

Card Type: GN Bar Code: 2410749103129503 Testing Instrument: 00000B4B8137 (VITEK2C)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0001410150040001
Organism Quantity:

Selected Organism: Pasteurella multocida

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	(+)	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

nstalled VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

Fig. 3 : *VITEK*® 2 compact identify isolate as *P.multocida*.

bioMérieux Customer:

Microbiology Chart Report

Printed Sep 12, 2019 15:08 CDT

Patient Name: 2, .
Location:
Lab ID: 2

Patient ID: dfhfd
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pasteurella canis

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Fig. 4 : *VITEK*® 2 compact identify isolate as *P.canis*.

bioMérieux Customer: **Microbiology Chart Report** Printed Sep 12, 2019 15:07 CDT

Patient Name: 1, .
Location:
Lab ID: 1

Patient ID: ryturjutr
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pasteurella canis

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	(-)	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Fig. 5 : *VITEK*® 2 compact identify isolate as *P.canis*.

4. Antibiogram profile

P. multocida isolates from tonsillar swabs were screened for antimicrobial susceptibility against Ampecilin, *Penicillin G*, Trimethoprim-Sulfamethoxazole, Streptomycin, Ampicillin/Sulbactam, Tetracyclin, Amoxicillin- Clavulanic acid, Chloramphenicol, Erythromycin, Clarithromycin, Doxycycline, Cefixime, Ceftriaxone, Gentamicin, Trimethoprim and Enrofloxacin as shown in table (2).

Table 2 : Antimicrobial susceptibility against *P.multocida* isolates.

No.	Antimicrobial type	Isolate No. 1	Isolate No.2	Isolate No. 3
1	AUG	S	S	S
2	PG	S	M S	S
3	SXT	S	S	S
4	Am	M S	M S	S
5	S	S	S	S
6	C	S	S	S
7	T	S	S	S
8	DXT	S	R	S
9	CXM	S	I	S
10	CRO	R	R	R
11	CN	R	S	S
12	E	S	I	I
13	SAM	S	R	S
14	TM	S	S	S
15	ENF	S	S	S
16	CLA	R	R	S

*S= sensitive , I= intermediate,MS= moderate sensitive and R = resistant

5. Molecular study

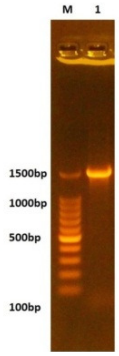

Fig. 6: Shows PCR product the band size 1500 bp. The product electrophoresed on 1% Agarose gel Which shows positive amplification of *Pasteurella miltocida.*

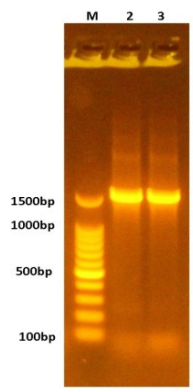

Fig. 7: Shows PCR product the band size 1500 bp. The product electrophoresed on 1% Agarose gel Which shows positive amplification of *Pasteurella miltocida.*

Figure (6-7) shows the amplification of DNA product 1500 base pair of *Pasteurella* spp. then analyzed with gel electrophoresis to estimate the DNA product size depending on DNA marker (100 base pair DNA ladder). Then the samples were sent for identification to the genus level by Sequencing (figure 8,9), Which reveal that the three isolates confirmed as *Pasteurella multocida*.

Sbjct 759
AAACAGGATTAGATACCCTGGTAGTCCACGCTGTAA
ACGCTGTCGATTT 807
NR_115138.1 →

Sbjct 153
ACCGCGTAATCTCTGAGGAGTAAAGGGTGGGACCT
CTTGGCCACCTGCCATAAGATGAGC 212

Sbjct 393
AAGGCCTTCGGGTTGTAAAGTTCTTTCGGTAATGAG
GAAGGGATGTTGTTAAATAGATGG 452
NR_115138.1 of second strain →

Sbjct 1438
CGCCCCCCCGAAGGTTAAGCTATCTACTTCTGGTAC
AACCCACTCCCATGGTGTGACGGG 1379
← NR_115138.1

Sbjct 1295
TGCAGACTCCAATCCGGACTTAGATGCACTTTCTGA
GATTCGCTCAAGCTCGCACTCTCG 1236

Sbjct 1115
CTAATCGCTGGCAACAAAGGATAAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATTTC 1056

Sbjct 1055
ACAACACGAGCTGACGACAGCCATGCAGCACCTGT
CTCAGAGCTCCCGAAGGCACACTCG 996

Sbjct 995
CATCTCTGCAAGCTTCTCTGGATGTCAAGAGTAGGT
AAGGTTCTTCGCGTTGCATCGAAT 936

Sbjct 635
CTCTAGACTCCCAGTCTGAAATGCAATTCCCAAGTT
AAGCTCGGGGATTTCACATCTCAC 576

Sbjct 455
ATGCCATCTATTTAACAACATCCCTTCCTCATTACCG
AAAGAACTTTACAACCCGAAGGC 396
NR_115138.1 of second strain

Fig. 8 : 16S ribosomal RNA, partial sequence Sequence ID: NR_115138.1Length: 1468Number of Matches: 1
Range 1: 33 to 612GenBankGraphics of two strain *Pasteurella multocida subsp. septica*

Query 123
TGCAGACTCCAATCCGGACTTAGATGCACTTTCTGA
GATTCGCTCAAGCTCGCACTCTCG 182

Sbjct 1291
TGCAGACTCCAATCCGGACTTAGATGCACTTTCTGA
GATTYGCTCAAGCTCGCACTCTCG 1232
← **AF326324.1**

Fig. 9 : 16S ribosome RNA, component sequence ID: NR_115138.1Length: 1468Number of Matches: 1
Range 1: 33 to 612GenBankGraphics of two strain *Pasteurella multocida subsp. gallicida*

The sequencing of 16s RNA gene amplified product from three Samples of tonsillar swabs of cats appeared that Sequence ID: NR 115138.1 for isolate No.1F has score 1383 and compatibility 99% for having one Transvertion (A>T) in the location 773 while No. 1R has score 1297 and compatibility 99% with standard in Gene Bank for having one Transition (C>T) in the location 1433 as shown in the figure (8 and 9). Sequence ID: AF 326324.1 for isolate No. 2F has score 1719 and compatibility 100% with standard in Gene Bank while 2R has score 1836 and compatibility 99% with standard in Gene Bank for having one Transition (Y>C) in the location 1251 as shown in the figure (8 and 9). Sequence ID: NR 115138.1 for isolate No. 3F has score 1020 and compatibility 99% with standard in Gene Bank for having four Transvertion (A>T, T>G, T>A and T>G) in the locations 161, 173, 189 and 190 respectively and two Transition (C>T and G>A) in the location 188 and 451 respectively while No. 3R has score 1831 and compatibility 98% with standard in Gene Bank for having 10 Transvertion (C>A, T>G, A>T, A>T, C>A, G>T, C>A, A>C, G>T, T>A) in the locations 1243, 1114, 1017, 999, 998, 996, 987, 985, 984 and 977 respectively and six Transition (G>A, C>T, A>G, C>T, A>G and C>T) in the locations 1016, 1013, 986, 978, 600, 45 respectively as shown in the figure (8 and 9).

6. Phylogenetic tree of *Pasteurella multocida* subsp. *septica* 16s rRNA

The Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0.Sequences of phylogenetic tree diagram that displayed the highest identity (>98%) and full coverage (> 99 per cent), by independent contrast. The adjacent tree was designed for phylogenetic analysis as shown (figure 10). According to the Phylogenetic tree and even the comparison table, the genetic element between Iraq and global isolates is comprehensive. When comparison between *Pasteurella multocida* subsp. *septica* isolated from different sources and reported in the National Center for Biotechnology Information (NCBI) have been sequenced (ID: EF579833.1, ID: AY299314.1, ID: NR_115138.1, ID: AF326325.1, ID: AF225205.1, ID: LR134514.1, ID: HM746990.1, ID: CP020347.1, ID: AY999017.1, ID: JF317218.1, ID: AY634646.1 and ID: MN517822.1) The highest identity (> 98 percent) and maximum coverage (> 99 percent) were consistent with both the isolation source respectively, and expect 0.0 with gene bank show in table(3).

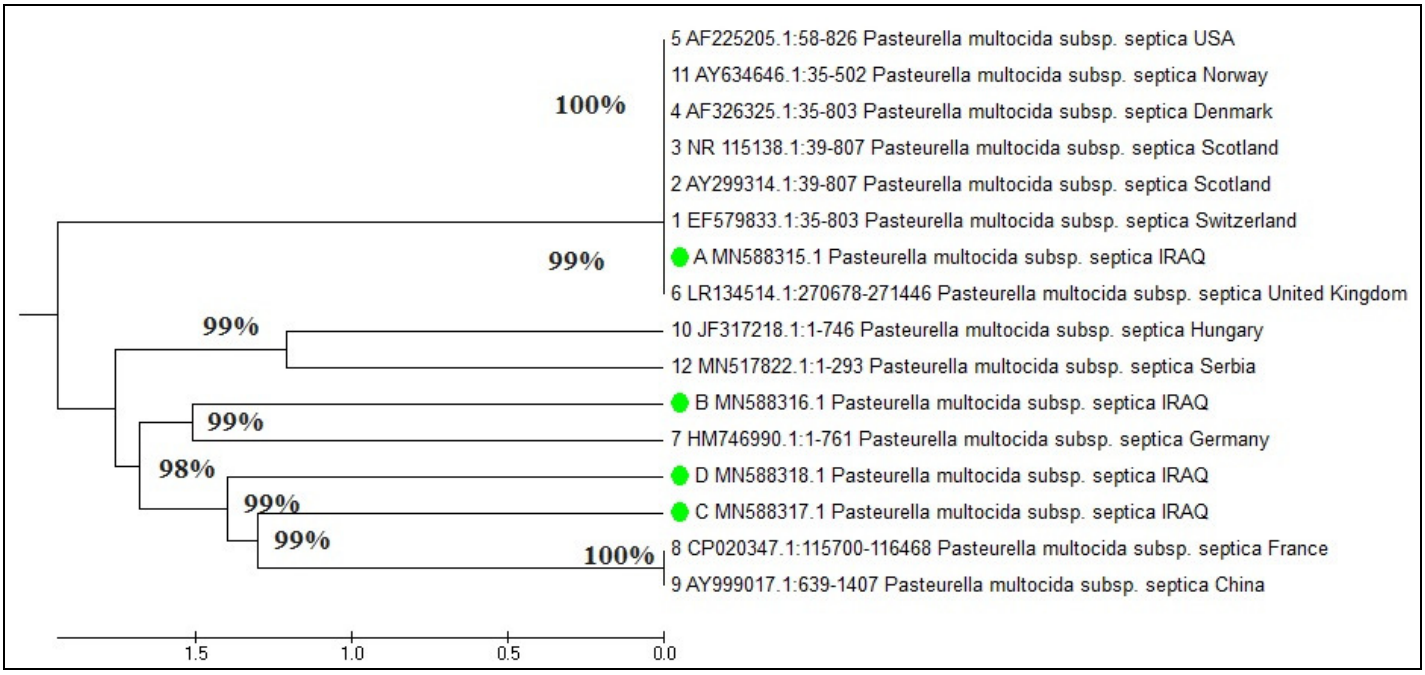

Fig. 10: Neighbor-joining tree

Table 3 : Illustrates the genetic relatedness isolates of among different countries

Accession	Country	Source	Compatibility
ID: EF579833.1	Switzerland	*Pasteurella* multocida subsp. septica	99%
ID: AY299314.1	Scotland	*Pasteurella* multocida subsp. septica	99%
ID: NR_115138.1	Scotland	*Pasteurella* multocida subsp. septica	99%
ID: AF326325.1	Denmark	*Pasteurella* multocida subsp. septica	99%
ID: AF225205.1	USA	*Pasteurella* multocida subsp. septica	99%
ID: LR134514.1	United Kingdom	*Pasteurella* multocida subsp. septica	99%
ID: HM746990.1	Germany	*Pasteurella* multocida subsp. septica	99%
ID: CP020347.1	France	*Pasteurella* multocida subsp. septica	99%
ID: AY999017.1	China	*Pasteurella* multocida subsp. septica	99%
ID: JF317218.1	Hungary	*Pasteurella* multocida subsp. septica	99%
ID: AY634646.1	Norway	*Pasteurella* multocida subsp. septica	99%
ID: MN517822.1	Serbia	*Pasteurella* multocida subsp. septica	99%

7: Phylogenetic tree of *Pasteurella multocida* subsp. *gallicida* 16s rRNA

The program Molecular Evolutionary Genetics Analysis (MEGA) edition 6.0.Phylogenetic structure map sequences displaying the highest identity (> 98 percent) and good coverage (> 99 percent) by independent comparison. The corresponding tree was planned to be analysed phylogenetically show in figure (11). Neighbor-joining tree was designed for studying phylogenetics. The genetic element between Iraq with the world's isolates is analyzed compared with the phylogenetic tree and the table of comparisons. When comparison between *Pasteurella multocida* subsp. *gallicida* derived and derived from different sources, registered in the National Center for Biotechnology Knowledge (NCBI) (ID: LR134298.1, ID: NR_115136.1, ID: AF326324.1, ID: AF326323.1, ID: NR_041811.1, ID: AF224297.1) respectively with the source of isolation showed compatibility the highest identity (>98%) and maximum coverage (>99%) and expect 0.0 with gene bank show in table (4).

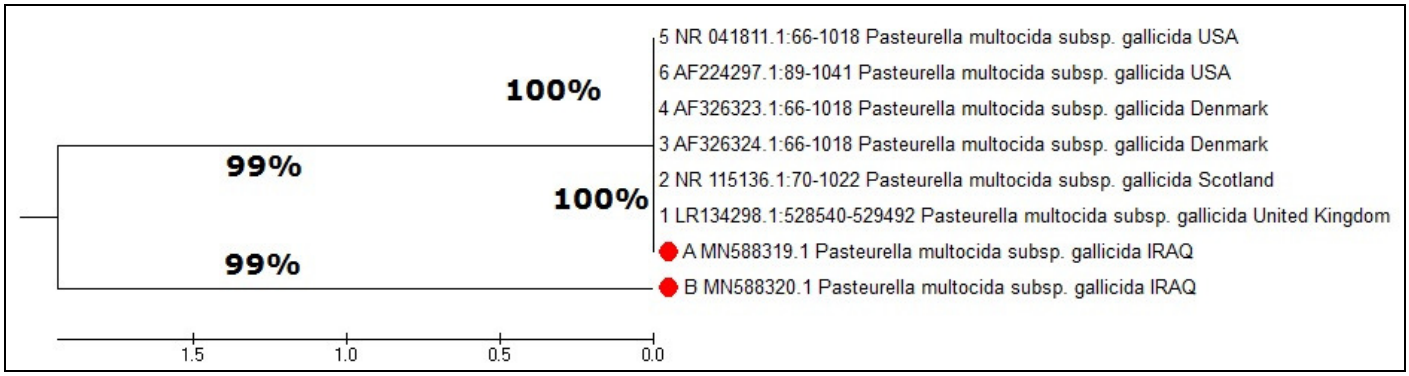

Fig. 11 : Neighbor-joining tree

Table 4 : Illustrates the genetic relatedness isolates of among different countries

Accession	Country	Source	Compatibility
ID: LR134298.1	United Kingdom	*Pasteurella* multocida subsp. gallicida	100%
ID: NR_115136.1	Scotland	*Pasteurella* multocida subsp. gallicida	100%
ID: AF326324.1	Denmark	*Pasteurella* multocida subsp. gallicida	99%
ID: AF326323.1	Denmark	*Pasteurella* multocida subsp. gallicida	100%
ID: NR_041811.1	USA	*Pasteurella* multocida subsp. gallicida	100%
ID: AF224297.1	USA	*Pasteurella* multocida subsp. gallicida	100%

Discussion

This is the first report from dogs and cats on isolation, genotype and phylogenic analysis of *Pasteurella multocida* in Iraq, the prevalence of *P.multocidaa* (7.5%, n=3/40) in cat found in this study that level agree with (Ewers *et a* 2006) although it was lower than the other recorded in the previous studies in the world (Baldrias *et al.,* 1988; Ganiere *et al.*, 1993). Muhairwa *et al.* (2001) found 51% prevalence in cat and 4% in dog, whereas Wilson and Ho. (2013) found 75 % prevalence in cat and Lefebvre *et al.* (2006) found 29% in dog. The lower rate of *P.multocidaa* observed in this analysis could be attributed to the age and breed differences of cats as well as the resistance of pet cat due to improved management, vaccination and nutrition.

28-90% of apparently normal dogs and cats were noted to carry *Pasteurella* in their oropharynx in different studies (Saphir *et al.,* 1976; Baldrias *et al.,* 1988; Ganiere *et al.*, 1993).

Dogs are the animal most often involved in bite cases, while cat bites cause secondary infections three times as often, It is interesting that all three isolates of the *Pasteurella* described in this report were recovered from mouth swabs of cat (Aghababian *et al.,* 1980; Butler, 2015).

Finally the three Gram-negative isolates two species were graded with positive catalase and oxidase reactions as *P.multocidaa* and one *as P.canis* in vitek 2 compact which has misidentified the species of *P.canis* the species name *P. canis* In Program Server Vitek 2 diagnosed as *P.multocidaa subsps gallicida* in PCR technique, On that basis, the Vitek 2 system database included only *P.multocidaa P. canis, P. aerogenes,* and *P.pneumotropica* for identification, and *P. multocidaa subsps gallicida* Until now, recommendations to producers to enhance their systems have not been included in the database; Nevertheless, Commercial identity systems such as Vitek 2 are restricted also relevant for standard laboratories of clinical microbiology Since not all laboratories have been equipped for molecular assay handling, this should definitely be of benefit to clinical microbiologists Vitek 2 also gives the biochemical differential characteristics of oxidase, catalase, ornithine decarboxylase, urease activity, and production of indole, although Vitek 2 actually contains In the registry *P. canis*., gallicidal strain had both been misidentified as *P. canis*. Such results have apparently shown that common identification systems are ineffective in the correct the description of the genus *Pasteurella*, In particular, *P.multocidaa subsp. gallicida*. Consequently, a remarkably underestimated isolation frequency of *P.multocidaa subsp gallicida*. Misidentification of the species may be attributed to *P.canis*, Where VITEK 2 did not perform well and could only classify the isolates (48.5 percent) (Zangenah *et al.,* 2013).

The two subspecies of *P.multocidaa* (subspecies multocida and septica) formed the majority of the strains. These two subspecies were also mainly recorded in other animals.

Gram stain does not detect *Pasteurella* species in about 50 per cent of cases (Satomura *et al.,* 2009).

In the present study, fluoroquinolone (enrofloxacin), Trimethoprim-Sulfamethoxazole, Chloramphenicol, Tetracycline, Streptomycin, Amoxicillin-Clavulanic acid and Trimethoprim-Sulfamethoxazole have been found to be more effective than moderately suscebtiple beta-lactams (penicillin, ampicillin). Hence for the therapy of *P.multocida*a disease, these agents are suggested as empirical antimicrobials. *P.multocida*a isolates previously reported resistance to ceftrioxone (San Millan *et al.,* 2009). Nonetheless, the present study showed a relatively higher tolerance, as this medication is the antibiotics most widely used in pets in Iraq, Its the recurrent use of tolerance levels in this study is reflected in such the use of antimicrobials cautiously and supplemented by high levels of doxycycline and gentamicin, ampicillin / sulbactam and clarithromycin tolerance by susceptibility tests Based on high rates of resistance to doxycycline and gentamicin, ampicillin / sulbactam and clarithromycin, such antimicrobial agents should be cautiously used and followed by sensitivity tests In addition, ongoing monitoring of the resistance for antimicrobials in respiratory illnesses like *P.multocida*, Because of this increased to the use of antimicrobial medication and the current resistant emerging strain. PCR offers a sensitive, accurate and rapid method for identifying *P.multocida* isolates, regardless of the sample purity (Townsend *et al.,* 1998). Polymerase chain reaction and sequence-based study of ribotyping Phenotypic replacements have now been rendered using universal primers for the 16sRNA gene or rpoB gene sequencing approaches to classify, describe and distinguish *P.multocida* and other *Pasteurella* spp. (Wilson *et al.,* 2013; Zbinden *et al.,* 2015).

P.multocidaa subsp. septica Isolated from various sources including cat and avain (Fegan *et al.,* 1995; Kuhnert *et al.,* 2000 and Petersen *et al.,* 2001) Probably as a result of their predatorprey relationship, cats have developed *P.multocida*a strains adapted to the avian (Davies, 2004).

The creation of genomic subtractive hybridization has revolutionized the quest for virulence genes in pathogenic bacteria by using a virulent and similar strain to improve pathogenicity-related DNA fragments isolation, Furthermore, this technique is capable of isolating species-specific sequences that are useful for identifying bacterial species. A modified magnetic cloning strategy involving the use of Dynabeads created a cloned fragment that was subsequently analyzed with hybridization (Townsend *et al.,* 1998)

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