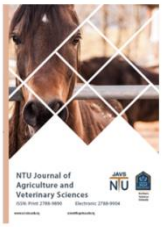
NTU Journal of Agricultural and Veterinary Sciences (2024) 4 (1) : 56-60
DOI: https://doi.org/10.56286/ntujavs.v2i2

IRAQI Academic Scientific Journals

JAVS NTU

P-ISSN: 2788-9890 E-ISSN: 2788-9904
NTU Journal of Agricultural and Veterinary Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JAVS/index



# Molecular determination of virulence factors of *Mycoplasma cynos* isolated from household dogs

1st Saba A. Hussein[1] 2nd Mohammad A. Hamad[2]
1,2Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

**Article Informations**

**Received:** 01-08- 2023,
**Accepted:** 02-10-2023,
**Published online**: 28-03-2024

**Corresponding author:**
Mohammad A. Hamad
Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq:
mahmah1073@uomosul.edu.iq

**Key Words:**
*Mycoplasma cynos*
Hemagglutinin
Neuraminidase
Respiratory infection and conjunctivitis
Household dogs.

**A B S T R A C T**

*Mycoplasma cynos* is a member of the class Mollicutes. It is more pathogenic, more host-specific than other canine *Mycoplasma*, and only associated with respiratory diseases in dogs due to producing many virulence factors, recently neuraminidase and hemagglutinin. The study was done to detect the neuraminidase and hemagglutinin of *Mycoplasma cynos* isolated from the upper respiratory tract and conjunctival infections in household dogs. *Mycoplasma* isolates obtained from nasal, oropharyngeal, and conjunctival swabs from household dogs suffering from respiratory diseases in different veterinary clinics in Mosul city. DNA extraction was prepared , PCR and sequencing of 16S rRNA, then *Mycoplasma cynos* was examined for neuraminidase and hemagglutinin using specific primers.The results showed the identification of *Mycoplasma cynos* strain "SM-MY-M23" under accession number "OQ446513" in percentage (67.6%), and detection of neuraminidase only. Results obtained are considered the first record of *M. cynos* and the important virulence factor (Neuraminidase) from household dogs in Iraq.

## Introduction

*Mycoplasmas* are the most pathogenic agent for respiratory disease in a wide range of animal species, humans, insects, and plants [1 , 2]. A recent study of canine *Mycoplasmas* found in the upper and lower respiratory tract noted a correlation of *M. cynos* with the canine infectious respiratory disease complex [3, 4]. *Mycoplasma cynos* was first secluded from the lungs of a dog with clinical pneumonia in 1972 [5]. The organism is extensive and more common in younger and kenneled dogs, and it often originates in dogs with respiratory disease, it is believed that *M. cynos* has a significant role in the association of bronchopneumonia and pleurisy in puppies [6] . In in addition to the identification of M. cynos in the laboratory, beagle dog have the respiratory disease by using PCR technique of the lung tissue [7, 8], as well as the isolation of *M. cynos* in pure culture from the tracheal wash of dogs have the respiratory disease in United States after performing the complete genome sequence to it [9, 10]. Neuraminidase (sialidase) is a pathogenic enzyme that stimulates the hydrolysis of sialic acid and contributes to microbial colonization, dissemination within the host, tissue invasion, and apoptosis for microorganisms [11], and it is identified as a virulence factor in canine *Mycoplasmas* like *M. canis*, *M. cynos* and *M. molare* [12 , 13], so the enzyme can cause an exact toxic effect on the host cells as well as affect host defense mechanisms [14]. *Mycoplasma* also possesses mechanisms for the variable expression of surface antigens, including hemagglutinins. Hemagglutinins are highly immunogenic surface lipoproteins that cause hemagglutination (sticking) of erythrocytes or their binding to Mycoplasma colonies (hemadsorption). Hemagglutinin, one of the immunodominant proteins produced by *M. canis* and *M. cynos* which are playing a critical role in infecting dogs [15, 16]. Rycroft and colleagues [17] also called a 45 kDa immunodominant protein in *M. cynos* when dog sera antibodies were linked with this protein. The aim of this study was to molecular determine neuraminidase and hemagglutinin as virulence factors of *Mycoplasma cynos* that isolated from the upper respiratory tract and conjunctival infections in household dogs in Mosul city/ Iraq. Thus, confirming the important pathogenicity of *Mycoplasma cynos* in causing respiratory infections in dogs.

## Material and Methods

**Ethical approval OR data collection permit**:

The endorsement certificate with the number (UM.VET.2022.059) was granted by the Commission of scientific morals, which also provided the moral consent to carry out this methodical activity in the College of Veterinary Medicine.

***Mycoplasma* isolates culture**

Samples for isolation of *Mycoplasma* obtained from nasal, oropharyngeal, and conjunctival swabs of the dogs that suffered from upper respiratory tract infections and conjunctivitis were cultured on *Mycoplasma* media and the isolated bacteria were identified according to the colonial characteristics, staining, and biochemical tests [18].

**DNA Extraction, PCR, and Sequencing of the 16S rRNA**

In a previous phase of the study [19], the DNA of the isolated *Mycoplasma* was extracted by following the extraction kit procedures (Geneaid, Presto TM Mini gDNA Bacteria Kit Quick Protocol, Korea), and the polymerase chain reaction of the samples was achieved through amplified *Mycoplasma*-specific primers for 16S rRNA [20-23]. The extracted DNA of the isolates underwent sequencing and was determined as M. cynos (SM-MY-M23) with accession number "OQ446513 " in the GenBank database. The DNA of these *M. cynos* isolates has undergone nextly to determine the virulence factors (Neuraminidase and hemagglutinin).

**Primers for virulence factors**

The primers and reaction mixture used in this study for neuraminidase and haemagglutinin detection are shown in (Table 1,2)

**Table 1.** Primers of neuraminidase (N) and haemagglutinin (H) of *Mycoplasma cynos*

| Primers/Sequence | Size | Annealing |
|---|---|---|
| *N F[GCATTGGTAAATTATTTGCGA] R[CGATATCTTTTCGCGCTTCT] | 786 | 55℃ |
| *H F[AGTATGATGTTAGTGAGCCGATTG] R[CTGTTCCTGGTGCAGGATTT] | 400 | 57℃ |

*Reference[16]

**Table 2.** The reaction mixture of neuraminidase and haemagglutinin

| Reagent | Vol. |
|---|---|
| 10x Taq Master Mix | 10 µl |
| PCR grade water | 6 µl |
| F-primer (10 µM) | 1 µl |
| R-primer (10 µM) | 1 µl |
| DNA (100 ng/µl) | 2 µl |
| Total | 20 µl |

## Results

The genome sequence of the 16S rRNA gene which indicated that *Mycoplasma cynos* strain "SM-MY-M23" from the nasal swabs in the household dogs suffering from respiratory signs has been submitted to the GenBank database of nucleotide sequences with entry number "OQ446513 ". Twenty-three (67.6%) out of thirty-four *Mycoplasma* isolates confirmed conventionally and molecularly as *M. cynos* in the previous phase of the study had appeared as proprietors of neuraminidase only that has (786)bp (Figure 1), but hemagglutinin is not detected .

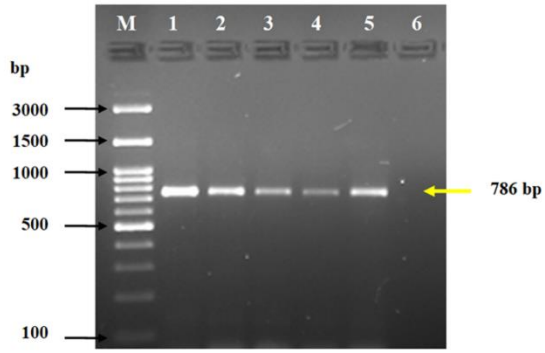



Figure 1. Polymerase chain reaction (PCR) of *Mycoplasmas cynos* neuraminidase gene. Lane M: 100 bp DNA ladder. Lanes 1-5 are positive samples. Lane 6 negative control.

## Discussion

*Mycoplasma cynos* is regarded as the most pathogenic among 14 canine *Mycoplasma* species [6 , 24], and its novel species was identified more than 40 years ago [25 , 26], so it is more host specific [24] when contrast with other canine *Mycoplasma*, like *Mycoplasma canis* which infect human and calves. *Mycoplasma cynos* was identified in the existing study as a result of the sequencing of the amplification products of 16S rRNA specific for the *Mycoplasma* genus and the confirmed *M. cynos* strain "SM-MY-M23" from nasal swabs was registered in GeneBank under accession number "OQ446513 " so this is consistent with those observations [7 , 27-29], who detected *M. cynos* in the nasal, oropharyngeal swabs from the dogs with respiratory infections. The strain obtained in the current study differs from the strain of the researcher [9] who identified *M. cynos* strain "C142" from tracheal wash and was registered in GeneBank under accession number "HF559394" in the United States, this difference was explained by the unknown in the distribution, importance of *M. cynos*, mechanisms of pathogenicity and nature of the immune response to this pathogen, this is due to the lack and neglect of studies related with canine *Mycoplasma* because of the difficulty in identifying the species and its mixing with other respiratory infections [30-32]. Our results indicated twenty-three (67.6%) *M. cynos*, these were contrast with researchers [8 , 33, 34], whose recorded *M. cynos* percentage as (29.2%, 33.9%, 44%) respectively. This is due to the difference in animal number, animal age, coinfections, oropharyngeal contamination and antibiotic pre-treatment of diseased dogs, which may have reduced the number of positive dogs[35-37].There is little information about the factors related to the pathogenicity of *M. cynos*, but two concerned studies confirmed that *M. cynos* produces neuraminidase enzyme as a virulence factor with approximatlely105kDa [14 , 38] . Hemagglutinin is the essential event for the attachment of *M. cynos* to the host cell [39]. this study couldnot detect the production of hemagglutinin from *M.cynos* by using specific primers, this differed from [15] who was the first to detect and characterize the hemagglutinin of *M. cynos*, and whether this in any way contributes to the increased ability to cause disease in dogs, followed by [16] who was able to produce hemagglutinin from *M.cynos* using the same primers. The reason for difference may be the ability to produce hemagglutination is a highly variable trait that varies between species and even strains and declines over time [16]. The present study also proved the production of neuraminidase from *M. cynos* in dogs with respiratory infections using specific primers, this agreed with other studies [14-16, 38] which were showed that the ability of *M. cynos* to produce the neuraminidase in dogs, so that this confirmed the role of neuraminidase in increase the pathogenicity of *M. cynos* to induce the infections in household dogs [39 , 40].

**Conclusion.** The current study proved the important role of *Mycoplasma cynos* in causing respiratory infections in household dogs by producing neuraminidase as virulence factors that accelerated the infections in dogs, so we recommend the proprietors of dogs must take care of them and provide necessary treatments and vaccinations to decrease infection with *Mycoplasma* in Mosul city.

**Acknowledgment.** The deanship of the Faculty of Veterinary Medicine and the Department of Microbiology at the University of Mosul is gratefully acknowledged by the authors.

**Competing Interests**

The authors should declare that there are no competing interests.

## References

[1] Stull, W.J., Sherding, R.G., O'Quin, J., Evason, M.D., Kasten, J.I., Hoet, A.E., Burkhard,

M.J., Weese, J.S. (2016). Risk reduction and management strategies to prevent transmission of infectious disease among dogs at dog shows, sporting events, and other canine group settings. J. American Vet. Med. Associ., 249(6):1-16 DOI: https://doi.org/10.2460/javma.249.6.612

[2] Yiwen, C., Yueyue, W., Lianmei, Q., Cuiming, Z., Xiaoxing, Y. (2021). Infection strategies of *Mycoplasma*: unraveling the panoply of virulence factors. Virul., 12(1):788-817. DOI:10.1080/21505594.2021.1889813

[3] Chalker, V.J., Owen, W.M.A., Paterson, C., Barker, E., Brooks, H., Rycroft, A.N., Brownlie, J.(2004). *Mycoplasmas* associated with canine infectious respiratory disease. Microbiol., 150:3491-3497. DOI: 10.1099/mic.0.26848-0

[4] Decaro, N., Mari, V., Larocca, V., Losurdo, M., Lanave, G., Lucente, M.S., Corrente, M., Catella, C., Bo S., Elia, G., Torra, G., Grandolfo, E., Martella, V., Buonavoglia, C.(2016). Molecular surveillance of traditional and emerging pathogens associated with canine infectious respiratory disease. Vet. Microbiol., 192: 21-25 DOI:10.1016/j.vetmic.2016.06.009

[5] Frieman, M., Baric, R.(2008). Mechanisms of severe acute respiratory syndrome pathogenesis and innate immunomodulation. Microbiol. Mol. Biol. Rev., 72:672–685.DOI: 10.1128/MMBR.00015-08

[6] Zeugswetter, H., Weissenböck, H., Shibly, S., Hassan, J., Spergser, J. (2007). Lethal bronchopneumonia caused by *Mycoplasma cynos* in a litter of golden retriever puppies. Vet. Reco., 161:626-628 DOI:10.1136/vr.161.18.626

[7] Hong, S., Kim, O.(2012). Molecular identification of *Mycoplasma cynos* from laboratory beagle dogs with respiratory disease. Lab. Anim. Res., 28(1):61-66 DOI:10.5625/lar.2012.28.1.61

[8] Sakmanoglu, A., Sayin, Z., Pinarkara, Y., Uslu, A., Ucan, U.S., Erganis, O. (2019).Evaluation of random amplified polymorphic DNA and multiple-locus variable number tandem repeat analyses for *Mycoplasma cynos*. J. Microbiol. Meth., 161:1-7 https://doi.org/10.1016/j.mimet.2019.04.004

[9] Walker, C.A., Mannering, S.A., Shields, S., Blake, D.P., Brownlie, J. (2013).Complete genome sequence of *Mycoplasma cynos* strain C142. Genom. Announc., 1(1):e00196-12 DOI:10.1128/ genomeA.00196-12

[10] Sakmanoglu, A., Sayin, Z., Ucan, U.S., Pinarkara, Y., Uslu, A. (2017).Comparison of five methods for isolation DNA from *Mycoplasma cynos.* J. Microbiol. Meth., 140:70-73. DOI:10.1016/j.mimet.2017.07.003

[11] Robinson, L.S., Lewis, W.G., Lewis, A.L. (2017).The sialate Oacetylesterase Esta from gut bacteroidetes species enables sialidase-mediated cross-species foraging of 9-O-Accetylated sialoglycans. J. Biol. Chem., 292(28):11861-11872 DOI: 10.1074/jbc.M116.769232

[12] Michaels, D.L., Moneypenny, C.G., Shama, S.M., Leibowitz, J.A., May, M.A. , Glass, J.I., Brown, D.R.(2019). Sialidase and N-acetylneuraminate catabolism in nutrition of *Mycoplasma alligatoris*. Microbiol., 165(6): 662-667. https://doi.org/10.1099/mic.0.000739.

[13] Perez, K., Mullen, N., Canter, J.A., Lay, D.H., May, M. (2020).Phenotypic diversity in an emerging Mycoplasmal disease. Microbial. Pathogen., 138:103798. DOI:10.1016/j.micpath. 2019.103798.

[14] Berčič, R.L., Cizelj, I., Benčina, M., Narat, M., Bradbury, J.M., Dovč, P., Benčina, D.(2012). Demonstration of neuraminidase activity in *Mycoplasma neurolyticum* and of neuraminidase proteins in three canine Mycoplasma species. Vet. Microbiol., 155(2-4):425-429DOI: 10.1016/j.vetmic.2011.08.026

[15] Kastelic, S., Cizelj, I., Narat, M., Tozon, N., Chalker, V. J., Lysnyansky, I., Spergser, J., Benčina, D. (2015). Molecular characterization of the *Mycoplasma cynos* haemagglutinin HapA. Vet. Microbiol., 175: 35-43 DOI: 10.1016/j.vetmic.2014.10.026

[16] Koprivec, S. (2016). Characteristics of neuraminidases and haemagglutinins from bacteria *Mycoplasma canis* and *Mycoplasma cynos*. Doctoral Dissertation, Ljubljani, Univerza V Ljubljani Biotehniška Fakulteta https://cris.cobiss.net/ecris/si/en/biblio?q=as%3D(34335)%20and%20td%3D(2.08)

[17] Rycroft, A.N., Tsounakou, E., Chalker, V. (2007). Serological evidence of *Mycoplasma cynos* infection in canine infectious respiratory disease. Vet. Microbiol., 120: 358-362. DOI: 10.1016/j.vetmic.2006.11.011

[18] Hussein, S.A., Hamad, M.A. (2022). *Mycoplasma* from the upper respiratory tract and conjunctival infections in household dogs. I. J. Vet. Sci., 36(1):137-141 DOI:10.33899/ijvs.2022.135824.2525

[19] Hussein, S.A., Hamad, M.A. (2023). Genetically diagnosis of *Mycoplasma* isolated from respiratory and conjunctival infections in household dogs. I. J. Vet. Sci., under press.

[20] Botes A., Peyrot B.M., Olivier A.J., Burger W.P., Bellstedt D.U.(2005). Identification of three novel *Mycoplasma* species from ostriches in South Africa. Vet. Microbiol. 111:159-169 DOI: 10.1016/j.vetmic.2005.10.017

[21] Han-Jouchim, D., Annette, D., Doris, E., Stefanie, G., Joe, K. (2006).PCR applications manual.3rd ed. Roche Diagnostics GmbH, Mannheim, Germany.pp.9-13. https://www.roche-applied-science.com.

[22] Leber, A.L.(2016).Clinical microbiology procedures handbook. 4th ed. ASM Press, USA. https://onlinelibrary.wiley.com/doi/book/10.1128/9781555818814

[23] Rahimee, I., Azeemi, M.Z. (2020). Polymerase chain reaction. Inter. J. Res. Biol. Pharm., 6(1):1-8 DOI: https://doi.org/10.53555/bp.v6i11.1456.

[24] Chalker, V.J.(2005). Canine mycoplasmas. Res. Vet. Sci., 79:1-8. DOI:10.1016/j.rvsc.2004.10.002

[25] Rosendal, S. (1973a). *Mycoplasma cynos*, a new canine *Mycoplasma* species. Intern. J. Syst. Bacteriol., 23: 1-49 https://www.microbiologyresearch.org/docserver/fulltext/ijsem/23/1/ijs-23-1-49.pdf?expires=1684710771&id=id&accname=guest&checksum=926CA640FAA47787FDFE266059D0BE78

[26] Rosendal, S. (1973b). Canine *Mycoplasma*. I. Cultivation from conjunctiva, respiratory and genital tracts. Acta. Pathol. Microbiol. Scandinavica Section B Microbiol. Immunol., 81(4):441-445 https://doi.org/10.1111/j.1699-0463.1973.tb02228.x

[27] Chvala, S., Benetka, V., Mostl, K., Zeugswetter, F., Spergser, J., Weissenböck, H. (2007). Simultaneous canine distemper virus, canine adenovirus2, and *Mycoplasma cynos* infection in dogs with pneumonia. Vet. Path., 44:508-512 DOI: 10.1354/vp.44-4-508

[28] Mannering, S.A., McAuliffe, L., Lawes, J.R., Erles, J.R., Erles, K., Brownlie, J. (2009). Strain typing of *Mycoplasma cynos* isolates from dogs with respiratory disease. Vet. Microbiol., 135:292-296 DOI:10.1016/j.vetmic.2008.09.058

[29] Priestnall, S.L., Mitchell, J.A., Walker, C.A., Erles, K. (2014). New and emerging pathogens in canine infectious respiratory disease. Vet. Path., 51(2):492-504 DOI:10.1177/ 0300985813511130

[30] Singh, V.K. (2017).Clinical veterinary microbiology. 1st ed. Replika Press Pvt. Ltd., India. https://www.amazon.com/Clinical-Veterinary-Microbiology-V-K-Singh/dp/9350848953

[31] Scott, D., Kennedy, M., Chengappa, M.M., Wikes, R. (2022).Veterinary microbiology. 4th ed. John Wiley and Sons, Inc., Oxford City. https://onlinelibrary.wiley.com/doi/book/10.1002/9781119650836

[32] Tille, P.M. (2022). Bailey & Scott's Diagnostic microbiology. 15th ed. Elsevier, Canada. https://evolve.elsevier.com/cs/product/9780323681056?role=student

[33] Lavan, R., Knesl, O. (2015). Prevalence of canine infectious respiratory pathogens in asymptomatic dogs presented at US animal shelthers. J. Small Anim. Pract., 56:572-576 DOI:10.1111/jsap.12389

[34] Michael, H.T., Waterhouse, T., Estrada, M., Seguin, M.A. (2021).Frequency of respiratory pathogens and SARS-CoV-2 in canine and feline samples submitted for respiratory testing in early 2020. J. Small Anim. Pract., 62:336-342 DOI:10.1111/jsap.13300

[35] Randolph, J.F., Moise, N.S., Searlett, J.M., Shin, S.J., Blue, J.T., Bookbinder, P.R. (1993). Prevalence of Mycoplasmal and Ureaplasmal recovery from tracheobronchial lavage and prevalence of Mycoplasmal recovery from pharyngeal swab specimens in dogs with or without pulmonary disease. Am. J. Vet. Res., 54(3):387-391.[available at https://www.pubmed.ncbi.nlm.nih.gov]

[36] Chan, C.M., Ridgway, M.D., Mitchell, M.A., Maddox, C.W. (2013). Association between *Mycoplasma*-specific polymerase chain reaction assay results and oral bacterial contamination of bronchoalveolar lavage fluid samples from dogs with respiratory tract diseases:121 cases (2005-2012). J. Am. Vet. Med. Assoc., 243(11):1573-1579. DOI: 10.2460/javma.243.11.1573

[37] Day, M.J., Carey, S., Clercx, C., Kohn, B., Marsillo, F., Thiry, E., Freyburger, L., Schulz, B., Walker, D.J. (2020). Aetiology of canine infectious respiratory disease complex and prevalence of its pathogens in Europe. J. Comp. Pathol., 176:86-108. DOI:10.1016/j.jcpa.2020.02.005

[38] May, M., Brown, D.R. (2009). Secreted sialidase activity of canine *Mycoplasmas*. Vet. Microbiol., 137(3-4):380-383 DOI:10.1016/j.vetmic.2009.01.009

[39] May, M.A., Brown, D.R. (2018). Virulence effectors of pathogenic *Mycoplasmas*. Preprints.org., 2018090533. DOI: 10.20944/preprints201809.0533.v1

[40] Dawood, A., Algharib, S.A., Zhao, G., Zhu, T., Qi, M., Delai, K., Hao, Z., Marawan, M.A., Shirani, I., Guo, A.(2022). *Mycoplasmas* as host pantropic and specific pathogens: clinical implications, gene transfer, virulence factors, and future perspectives. Frontiers Cellul. Infect. Microbiol., 12:1-40. DOI:10.3389/fcimb.2022.855731