Genetically diagnosis of mycoplasma isolated from respiratory and conjunctival infections in household dogs

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Abstract

Genetic depiction of Mycoplasma isolates was done by a PCR approach from the upper respiratory tract and conjunctival infections in domestic dogs in Mosul City. This was accomplished by amplifying the 16S rRNA gene, which is specific to the Mycoplasma genus. 100 domestic dogs of various ages, sexes, and breeds participated in the study from 1/2/2022 to 1/1/2023. There were 300 swabs total, including conjunctival, nasal, and oropharyngeal swabs. The swabs were subjected to mycoplasmal culturing in suitable conditions. The growing colonies were examined by light and a dissecting microscope. The DNA of growing colonies was extracted and amplified then migrated in agarose gel to observe the bands. The study's findings revealed the isolation of 58 Mycoplasma isolates, with an overall swab isolation rate of around 58% and a high rate of 68% from nasal swabs, then from oropharyngeal swabs and conjunctival swabs 58 and 45% sequentially. PCR technique showed that 34 isolates from the total of 58 isolates were assured for the 16S rRNA gene and yielded a band at 270bp, these findings are regarded as distinctive for the genus Mycoplasma. DNA Sequencing results of the 16S rRNA gene revealed that twenty-three 67.65% out of thirty-four Mycoplasma isolates were indicated as Mycoplasma cynos strain SM-MY-M23 which was dumped in the GenBank nucleotide sequence database under accession number OQ446513, so current results are considered as the first record of Mycoplasma cynos in dogs in Iraq.

Introduction

Mycoplasmas are members of the class Mollicutes (soft skin) insinuating the absence of the robust bacterial cell wall and are the minutest and most modest self-replicating prokaryotes (1,2). They are fastidious and demand sterol, or urea for growing. They require enriched media for growth supplemented with serum and yeast extract which delivers amino acids with vitamins, furthermore, penicillin and thallium acetate are added to block the contaminant bacteria and fungi (3). Certain varieties of Mycoplasma, which are facultative anaerobes, thrive best in an environment with 5-10% CO₂ and forming characteristically umbonate microcolonies when illuminated obliquely and a (fried egg appearance) in transmitted light (4). Domestic dogs are one of the two (the second one is the cat) most prevalent and familiar domestic animals worldwide. It has cohabited with humans for more than thousands of years as a hunted animal, a source of protection, a favorite, and a buddy (5-8). Notwithstanding the fact that not all Mycoplasma infections result in clinical illness, dogs were susceptible to a variety of Mycoplasma species (9,10). It is thought that Mycoplasma is one of the microbiomes of the upper airways in dogs (11,12), yet, a recent analysis of the normobiota of apparently healthy dogs revealed that Mycoplasma regularly moved through the nasal passages to the lower airways. This may propose that Mycoplasma habitation of the upper airways led to the dominance of the lower respiratory tract (13-18). Furthermore, Mycoplasma is a significant player in complex respiratory disease in canine (19-24). Many mycoplasmal...
species are significant veterinary pathogens that settle on the red blood cells or respiratory, and genital mucosal membranes and cause respiratory infections, mastitis, conjunctivitis, arthritis, and occasionally miscarriage (1,2,25).

The current work sought to identify and classify Mycoplasma isolated from respiratory infections and conjunctivitis in domestic dogs through molecular techniques with an observation of DNA sequencing.

Materials and methods

Ethical approval

The endorsement certificate with the number UM.VET.2021.071 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.

Samples and cultivation

The collected samples underwent standard methodological processing according to Hussein and Hamad (26). The growing colonies were microscopically examined with light and a dissecting microscope. The growing bacteria were examined for modified Diene’s stain, and morphological and biochemical tests (25-29). Then the bacterial colonies underwent molecular processing.

DNA extraction

The suspected colonies were subcultured in Mycoplasma broth medium for 7 days at 37°C. The extraction was accomplished according to the guidelines of the kit manufacturing company (Geneaid, Presto(TM) Mini) (Table 1). The Macrogen Company (South Korea) produced this primer, in a lyophilized form. As a stock solution, primer was dissolved in nuclease-free water to an eventual concentricity of 100 pmol/l. This primer's working solution was created by mixing 90µl of nuclease-free water with 10µl of primer stock solution, which was kept at -20 Celsius degrees in the freezer (32,33).

Preparing the primer

The primer sequence for the amplification of the 16S rRNA gene for Mycoplasma (31) (Table 1). The reaction mixture for PCR was prepared in a total volume of 20µl for the 16S rRNA gene (33).

PCR detection of 16S rRNA gene of Mycoplasma

The reaction mixture for PCR was prepared in a total volume of 20µl for the 16S rRNA gene (33).

PCR amplification condition

The 16S rRNA gene amplification was done in a thermal cycler (An Analytik Jena/Biometra, Germany) and the using program included three stages, the first stage was the initial denaturation at 94°C for 10 minutes, the second stage included 30 repeated cycles of three steps (Denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute), while the last stage was the final extension at 72°C for 5 minutes.

Table 1: Primer used in the existing study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ 3’)</th>
<th>Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA gene</td>
<td>F TGGGGGACAAACAGGATTAGATACC</td>
<td>270</td>
<td>(31)</td>
</tr>
<tr>
<td></td>
<td>R TGCACCATCTGACTGTATACCTC</td>
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Agarose gel electrophoresis

The fineness of the extricated DNA was examined by employing electrophoresis in 1.5% agarose gel (Biobasic Canda Inc, Canada) mixed with 3 µl of Ethidium Bromide stain (Promega, USA) by adding 1µl of DNA loading dye (Promega, USA) to 5µl of extracted DNA. In order to segregate the amplified products, 1.5% agarose gel electrophoresis was also employed. Each PCR result was placed into the agarose gel's well at a volume of 5 µl. The electrophoresis was performed using a power supply MP
300V at 80 V for one hour, and an electrophoresis tank (BioRad, USA) holding 1X TBE buffer (Promega, USA). A 100 bp DNA ladder, 4 µl (Promega, USA) served as a common molecular weight indicator. Lastly, the band was saw under the UV transilluminator (An Analytik Jena/Biometra, Germany) (34,35), and the photo of the gel was captured using a digital camera.

Sequencing 16S rRNA gene

Sanger dideoxy sequencing was used to assess the sequencing data after PCR product purification and sequencing, and the BLAST algorithm at the NCBI server [available at]. The sequence data were deposited in GenBank.

Results

Bacterial isolation and identification

Results of the culture of *Mycoplasma* isolates showed that these isolates were pure and formed characteristically umbonate microcolonies when illuminated obliquely and had a fried egg appearance (Figure 1).

Isolation rates

The existing investigation found that 58% of samples were affirmative for *Mycoplasma* isolation. Oropharyngeal swabs and conjunctival swabs came in second and third, respectively, to nose swabs in terms of isolation 68, 58, and 45%.

DNA extraction

The confirmation of *Mycoplasma* isolates was done by detecting the 16S rRNA gene using PCR and agarose gel. The findings exhibited that 34 isolates (from a total of 58 isolates) had the Mycoplasma 16S rRNA gene which has a 270bp product size, (Figure 2). These results confirmed that 34 isolates were *Mycoplasma* and pure.

![Figure 1: Fried egg appearance characteristics for Mycoplasma colony.](Image)

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<th>Lane</th>
<th>Description</th>
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<tbody>
<tr>
<td>M</td>
<td>100 bp DNA ladder</td>
</tr>
<tr>
<td>1-11</td>
<td>10 positive samples</td>
</tr>
<tr>
<td>11</td>
<td>Negative control</td>
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</tbody>
</table>

![Figure 2: Amplification of Mycoplasma 16S rRNA gene.](Image)

Nucleotide sequence accession number

The genome sequence of the 16S rRNA gene revealed that twenty-three 67.65% out of thirty-four *Mycoplasma* isolates were indicated as *Mycoplasma cynos* strain SM-MY-M23 from the nasal swabs in the household dogs suffering from respiratory signs have been submitted to the GenBank database of nucleotide sequences with entry number OQ446513.

Discussion

Respiratory diseases are frequent in dogs (10), and dogs are more sensitive to numerous types of respiratory infectious diseases (12), including *Mycoplasma* which was a pathogenic agent of respiratory disease in dogs (35,36). The flourishing bacteria in the contemporary investigation were matching to *Mycoplasma* when grownup on the medium, and bare the fried egg exterior as recounted in former reference books (3,25), which showed that in vitro culturing was the ideal style for identifying and isolating *Mycoplasma*, yet, the leisurely growth of *Mycoplasma* renders this technique time-wasting unto precise laboratories (3,13).

The existing study's 58% *Mycoplasma* isolation rate deviates significantly from the data stated by Sakmanoglu et al. (37) who documented 27% and Randolph et al. (38) and Barreto et al. (39) who chronicled 29.96%. The variances are perhaps imputed to the methods of sample gathering, isolation circumstances, case history, and specimen gathering time (3). The greater Mycoplasmal isolation ratio was recorded in nasal swabs 68%, then in the other two types of swabs (oropharyngeal swabs 58%, and conjunctival swabs 44%, however, various findings were established by former articles Randolph et al. (38) and Mitchall et al. (40) that showed oropharyngeal swabs 42.86% arranged at the peak of isolation and approved to them that conjunctival swabs 18.30% were the lowermost of isolation. The high rate of isolation from oropharyngeal swabs confirms that...
**Mycoplasma** is a habitual symbiont of the upper respiratory tract. Whenever weary events occur, **Mycoplasma** transforms into infectious and the disease emerges. The weakened Mycoplasmal isolation from the analogous swab kinds of apparently healthy dogs also provides evidence for this by Barreto et al. (39). The output of the amplicon band at 270 bp., a positive band of isolates of **Mycoplasma**, exposes the importance and vast role of this technique in the detection of **Mycoplasma** species, determination of virulence factors, sequencing and identification of phylogenetic tree of **Mycoplasma** isolated in domestic dogs (41). The genome sequence of the 16S rRNA gene specialized for **Mycoplasma** genus achieved in the existing study was indicated **M. cynos** strainSM-MY-M23 from nasal swabs in the household dogs suffering from respiratory signs and was recorded in accession number OQ446513 in GenBank, so these results have differed from the data recorded by Walker et al. (42) who indicated **M. cynos** strain (C142) from tracheal wash in dogs, in accession number HF559394 in the United States, this confirms the part of **M. cynos** in the occurrence of respiratory diseases in household dogs, with its multiple virulence factors that will be detected by our subsequent study. Thus, the current study is one of the first studies in recording **Mycoplasma cynos** strainSM-MY-M23 isolated from upper respiratory and conjunctival infections in household dogs in Iraq, especially in Mosul city.

**Conclusion**

The output of the existing investigation denoted **Mycoplasma cynos** by sequencing of 16S rRNA gene specialized for **Mycoplasma** genus so further studies must be done to know the virulence determinants of **Mycoplasma cynos** in dogs to preclude the dispersal of infective strains of **Mycoplasma cynos** between dogs.

**Acknowledgments**

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

**Conflict of interest**

Regarding the publishing of the current investigation, the authors thus state that yonder is no conflict of advantage.

**References**


تشخيص البؤس للعِنْبُكَات المعزولة من إصابات التنفسية والملتحمة العين في الكلاب المنزلية

صب عبد الرحيم حسين محمد علي حد

جاري وصف الأساليب الوراثية للعِنْبُكَات المعزولة من إصابات التنفسية والملتحمة العين في الكلاب المنزلية في مدينة الموصل باستخدام تقنية تعقب اللمبرة المتسائلة والتي تم إنجازها عن طريق تضخيم الجين الرديفوزيمي الخاص بتحديد الجنس المبكر للعِنْبُكَات ومن ثم متابعة التسلسل الجيني. اتخذت المسحات من 100 عزل من كل الجنسين وأعمار وأنواع مختلفة في الدراية للفترة من 1/1/2021 إلى 1/1/2022، حيث جمعت 300 مسحة تمثلت على مسحات من الأنف، البلعوم، اللثة، العين، واستخرجت النسب المئوية من الانخفاض في الباوث والظروف المجاورة لها. أكدت المستعمرات الناجحة في,last_smithed البرية والتحليليات. بعد ذلك استخلص الباوث، وجاءت النتائج التحليلية. بعد ذلك استخلص الباوث ل فصيلة من فصيلة الأكثر الانتشار، واستخلص عينة سلسة للعِنْبُكَات الناجحة والمتضخمة، وتم تضخيمها وترحيلها على الأكاروز لملاحظة الاستعمرات الناجحة وتم تضخيمها وترحيلها على الأكاروز لتشخيصها في الدراسة للفترة من 1/1/2021 إلى 1/1/2022. حيث تمت عزل 58 عزلة من العِنْبُكَات والباقيات بنسبة 68% من العليل العِنْبُكَات الناجحة، وتأتي من مجموع 300 مسحة كانت مؤكدة بإنتاج الجين الرديفوزيمي، ولم الخيار على حزم بحمض 300 زوج قاعدي. ونتيجة هذا النتائج ميزة لعِنْبُكَات الناجحة، وتثبت في قاعدة بيانات ميکروپلازماس نقيدين الجينات MY-SM، وتم توثيقها في قائمة موضوع العِنْبُكَات الناجحة - OQ446513. واعتبرت هذه النتائج كتسجيل أول لهذه النوع من العِنْبُكَات في العراق.

117