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Virulence, antimicrobial resistance and phylogenetic analysis of zoonotic walking pneumonia Mycoplasma arginini in the one-humped camel (Camelus dromedarius)

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ABSTRACT

In the scientific literature, a small amount of information is found concerning mycoplasmosis in camel species. A variety of pathogens could be causative agents for pneumonia, but walking pneumonia is mostly caused by Mycoplasma with slow development and mild symptoms. The aim of this study was to identify mycoplasmas from camels (Camelus dromedarius) and extending the arsenal of factors implicated in pathogenicity of M. arginini to shed light on the current knowledge gap. 460 lung samples (pneumonic; n=210 and apparently healthy; n=250) were randomly collected from the one-humped camels (C. dromedarius) that have been imported from Sudan and slaughtered at Cairo Slaughterhouse. 48 out of 210 isolates (22.9%) recovered from the pneumonic lungs were recorded as M. arginini. Positive PCR results were obtained for all 48 isolates. On the other hand, infection with the organism was not detected in the apparently healthy lungs. Hemolysis and hydrogen sulphide (H2S) production, a compound that has previously not been identified as a virulence factor in M. arginini, was evident in 100% of the isolates. The 48 M. arginini isolates were weak in their ability to form biofilm on poly-styrene surfaces. All isolates were 100% susceptible to florfenicol and streptomycin and 100% resistant to ciprofloxacin. Resistance to lincomycin, spiramycin, tylosin, doxacyclin and erythromycin was observed at different frequencies. 13 different combinations of antibiotics representing one to four classes were evident with the Macrolide erythromycin being the most represented. It also should be noted that the ciprofloxacin, doxacyclin, lincomycin, erythromycin combination was the most noted in 21/48 isolates. Surprisingly, none of the virulence genes (vsp, uvrC and gapA) and quinolone resistance genes (parC and gyrA) were detected by PCR.

1. Introduction

The one-humped camel, dromedary (Camelus dromedarius), has a historical presence in civilization, though they were not used until more recent times. The camel was used primarily as means of transportation in the desert, though they are now also used for wool, milk, and farming. To date, the camels are widespread and are located anywhere from Egypt to Chad, Sudan, Ethiopia, Eritria, Somalia and Libya (Roess et al., 2015; Napp et al., 2018).

Interestingly, dromedary camels are associated with various infectious diseases. One major disease is Middle East Respiratory Syndrome, which is a camel-to-human spread coronavirus that can originate in camel abattoirs, camel meat markets, camel farms and contribute to the virus spread (Roess et al., 2015; Napp et al., 2018). Unfortunately, there are other infectious diseases that are less well-understood, but also associated with camels. One such infection could be Mycoplasma species.

While most Mycoplasma species are host specific, there are reports of the presence of Mycoplasma in hosts that are not perceived as their normal habitat. In fact, since its first description in 1968 (Barile et al., 1968), M. arginini has been recovered from tissues and secretions of various mammals (Barile et al., 1968; Leach, 1970; Tan and Miles, 1974; al-Aubaidi et al., 1972; Hill, 1975; St George and Carmichael, 1975; Tan et al., 1977b; Goltz et al., 1986; Brogden et al., 1988; Hassan et al., 1997; Elfaki et al., 2002; Thomas et al., 2002; Navidmehr et al., 2009; Gonçalves et al., 2010; Navidmehr et al., 2011;...
Mederos-Triarte et al., 2014; Alaa et al., 2018; Göçmen et al., 2020). Moreover, Silló et al. (2012) and Watanabe et al. (2012) wrote two separate reports that provided supportive evidence of a fatal cases of human infection with \textit{M. arginini}. Each affected patient had an apparent history or possibility of exposure to a wild animal or animal products, thereby suggesting a zoonotic transmission of \textit{M. arginini} to humans. That said, only three cases of \textit{M. arginini} isolation occurred, though all people affected by the disease were exposed to various animals in different settings (e.g. slaughterhouse, lion attack, etc.) (Yechouron et al., 2006). Moreover, Silló et al. (2012) and Watanabe et al. (2012) wrote two national terminology for defining multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR), which was initially created through a joint initiative by the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention (Magiorakos et al., 2012).

2. Materials and Methods

2.1. Sample collection

There were 460 samples randomly recovered from pneumonic (n = 210) and apparently healthy (n = 250) lungs of imported one-humped camels between February to April 2018 from Sudan. The animals were submitted for routine slaughter. For the purposes of this study ‘pneumonic lungs’ was referred to those lungs that had gross lesions such as consolidation, fibrin deposition on the pleura, pleurisy, and/or adhesion; alternatively, ‘apparently healthy lungs’ was used to describe lungs without any gross lesions. Specimens were obtained aseptically using a sterile scalpel while taking precautions to prevent surface contamination. Following collection, the samples were transported to the microbiology laboratory in special ice-filled containers within 2 hours of sampling. Pieces of tissue from the border area between disease lesions and healthy lung were suspended by pounding in a mortar with sand and liquid growth medium. The suspension was inoculated into broth growth media with added Mycoplasma Supplement G. Dilutions of up to 10 − 5 of the liquid medium was prepared. The inoculated medium was incubated 7−10 days at 37°C in an incubator with 5 % CO2. The growth medium was checked daily for growth. A loopful of the broth cultures showing visible growth or turbidity was inoculated onto Mycoplasma Agar Base media in a 95 % Na2 and 5 % CO2 humidified atmosphere at 37 °C; the petri dishes were examined in steremicroscopy at the end of incubation. Colonies suspected for Mycoplasma were identified by routine methods.

Digitonin sensitivity test was carried out to differentiate between \textit{Mycoplasma} and \textit{Acholeplasma} genera using filter paper discs impregnated with 0.2 mL of 1.5% (W/V) ethanol solution of digitonin and dried overnight. \textit{Mycoplasma} spp. show digitonin sensitivity while \textit{Acholeplasma} spp. are resistant (Freundt et al., 1973). Biochemical identification was used for further testing of \textit{Mycoplasma} sp. Glucose fermentation, arginine deamination and urea hydrolysis tests were also performed (Erno and Stipkovits, 1973; Howard et al., 1994). Serological confirmation of \textit{Mycoplasma} sp. was conducted per methods of Clyde (1964), while the species-specific identification was performed with anti-\textit{M. bovis} hyperimmune serum (Lauerman et al., 1994) by the growth inhibition test utilizing dried antisemum impregnated paper discs. Final identification of the isolates was achieved by PCR.

2.2. Phenotypic virulence traits

There were four assays used to demonstrate the phenotypic virulence potentiality of the 48 \textit{M. arginini} isolates. This was conducted using the following: 1) the catalase enzyme activity was performed according to the procedure of Pritchard et al. (2014); 2) the hemolytic and hemoxidative activity of \textit{M. arginini} was determined by the method adopted by Großhennig et al. (2016) using washed 2% sheep RBCs in PBS with or without supplements in a final volume of 1 ml; 3) the hydrogen sulfide (H2S) produced by the 48 \textit{M. arginini} isolates was determined using lead acetate detection strips (Großhennig et al., 2016); and 4) biofilms grown on glass coverslips and in microtitre plates were quantified by measuring the absorbance (560 nm) of 100 ml of the solubilized crystal violet in a microtitre plate (McAuliffe et al., 2006).

2.3. Phenotypic susceptibility test

The 48 \textit{M. arginini} isolates were tested for susceptibility to eight antimicrobial agents, which represented six classes; this was accomplished by the disc diffusion method pursuant to procedures described by Clinical and Laboratory Standards Institute (CLSI, 2012). The M.\textsubscript{RD} was calculated by the equation a/(b x c), where ‘a’ was the aggregate antibiotic resistance score of all the isolates from the sample, ‘b’ was the number of antibiotics used (n = 8 via this study) and ‘c’ was the number of isolates that originated from the sample (Krumpeman, 1983). The criteria was followed for standardized international terminology for defining multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR), which was initially created through a joint initiative by the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention (Magiorakos et al., 2012).

2.4. Molecular Identification

2.4.1. DNA extraction

The bacterial lysates used as templates for the PCR were prepared. This was accomplished by gathering a loopful of bacteria from a fresh overnight culture on a tryptic soy agar plate, which was re-suspended homogeneously in 200 µl of sterile water. Then, the mixture was boiled at 100°C for five minutes to release the DNA and centrifuged. The supernatant was used as a template for PCR mixture. Isolates that only reacted with \textit{M. arginini} antisera were selected.

2.4.2. 16S rRNA identification

Positive isolates were further confirmed for mycoplasmas by PCR amplification of the 16S rRNA gene using \textit{Mycoplasma} specific primers (Table 1). PCR reaction was performed in a 50 µl volume for each isolate, consisting of 5 µl of 50 ng of \textit{Mycoplasma} DNA, 10 µl of 10 x Tq buffer (10 mM tris- HCl [pH 8.8], 50 mM KCl), 1 µl of 50 µM of forward and reverse primers, 1.5 mM MgCl2, 1 µl of 2U of Tq polymerase, 1 µl of 50 uM of each dNTP, and 31µl of DNase- RNase- free, deionized water. The PCR reaction was performed in a thermal cycler (Biometra TRIO, Jena, Germany) with an intial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1.5 minutes with a final extention at 72°C for 10 minutes.

The same procedure for 16S rRNA PCR reaction as described above was used for \textit{Mycoplasma} species identification, but with the following PCR modifications: 40 cycles in denaturation at 94°C for 60 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1.5 minutes with a final extention at 72°C for 10 minutes.

2.4.3. \textit{Mycoplasma arginini} typing

Positive \textit{M. arginini} isolates were further confirmed by PCR reaction. The PCR conditions for this reaction were denaturation 94°C for 45 seconds, primer annealing at 60°C for 1 minute, and extension at 72°C for 2 minutes. The reactions were performed for 30 or 35 cycles with a final extention at 72°C for 3 minutes.

2.4.4. Detection of virulence genes

PCR was performed to detect three virulence genes of \textit{M. arginini}
including the variable surface lipoprotein gene (vsp), cytadhesin (gapA) and the uvrC, which encodes a protein involved in DNA excision and repair.

2.4.4.1. Detection of vsp, uvrC and gapA genes. The detection of vsp, uvrC and gapA genes was performed (Table 1). PCR reactions were performed in a 20 µl volume for each isolate. The PCR condition for detection of the vsp gene was initial denaturation 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1.5 minutes with a final extension at 72°C for 10 minutes. The PCR condition for detection of the uvrC and gapA genes had an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds for gapA and at 52°C for 30 sec for uvrC, and extension at 72°C for 2 min with a final extension 72°C for 5 min.

2.4.5. Detection of quinolones resistance (QRDR) genes

Detection of genes (gyrA and parC) encoding for quinolone resistance (QRDRs) was carried out by PCR. The PCR reactions were performed in 50 µl volume for each isolate, with 30 pmol/µl of each primer and 100 ng DNA. Conditions for the PCR were as follows: 95°C for 3 minutes, 30 cycles of denaturation for 30 seconds at 95°C, followed by annealing of 30 sec at 56°C and extension at 72°C for 45 seconds with a final extension at 72°C at 10 minutes.

2.5. Nucleotide sequencing and sequence analysis

Sequencing of the PCR amplified product was conducted by GATC Company using ABI 3730xl DNA sequencer and forward/reverse primers (Table 1). For M. arginini, the traditional Sanger technology was combined with the novel 454 technology for sequencing 326 bp PCR product of the specific gene. In order to establish sequence identity to GenBank accessions, a BLAST® analysis (Basic Local Alignment Search Tool) was initially performed. Published data of international reference strains from the GenBank were available from the National Center for Biotechnology Information (NCBI) ([https://www.ncbi.nlm.nih.gov/nucore/?term= infectious + bursal + disease + virus]). The comparisons of obtained nucleotide 326 sequences and multiple alignments were performed using the BioEdit sequence alignment editor (7.1.5) for multiple sequence alignment. Sequences were then submitted to NCBI GenBank using BankIt [see: http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank] under the accession numbers:

MK271638 Mycoplasma arginini strain WA 1.Egypt, MK271639 Mycoplasma arginini strain WA 2.Egypt, MK271640 Mycoplasma arginini strain WA 4.Egypt, and MK271641 Mycoplasma arginini strain WA 5.Egypt.

2.6. Mycoplasma arginini specific gene phylogenetic tree

The 16S phylogenetic tree was inferred by the distance-based neighbor-joining (NJ) method using MEGA version 7.

3. Results

3.1. Prevalence of M. arginini in the samples recovered from lungs of camels

A total of 460 lung samples (pneumonic n = 210; and apparently healthy n = 250) samples were randomly collected from lungs of imported one humped camels that were slaughtered at Cairo Slaughter house. Of these, 48 out of the 210 pneumonic lung samples (22.9%) were recorded to be positive for putative mycoplasmas. All isolates that were identified as mycoplasmas formed typical ‘fried-egg’ and/or centerless granular colonies. They were all diguitin-sensitive, glucose-positive and arginine-negative. A positive PCR result for M. arginini was obtained for all 48 isolates. Alternatively, infection with the organism was not detected in the apparently healthy lungs.

3.2. The catalase enzyme activity of M. arginini

The catalase activity of M. arginini was examined and indicated by the generation of bubbles. There were nine M. arginini (19%) samples that tested catalase positive.

3.3. The hemolytic activity of M. arginini

The effect of M. arginini on RBCs was tested in a liquid environment. In this assay, the erythrocytes remained intact, though a red to brown color change of the blood cells for α-haemolysis did occur due to modification of hemoglobin. This confirms that all 48 isolates were phenotypically hemolytic.

3.4. Hydrogen sulfide production

To test whether sulfide ions caused hemolysis, RBCs were incubated in the presence of sodium sulfide (0.625% Na₂S) and tested for the formation of H₂S using lead acetate strips. The white strips changed to black when H₂S was added, indicating production of lead sulfide.

Table 1

| Species | Sequence | Target | References |
|---------|----------|--------|------------|
| 16SrRNA. For Mycoplasma | 5′- AGA CTC CTA CTA GAG CCA GCA -3′ | 1000bp | Alberto et al. (2006) |
| | 5′- ACT AGC GAT TCC GAC TCC AGT -3′ | | |
| Mycoplasma group- specific | 5′- GGGAGAAACAAGGATAGACTCTC-3′ | 280bp | VanKuppeved et al. (1994) |
| | 5′- TGGACATCTGCTACATGTTAACCCTC-3′ | | |
| M. arginini | 5′- TATCTCGATGCTCAGAATGCT-3′ | 326bp | Vahid et al. (2009) |
| Virulence genes | 5′- TTAAGGAAAGGACCCCTATTGAT-3′ | 1626 bp | Perez-Casal et al. (2007) |
| uvrC | 5′- CTTGGATCAGTGGCTTCATTAGC-3′ | 1007 bp | |
| gapA | 5′- TGGAGGATCAGAAGAATAGCTATCAAGGTTGGGACG-3′ | 400bp | Alberto et al. (2006) |
| vsp | 5′- CTTGGAATCTGGGTCCATTAGC-3′ | 531bp | Lysnya et al. (2009) |
| Fluorquinolone resistance | 5′- GAGCTAGTCAAGTGGGTCCATTAGC-3′ | 488bp | |
| genes | 5′- CTTGGAATCTGGGTCCATTAGC-3′ | | |
| gyrA | 5′- GACGAATCTAGTCGGTGGAGAGTTC-3′ | | |
| parC | 5′- GTCATCATGGGACGGATTCGGGCTTC-3′ | | |
| Antibiotics | Ciprofloxacin (5 μg) | Doxacyclin (30 μg) | Lincomycin (2 μg) | Florfenicol (30 μg) | Spiromycin (100 μg) | Tylosin (30 μg) | Erythromycin (15 μg) | Streptomycin (10 μg) | MAR index |
|-------------|---------------------|--------------------|-------------------|---------------------|---------------------|----------------|---------------------|---------------------|-----------|
|             | R                   | S                  | R                 | R                   | R                   | R             | R                   | R                   | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |
|             |                     |                    |                   |                     |                     |                |                     |                     | R         |

R: resistant; I: intermediate; S: susceptible. MDR: non-susceptibility to at least one agent in three or more antimicrobial categories; XDR: non-susceptibility to at least one agent in all but ≤2 antimicrobial categories.
3.5. Biofilm formation capability

*M. arginini* isolates (n = 48) were found to be weak in their ability to adhere to polystyrene multiwell plates. A very low spectrophotometric values (A590 0.0440-0.1) were detected for these isolates that had poor adhesion and biofilm formation abilities.

3.6. Antimicrobial resistance among the *M. arginini* isolates

The antimicrobial resistance profiles of *M. arginini* isolates obtained in this study were tested (Table 2). All isolates were 100% susceptible to florfenicol and streptomycin and 100% resistant to ciprofloxacin. Resistance to lincomycin, tylosin, doxacyclin and erythromycin was observed at different frequencies. On the other hand, there were 13 different combinations of antibiotics representing one to four classes, with the Macrolide erythromycin being the most obviously seen in 10 out of the 13 combinations (Table 3). It also should be noted that the Ciprofloxacin, Doxacyclin, Lincomycin, Erythromycin combination was the most noted in 21/48 isolates; moreover, the multidrug-resistant (MDR), and extensively drug-resistant (XDR) isolates were obvious in 31/48 (64.59%) and 8/48 (16.67%) isolates respectively.

3.7. Distribution of the virulence and QRDR genes in the *M. arginini* isolates

Interestingly and unexpectable, the *vsp, uvrC, parC* virulence genes and the *gyrA* and *gapA* QRDR genes were not detected by PCR in the 48 *M. arginini* isolates (Table 1).

3.8. Sequence analysis of *M. arginini* specific gene

From the sequence of *M. arginini* specific gene (Figs. 1 & 2), this study isolated MK271639.1, MK271638.1, MK271640.1 and MK271641.1. These had high nucleotide identity of 100%, with 99% occurring in other Egyptian isolates including: JN543264.1 and HM635904.1, European isolates GQ409971.1 (England), NR.041743.1 (Sweden), African isolatesHQ661827.1, HQ661828 (South Africa), and American isolates JN935883.1, JQ903578.1 and JQ903580.1 (USA). Some had low nucleotide identity at 24%, 31% and 48% with Egyptian isolates MH685445.1, MG564232.1 and JQ859817.1, respectively (Figs. 3 & 4).

4. Discussion

Pneumonia can be caused by bacteria, virus or fungi; that said, walking pneumonia is mostly caused by the bacteria *Mycoplasma*, which slowly progresses with mild symptoms that can evolve into more severe symptoms (Stamm and Stankewicz, 2020). In humans, walking pneumonia usually is caused more specifically by *M. pneumoniae* (CDC, 2018). Other forms of bacteria can cause pneumonia, though, and should not be dismissed as irrelevant. For example, *M. arginini* can modulate inflammatory response and energy metabolism on the transcription level in B-lymphoid cells with HTT mutation (Krysztof-Russjan et al., 2016). Additionally, *M. arginini* is particularly in-crinated to be involved in oncogenesis and infections in different human cell lines (e.g. fibroblast, embryonic kidney, breast cancer, colorectal carcinoma) and mouse fibroblasts (Zella et al., 2018).

Mycoplasmas are commensal bacteria found widely in association with mucous membranes of all mammalian species (Sykes, 2014) and can be isolated from normal human and animal respiratory mucosa (Thomas and Smith, 1972; Koshimizu and Ogata, 1974; Aroch et al., 2008; Gabiainitiene et al., 2011; Zhou and Li, 2014; Ericsson et al., 2016; Peek and Divers, 2017; Jambhekar et al., 2019; Moboni et al., 2019; Mead et al., 2019). Several non-*M. bovis* species are of interest in cattle with varying degrees of clinical significance of which *M. arginini* comes to our interest (Parkar et al., 2018). There is debate on whether these are truly new emerging pathogens or pre-existing pathogens that are now easier to detect due to the advent of sophisticated molecular diagnostic tools and more frequent diagnostic testing. For decades, the role of other bacterial agents such as *M. arginini* has been questioned (Yechouron et al., 1992; Watanabe et al., 2012). It is unknown whether certain *Mycoplasma* species such as *M. arginini* act as a medical hazard. It is of significance to emphasize that, the 48 *M. arginini* isolates were from the pneumonic lungs and not from the healthy lungs. This was also encountered in other animals reflecting that most mycoplasmas are normal inhabitants of the upper respiratory tract but do not appear in the lungs of healthy animals (Thomas and Smith, 1972; Tan et al., 1977a; Thomas et al., 2002; Aroch et al., 2008; Mohamed et al., 2018).

With the work presented in this study, an arsenal of factors were implicated in the pathogenicity of *M. arginini*. The overlapping, but distinct effects of H2S2, indicated that the bacteria possess a set of virulence determinants that synthesize and allow the bacteria getting efficient access to the host’s resources. Großhennig et al. (2016) shared results confirming H2S production and hemolysis were correlated in *M. pneumoniae*. This was the first time that correlation was recorded for *M. arginini* isolated from camels, where H2S was not previously identified as a virulence factor in lung pathogens in camels. This study also concluded that H2O2 was the responsible agent for the oxidation of heme, but not for lysis of RBCs (Großhennig et al., 2016).

Contrary to the assumptions based on the effects of catalase and catalase inhibitors on erythrocyte hemolysis by *M. pneumoniae*, Großhennig et al. (2016) also found that a mutant of *M. pneumoniae*, which was unable to produce H2O2, could still lyse erythrocytes in a blood agar overlay via β-hemolysis. The research conducted in this study concluded that catalase played only a minor role in hemolysis by *M. arginini*, similar to the property found in *M. pneumoniae* and that the discoloration of hemoglobin was specifically attributed to cysteine-dependent formation of H2S2 ions. This data was also in accordance to the

Table 3

| Antibiotics                        | n = resistant antibiotics | n = antibiotic classes | n = of isolates |
|-----------------------------------|--------------------------|------------------------|-----------------|
| Ciprofloxacin                     | 1                        | 1                      | 8               |
| Ciprofloxacin, Erythromycin       | 2                        | 2                      | 1               |
| Ciprofloxacin, Doxacyclin, Erythromycin | 3                    | 3                      | 4               |
| Ciprofloxacin, Doxacyclin, Lincomycin | 3                    | 3                      | 1               |
| Ciprofloxacin, Spiromycin, Tylosin | 3                        | 2                      | 1               |
| Ciprofloxacin, Lincomycin, Erythromycin | 3                    | 3                      | 1               |
| Ciprofloxacin, Lincomycin, Erythromycin | 4                    | 4                      | 21              |
| Ciprofloxacin, Spiromycin, Tylosin, Erythromycin | 4                        | 2                      | 1               |
| Doxacyclin, Lincomycin, Tylosin, Erythromycin | 4                    | 3                      | 1               |
| Ciprofloxacin, Lincomycin, Spiromycin, Tylosin, Erythromycin | 5                        | 3                      | 2               |
| Ciprofloxacin, Doxacyclin, Lincomycin, Tylosin, Erythromycin | 5                        | 3                      | 1               |
| Ciprofloxacin, Doxacyclin, Lincomycin, Spiromycin, Tylosin, Erythromycin | 6                        | 3                      | 1               |
findings of Großhennig et al. (2016). On the other hand, the induced weak biofilm formation by *M. arginini* in this study could be attributed as a reflection to the weak production of catalase, which is considered as an enhancement factor for biofilm growth and formation (Simmons et al., 2015).

The MAR indexes of the *M. arginini* isolates were decided based on eight different antibiotics. *M. arginini* demonstrated MAR indexes ranging from 0.0 and 0.625. Those isolates with a MAR indexes above 0.2 suggested that the bacteria isolates were from a high-risk source, where antibiotics were frequently used. Drug resistance in isolates originating from wildlife, which are not influenced by selective pressures of antimicrobial drugs, are believed to be low (Gufe et al., 2019). The values for all the strains between 0 and 0.625 suggested a diverse origin from a high-risk source of contamination where antibiotics are often used or encountered during transport from native countries (e.g. Sudan, Ethiopia and Chad) to Egypt.

The 16S rRNA gene is one of the most common genes targeted for bacterial identification because of its presence in all bacteria and unchanged function over time (Janda et al., 2007). The specificity of these PCR assays targeting the 16S rDNA PCR/DGGE and 16S-23S rRNA spacer region appeared to be adequate against most *Mycoplasma* species and *Acholeplasma* spp. (Tang et al., 2000; McAuliffe et al., 2005; Jozefova et al., 2014). This approach enabled us to identify and differentiate the 48 *M. arginini* species that were isolated from the pneumatic lungs of the camel. We also followed the steps of Gioia et al. (2016) by sequence analysis, which allowed identification and discrimination of several *Mycoplasma* and *Acholeplasma* spp., including *M. arginini*, *M. alkalescens*, *M. bovicenitalium*, *M. canadense*, *M. bovirhinis*, *M. californicum*, *A. laidlawii*, and *Acholeplasma oculi*, when the *Mycoplasma* 16-23S rDNA and *Acholeplasma* 16-23S rDNA were targeted.

Compared to other bacterial pathogens, the current knowledge of the molecular basis of pathogenicity of mycoplasmas is limited, and disruption at the molecular and cellular level remain to be elucidated. Several studies in the past years have shown that pathogenic mycoplasmas are equipped with sophisticated genetic systems, which allow these agents to spontaneously change their surface antigenic make-up (Jeqchlinger et al., 2004; Chopra-Dewasthaly et al., 2017). It is implicated that these variable surface components provide the wall-less mycoplasmas with a means to avoid the host immune response and
Fig. 2. Alignment of nucleotides of 16s rRNA gene of *M. arginini*.
>KU746836.1 Mycoplas 48.................................
>MH685445.1 Mycoplas 51C.TT.TCCGG.ATT.....GGC.TA.AG..TT.GTAG.C..TTTAT.AA.T
>MG564233.1 Mycoplas 48.................................
>MG564232.1 Mycoplas 48.................................
>MG564231.1 Mycoplas 48.................................
>MG564230.1 Mycoplas 48.................................
>JQ859817.1 Mycoplas 51C.TT.TCCGG.ATT.....GGC.TA.AGG.TT.GTAG.C..TTTAT.AA.T
>JQ820403.1 Mycoplas 48...C.................................
>JN543264.1 Mycoplas 48.................................
>HM635904.1 Mycoplas 48.................................
>GQ409971.1 Mycoplas 48.................................
>NR_041743.1 Mycoplas 48.................................
>HQ661827.1 Mycoplas 48.................................
>HQ661828.1 Mycoplas 48.................................
>JN935883.1 Mycoplas 48.................................
>JQ903578.1 Mycoplas 48.................................
>JQ903580.1 Mycoplas 48.................................

110 120 130 140 150

---|---|---|---|---|---|

>MK271641 Mycoplasm 98 TTAATTTGAAAGATACGCGGAGAACCTTACCTTACCTGGACATCCTTTCG-
>MK271638 Mycoplasm 96 .....................................
>MK271639 Mycoplasm 96 .....................................
>MK271640 Mycoplasm 97 .....................................
>KP972459.1 Mycoplas 98..................................
>KP972458.1 Mycoplas 98..................................
>MF101758.1 Mycoplas 98.............T........C.C........T.CT..
>KU746836.1 Mycoplas 98.................................
>MH685445.1 Mycoplas 101C.GG.G.CA..TCCCA.G.CTC....C.GG.TCGCT..GATA.TGGTAA
>MG564233.1 Mycoplas 98.................................
>MG564232.1 Mycoplas 98.................................
>MG564231.1 Mycoplas 98.................................
>MG564230.1 Mycoplas 98.................................
>JQ859817.1 Mycoplas 101C.GG.G.CA..TCCCA.G.CTC....C.GG.TCGCT..GATA.TGGTAA
>JQ820403.1 Mycoplas 98.............T.............T.-
>JN543264.1 Mycoplas 98.................................
>HM635904.1 Mycoplas 98.................................
>GQ409971.1 Mycoplas 98.................................

**Fig. 2.** (continued)
| Accession | Species    | Sequence                                                                 |
|-----------|------------|--------------------------------------------------------------------------|
| NR_041743.1 | Mycoplasma | >MK271641 Mycoplasma 146-CAATGCTATAGATATA-GCGGAGGTTAACGGAGTGACAGATGGTGC |
| HQ661827.1   | Mycoplasma | >MK271638 Mycoplasma 144-                                                  |
| HQ661828.1   | Mycoplasma | >MK271639 Mycoplasma 144-                                                  |
| JN935883.1   | Mycoplasma | >MK271640 Mycoplasma 145-                                                  |
| JQ903578.1   | Mycoplasma | >KP972459.1 Mycoplasma 146-                                                |
| JQ903580.1   | Mycoplasma | >KP972458.1 Mycoplasma 146-                                                |
| MF101758.1   | Mycoplasma | >MF101758.1 Mycoplasma 146-A--------T--------AC,G--------             |
| KU746836.1   | Mycoplasma | >KU746836.1 Mycoplasma 146-A--------TC----------                        |
| MH869454.1   | Mycoplasma | >MH869454.1 Mycoplasma 151ACT,G,A,G,G,A,G,G,A,G,G,A,GATGAG,C----------- |
| MG564233.1   | Mycoplasma | >MG564233.1 Mycoplasma 146-                                                  |
| MG564232.1   | Mycoplasma | >MG564232.1 Mycoplasma 144-                                                  |
| MG564231.1   | Mycoplasma | >MG564231.1 Mycoplasma 146-                                                  |
| MG564230.1   | Mycoplasma | >MG564230.1 Mycoplasma 146-                                                  |
| JQ859817.1   | Mycoplasma | >JQ859817.1 Mycoplasma 151ACT,G,A,G,G,A,G,G,A,G,G,A,GATC,T,GAA.CGGTGAAA... |
| JQ820403.1   | Mycoplasma | >JQ820403.1 Mycoplasma 146-A--------A----------                        |
| JN543264.1   | Mycoplasma | >JN543264.1 Mycoplasma 146-                                                  |
| HM635904.1   | Mycoplasma | >HM635904.1 Mycoplasma 146-                                                  |
| GQ409971.1   | Mycoplasma | >GQ409971.1 Mycoplasma 146-                                                  |
| NR_041743.1   | Mycoplasma | >NR_041743.1 Mycoplasma 146-                                                  |
| HQ661827.1   | Mycoplasma | >HQ661827.1 Mycoplasma 146-                                                  |
| HQ661828.1   | Mycoplasma | >HQ661828.1 Mycoplasma 146-                                                  |
| JN935883.1   | Mycoplasma | >JN935883.1 Mycoplasma 146-                                                  |
| JQ903578.1   | Mycoplasma | >JQ903578.1 Mycoplasma 146-                                                  |
| JQ903580.1   | Mycoplasma | >JQ903580.1 Mycoplasma 146-                                                  |

Fig. 2. (continued)
| Accession | Organism       | Length |
|-----------|----------------|--------|
| MK271639  | Mycoplasma     | 192    |
| MK271640  | Mycoplasma     | 193    |
| KP972459.1| Mycoplasma     | 194    |
| KP972458.1| Mycoplasma     | 194    |
| MF101758.1| Mycoplasma     | 185    |
| KU746836.1| Mycoplasma     | 194    |
| MH685445.1| Mycoplasma     | 187    |
| MG564233.1| Mycoplasma     | 160    |
| MG564232.1| Mycoplasma     | 144    |
| MG564231.1| Mycoplasma     | 158    |
| MG564230.1| Mycoplasma     | 179    |
| JQ859817.1| Mycoplasma     | 201    |
| JQ820403.1| Mycoplasma     | 194    |
| JN543264.1| Mycoplasma     | 194    |
| HM635904.1| Mycoplasma     | 194    |
| GQ409971.1| Mycoplasma     | 194    |
| NR_041743.1| Mycoplasma   | 194    |
| HQ661827.1| Mycoplasma     | 194    |
| HQ661828.1| Mycoplasma     | 194    |
| JN935883.1| Mycoplasma     | 194    |
| JQ903578.1| Mycoplasma     | 194    |
| JQ903580.1| Mycoplasma     | 194    |
|            |                | 260    |
|            |                | 270    |
|            |                | 280    |
|            |                | 290    |
|            |                | 300    |
| MK271641  | Mycoplasma     | 244    |
| MK271638  | Mycoplasma     | 242    |
| MK271639  | Mycoplasma     | 242    |
| MK271640  | Mycoplasma     | 243    |
| KP972459.1| Mycoplasma     | 244    |
| KP972458.1| Mycoplasma     | 244    |
| MF101758.1| Mycoplasma     | 199    |
| KU746836.1| Mycoplasma     | 244    |
| MH685445.1| Mycoplasma     | 187    |
| MG564233.1| Mycoplasma     | 160    |
| MG564232.1| Mycoplasma     | 144    |
| MG564231.1| Mycoplasma     | 158    |
| MG564230.1| Mycoplasma     | 179    |

Fig. 2. (continued)
>JQ58917.1 Mycoplas 251CTGA.GCTGAGGGGA.GAA..CGTGCGGA.CAAAAC.GAT--

>JK820403.1 Mycoplas 244.C....C........C.T...................C.....

>JK543264.1 Mycoplas 244.................................C.....

>HK635904.1 Mycoplas 244.................................C.....

>QG409971.1 Mycoplas 244.................................C.....

>N0_041743.1 Mycoplas 244.................................C.....

>RE671827.1 Mycoplas 244.................................C.....

>RE671828.1 Mycoplas 244.................................C.....

>JN935883.1 Mycoplas 244.................................C.....

>JQ903578.1 Mycoplas 244.................................C.....

>JQ903580.1 Mycoplas 244.................................C.....

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>MK271641 Mycoplasm 294 GATA.C

>MK271638 Mycoplasm 292.....

>MK271639 Mycoplasm 292.....

>MK271640 Mycoplasm 293.....

>KP972459.1 Mycoplas 294.....

>KP972458.1 Mycoplas 294.....

>MF101581.1 Mycoplas 199----

>KU746836.1 Mycoplas 294.....

>MH685445.1 Mycoplas 187----

>MG564233.1 Mycoplas 160----

>MG564232.1 Mycoplas 144----

>MG564231.1 Mycoplas 158----

>MG564230.1 Mycoplas 179----

>JQ859817.1 Mycoplas 289----

>JQ820403.1 Mycoplas 294.....

>JN543264.1 Mycoplas 294.....

>HK635904.1 Mycoplas 294.....

>QG409971.1 Mycoplas 294.....

>N0_041743.1 Mycoplas 294.....

>HQ61827.1 Mycoplas 294.....

>HQ61828.1 Mycoplas 294.....

>JN935883.1 Mycoplas 294.....

>JQ903578.1 Mycoplas 294.....

>JQ903580.1 Mycoplas 294.....

Fig. 2. (continued)
Fig. 3. Identity % of 16s rRNA gene of M. arginini isolates.

![Identity % of 16s rRNA gene of M. arginini isolates](image)

Fig. 4. Phylogenetic tree of 16s rRNA gene of M. arginini isolates.

![Phylogenetic tree of 16s rRNA gene of M. arginini isolates](image)
promote host colonization (Jeqchlinger et al., 2004). The uvrC gene is well conserved and an enzyme that is essential for replication, as it is involved with DNA repair, making it a highly stable gene (Thomas et al., 2004) and a much more specific target gene than 16S rRNA (Subramaniam et al., 1998). In our investigation this was not the case and the uvrC gene failed to be evident in addition to the other two virulence genes, vsp and gapA. There were 16 genes found to be essential in previous studies on other mycoplasma species that were found to be dispensable (Sharma et al., 2014). Of these, five genes have previously been predicted to be part of the core set of 153 essential genes in mycoplasmas (Sharma et al., 2014).

The absence of these three virulence genes was also reflected to the two gyrA and parC genes encoding for quinolone resistance. A strange and unexpected situation occurred, which raises the question of the dispensability of these genes in M. arginini isolated from camel. Moreover, this study could extend the list of non-essential genes of mycoplasmas that were previously generated by studies in other species (Sharma et al., 2014).

This study also has some limitations with substantial outputs. First, the number of isolates included in the study was relatively small. Second, isolates were selected from imported camels, thereby suggesting a further analysis conducted with domestic camel isolates. Third, the potential of whole genome sequencing (WGS) must be implemented to provide more detailed information than traditional molecular typing methods. This further exploration of genomic diversity among M. arginini isolates can advance the epidemiological information specific to at-risk populations for an important, yet often underestimated, respiratory pathogen. This information can highlight biological risks from a public health perspective as well as identify the emergence of new variants or genetic features that may impact transmission or virulence.

5. Conclusion

The live camel trade is a major industry in Egypt, which is also a source of pathogens dissemination and transmission of infectious diseases. Because of this significant presence and risks associated with it, this study was conducted to detect, isolate, and identify mycoplasmas from camels (C. dromedarius). To date, several non-M. bovis species are of interest in cattle with varying degrees of clinical significance, which was another factor reaffirming the importance of studying pathogens in M. arginini.

This study employed an arsenal of factors implicated in pathogenicity of M. arginini to shed light on current knowledge gaps. By better understanding H2S and investigating other potential virulence M. arginini of interest in cattle with varying degrees of clinical significance, which M. bovis this study was conducted to detect, isolate, and identify mycoplasmas source of pathogens dissemination and transmission of infectious diseases or virulence.

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