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. domedarius*) 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 (H_2S) 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 polystyrene surfaces. All isolates were 100% susceptible to florfenicol and streptomycin and 100% resistant to ciprofloxacin. Resistance to lincomycin, spiromycin, 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, uvr*C and *gap*A) and quinolone resistance genes (*par*C and *gyr*A) 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, Ethopia, 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;

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Mederos-Iriarte 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 *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 *M. arginini* to humans. That said, only three cases of *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., 1992; Prayson et al., 2008; Silló et al., 2012).

Therefore, the aim of the study was to evaluate *M. arginini* isolated from the dromedary camel, which could act as a host of the disease and possibly contribute to disease perpetuation. This was accomplished by analyzing the hydrogen sulfide production by *M. arginini* as a potential virulence factor in addition to the hemolysis activity as well as its ability to produce biofilm and identification of two genes responsible for quinolone resistance (QRDR). This study also investigated the phylogenetic tree of the 16SRNA sequences in the *M. arginini* camel isolates.

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 % CO_2. The growth medium was checked daily for growth. A loopful of the broth cultures showing *Mycoplasma* growth or color change were inoculated onto *Mycoplasma* Agar Base media in a 95 % N_2 and 5 % CO_2 humidified atmosphere at 37°C; the petri dishes were examined in stereomicroscopy at the end of incubation. Colonies suspect for *Mycoplasma* were identified by routine methods.

Digitonin sensitivity test was carried out to differentiate between *Mycoplasma* and *Acholeplasma* genera using filter paper discs impregnated with 0.2 mL of 1.5% (W/V) ethanol solution of digitonin and dried overnight. *Mycoplasma* spp. show digitonin sensitivity while *Acholeplasma* spp. are resistant (Freundt et al., 1973). Biochemical identification was used for further testing of *Mycoplasma* sp. Glucose fermentation, arginine deamination and urea hydrolysis tests were also performed (Erno and Stipkovits, 1973; Howard et al., 1994). Serological confirmation of *Mycoplasma* spp. was conducted per methods of Clyde (1964), while the species-specific identification was performed with anti-*M. bovis* hyperimmune serum (Lauerman et al., 1994) by the growth inhibition test utilizing dried antiserum 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 *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 *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 (H_2S) produced by the 48 *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 *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 MAR_{index} 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 *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 *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 *Mycoplasma* DNA, 10 μl of 10 x Taq buffer (10 mM tris- HCl [pH 8.8], 50 mM KCl), 1 μl of 50 pM of forward and reverse primers, 1.5 mM MgCl2, 1 μl of 2U of Taq 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 *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 2 minutes.

2.4.3. Mycoplasma arginini typing

Positive *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 *M. arginini*

Table 1

Oligonucleotide primers used for detection of *Mycoplasma* spp., *M. arginini,* virulence genes, and fluoroquinolone resistance (QRDR) genes.

Species	Sequence	Target	References
16SrRNA. For *Mycoplasma*	5′- AGA CTC CTA CGG GAG GCA GCA -3′	1000bp	Alberto et al. (2006)
	5′- ACT AGC GAT TCC GAC TTC ATG -3′		
***Mycoplasma* group- specific**	5′-GGGAGCAAACAGGATAGATACCCT-3′	280bp	VanKuppeved et al. (1994)
	5′-TGCACCATCTGTCACTCTGTTAACCTC-3/		
M. arginini	5′- TGATCATTAGTCGGTGGAGAGTTC-3′	326bp	Vahid et al. (2009)
	5′- TATCTCTAGAGTCCTCGACATGACTC-3′		
Virulence genes			
*uvr*C	5′-TTACGCAAGAGAATGCTTCA-3′	1626 bp	Perez-Casal et al. (2007)
	5′-TAGGAAAGCACCCTATTGAT-3		
gapA	5′-ATAGGAGGATCCAAAAGAGTCGCTATCAATGGTTTTGGACG-3′,	1007 bp	
	5′-GGAAATGGTACCTTACTTAGTTAGTTTAGCAAAGTATGTTAATG-3′		
vsp	5/-CTTGGATCAGTGGCTTCATTAGC-3/	400bp	Alberto et al. (2006)
	5/-GTCATCATGCGGAATTCTTGGGT-3/		
Fluoroquinolone resistance genes			
gyrA	5′-GACGAATCATCTAGCGAG-3′	531bp	Lysnyansky et al. (2009)_
	5′-GCCTTCTAGCATCAAAGTAGC-3′		
parC	5′-GAGCAACAGTTAAACGATTTG-3′	488bp	
	5′-GGCATAACAACTGGCTCTT-3′.		

including the variable surface lipoprotein gene (*vsp*), cytadhesin (*gap*A) and the *uvr*C, 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, uvr*C and *gap*A 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 for1 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 *uvr*C and *gap*A 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 *gap*A and at 52C for 30 sec for *uvr*C, and extension at 72°C for 2min with a final extension 72°C for 5 min.

2.4.5. Detection of quinolones resistance (QRDR) genes

Detection of genes (*gyr*A and *par*C) 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/nuccore/?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 digitonin-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 formed a loose pellet and 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% Na_2S) and tested for the formation of H_2S using lead acetate strips. The white strips changed to black when H_2S was added, indicating production of lead sulfide.

Table 2
Investigation of phenotypic antibacterial resistance, phenotypic virulence traits, virulence and Fluoroquinolone Resistance genes in *M. arginini* species isolated from camel pneumonic lungs.

Antibiotics										Phenotypic Virulence Traits					Virulence genes			Fluoroquinolone Resistance genes	
Ciprofloxacin (5 ug)	Doxacyclin (30 ug)	Lincomycin (2 ug)	Florafenicol (30 ug)	Spiromycin (100 ug)	Tylosin (30 ug)	Erythromycin (15 ug)	Streptomycin (10ug)		MAR_{index}	Hemolysis	Catalase	H_2S	Biofilm	vsp	parC	uvrC	gyrA	gqrA
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.051	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.050	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	+	+	0.048	-	-	-	-	-
S	S	I	I	I	I	R	S	MDR	0.625	+	-	+	0.049	-	-	-	-	-
R	R	S	S	S	S	R	S	XDR	0.375	+	-	+	0.050	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	+	+	0.051	-	-	-	-	-
R	S	R	S	S	I	R	S	MDR	0.625	+	-	+	0.064	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	+	+	0.069	-	-	-	-	-
R	R	S	S	S	S	R	S	XDR	0.375	+	-	+	0.063	-	-	-	-	-
I	R	R	S	I	I	R	S	XDR	0.25	+	+	+	0.066	-	-	-	-	-
R	R	R	S	S	I	R	S	MDR	0.75	+	-	+	0.078	-	-	-	-	-
R	R	S	S	S	S	S	S	XDR	0.375	+	-	+	0.095	-	-	-	-	-
R	R	R	S	S	S	R	S	-	0.125	+	-	+	0.046	-	-	-	-	-
R	R	S	S	S	S	S	S	MDR	0.5	+	+	+	0.060	-	-	-	-	-
R	R	S	S	S	S	R	S	-	0.125	+	-	+	0.047	-	-	-	-	-
R	R	R	S	S	S	S	S	MDR	0.5	+	+	+	0.050	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.083	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.069	-	-	-	-	-
I	S	S	S	S	S	R	S	-	0.125	+	+	+	0.061	-	-	-	-	-
R	S	S	S	I	I	R	S	XDR	0.5	+	-	+	0.049	-	-	-	-	-
S	R	I	S	I	S	S	S	MDR	0.625	+	+	+	0.097	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.081	-	-	-	-	-
R	R	S	S	S	S	S	S	-	0.125	+	+	+	0.066	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.079	-	-	-	-	-
S	R	R	S	S	S	S	S	MDR	0.5	+	-	+	0.058	-	-	-	-	-
I	R	R	S	S	S	R	S	-	0.125	+	-	+	0.066	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.057	-	-	-	-	-
R	S	S	S	S	S	S	S	-	0	+	-	+	0.060	-	-	-	-	-
R	S	R	S	S	S	R	S	MDR	0.5	+	-	+	0.053	-	-	-	-	-
S	R	R	S	I	S	S	S	-	0.125	+	+	+	0.068	-	-	-	-	-
R	R	S	S	S	S	R	S	-	0.5	+	-	+	0.059	-	-	-	-	-
I	S	S	S	I	I	S	S	XDR	0.375	+	+	+	0.067	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.055	-	-	-	-	-
R	R	R	S	S	S	S	S	MDR	0.5	+	-	+	0.060	-	-	-	-	-
R	S	S	S	S	S	S	S	-	0.125	+	-	+	0.095	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.100	-	-	-	-	-
S	S	I	S	I	S	R	S	MDR	0.5	+	-	+	0.046	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	+	+	0.073	-	-	-	-	-
R	S	R	S	S	S	R	S	MDR	0.375	+	-	+	0.057	-	-	-	-	-
R	R	S	S	S	S	R	S	XDR	0.375	+	-	+	0.051	-	-	-	-	-
S	R	R	S	S	I	S	S	MDR	0.5	+	-	+	0.044	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.625	+	-	+	0.055	-	-	-	-	-
R	S	S	S	S	S	S	S	-	0.125	+	-	+	0.065	-	-	-	-	-
R	R	R	S	S	S	R	S	MDR	0.5	+	-	+	0.057	-	-	-	-	-
I	R	S	S	S	S	R	S	XDR	0.375	+	-	+	0.071	-	-	-	-	-
R	S	R	S	I	S	R	S	MDR	0.5	+	-	+	0.062	-	-	-	-	-
R	R	I	S	I	S	R	S	MDR	0.625	+	-	+	0.055	-	-	-	-	-
S	R	R	S	S	I	R	S	MDR	0.5	+	-	+	0.045	-	-	-	-	-

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 two or fewer antimicrobial categories (non-susceptible to ≥1 agent in all but ≤2 categories).

3.5. Biofilm formation capability

M. arginini isolates ($n = 48$) were found to be weak in their ability to adhere to polystyrene multiwall plates. A very low spectrophotometric values (A_{560} (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 were13 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

Interestedly and unexpectable, the *vsp*, *uvr*C, *par*C virulence genes and the *gyr*A and *gap*A 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 (Krzysztoń-Russjan et al., 2016). Additionally, *M. arginini* is particularly incriminated to be involved in oncogenesis and infections in different human cell lines (e.g. fibroblast, embryonic kidney, breast cancer, colorectal carcinoma) and mouse fibroblasts (Zellaa 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; Gabinaitiene et al., 2011; Zhou and Li, 2014; Ericsson et al., 2016; Peek and Divers, 2017; Jambhekar et al., 2019; Maboni et al., 2019; Mead et al., 2019; Thomas 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 (Parker 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 H_2S, 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 H_2S production and hemolysis were correlated in *M. pneumonia*. This was the first time that correlation was recorded for *M. arginini* isolated from camels, where H_2S was not previously identified as a virulence factor in lung pathogens in camels. This study also concluded that H_2O_2 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 H_2O_2, 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 H_2S_2 ions. This data was also in accordance to the

Table 3
The antimicrobial resistance combinations of *Mycoplasma arginini* to various antibiotics.

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, Doxacyclin, Lincomycin, Erythromycin	4	4	21
Ciprofloxacin, Spiromycin, Tylosin, Erythromycin	4	2	1
Ciprofloxacin, Lincomycin, Spiromycin, Erythromycin	4	3	1
Doxacyclin, Lincomycin, Tylosin, Erythromycin	4	3	2
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

MK271639.1 *Mycoplasma arginini* strain WA 2 16S ribosomal RNA gene, partial sequence

AGCTAACGCATTAAATGATCCGCCTGAGTAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGA

CCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATCC

TTCGCAATGCTATAGAGATATAGCGGAGGTTAACGGAGTGACAGATGGTGCATGGTTGTCGTCAGCTCGT

GTCGTGAGATGTTTGGTCAAGTCCTGCAACGAGCGCAACCCCTATCTTTAGTTACTAACGAGTCATGTCG

AGGATCCTAGAGATAC

>MK271638.1 *47*strain WA 1 16S ribosomal RNA gene, partial sequence

AGCTAACGCATTAAATGATCCGCCTGAGTAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGA

CCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATCC

TTCGCAATGCTATAGAGATATAGCGGAGGTTAACGGAGTGACAGATGGTGCATGGTTGTCGTCAGCTCGT

GTCGTGAGATGTTTGGTCAAGTCCTGCAACGAGCGCAACCCCTATCTTTAGTTACTAACGAGTCATGTCG

AGGATTCTAGAGATAC

MK271640.1 *Mycoplasma arginini* strain WA 4 16S ribosomal RNA gene, partial sequence

CAGCTAACGCATTAAATGATCCGCCTGAGTAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGG

ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATC

CTTCGCAATGCTATAGAGATATAGCGGAGGTTAACGGAGTGACAGATGGTGCATGGTTGTCGTCAGCTCG

TGTCGTGAGATGTTTGGTCAAGTCCTGCAACGAGCGCAACCCCTATCTTTAGTTACTAACGAGTCATGTC

GAGGATTCTAGAGATAC

MK271641.1 *47u=*strain WA 5 16S ribosomal RNA gene, partial sequence

GCAGCTAACGCATTAAATGATCCGCCTGAGTAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGG

GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACAT

CCTTCGCAATGCTATAGAGATATAGCGGAGGTTAACGGAGTGACAGATGGTGCATGGTTGTCGTCAGCTC

GTGTCGTGAGATGTTTGGTCAAGTCCTGCAACGAGCGCAACCCCTATCTTTAGTTACTAACGAGTCATGT

CGAGGATTCTAGAGATAC

Fig. 1. DNA sequence of *M. arginini* isolates.

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$_{index}$ 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 the16S rDNA PCR/DGGE and16S-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 pneumonic 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. bovigenitalium, 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

```
              10      20      30      40      50
           ....|....|....|....|....|....|....|....|....|
>MK271641 Mycoplasm  1  GCAGCTAACGCATTAAATG—ATCCGCCTGAGTAGTATGCTCG-CAAGAG
>MK271638 Mycoplasm  1  —................—......................-......
>MK271639 Mycoplasm  1  —................—......................-......
>MK271640 Mycoplasm  1  -................—......................-......
>KP972459.1 Mycoplas 1.................—......................-......
>KP972458.1 Mycoplas 1.................—......................-......
>MF101758.1 Mycoplas 1.................--................C.T...-.....A
>KU746836.1 Mycoplas 1.................—......................-......
>MH685445.1 Mycoplas 1AGC.A.GG.TA.C..TG..CC.G.A...GCG...A..CATAG.T.GCA..
>MG564233.1 Mycoplas 1.................—......................-......
>MG564232.1 Mycoplas 1.................—......................-......
>MG564231.1 Mycoplas 1.................—......................-......
>MG564230.1 Mycoplas 1.................—......................-......
>JQ859817.1 Mycoplas 1AGC.A.GG.TA.C..TG..CC.G.A...GCG...A..CATAG.T.GCA..
>JQ820403.1 Mycoplas 1...T.C.............--...........A..A........-...A..
>JN543264.1 Mycoplas 1.................—......................-......
>HM635904.1 Mycoplas 1.................—......................-......
>GQ409971.1 Mycoplas 1.................--......................-......
>NR_041743.1 Mycopla 1.................--......................-......
>HQ661827.1 Mycoplas 1.................--......................-......
>HQ661828.1 Mycoplas 1.................--......................-......
>JN935883.1 Mycoplas 1.................—......................-......
>JQ903578.1 Mycoplas 1.................--......................-......
>JQ903580.1 Mycoplas 1.................--......................-......


              60      70      80      90     100
           ....|....|....|....|....|....|....|....|....|
>MK271641 Mycoplasm 48 TGAAACTTAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGG
>MK271638 Mycoplasm 46 ..................................................
>MK271639 Mycoplasm 46 ..................................................
>MK271640 Mycoplasm 47 ..................................................
>KP972459.1 Mycoplas 48..................................................
>KP972458.1 Mycoplas 48..................................................
>MF101758.1 Mycoplas 48.A..........................C.......C...........
```

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.CC.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 Mycopla  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  TTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATCCTTCG-
>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*)

>NR_041743.1 Mycopla 98..................................-

>HQ661827.1 Mycoplas 98..................................-

>HQ661828.1 Mycoplas 98..................................-

>JN935883.1 Mycoplas 98..................................-

>JQ903578.1 Mycoplas 98..................................-

>JQ903580.1 Mycoplas 98..................................-


```
      160    170    180    190    200
      ....|....|....|....|....|....|....|....|....|
```

>MK271641 Mycoplasm 146 --CAATGCTATAGAGATATA-GCGGAGGTTAACGGAGTGACAGATGGTGC

>MK271638 Mycoplasm 144 --..................-.............................

>MK271639 Mycoplasm 144 --..................-.............................

>MK271640 Mycoplasm 145 --..................-.............................

>KP972459.1 Mycoplas 146--..................-.............................

>KP972458.1 Mycoplas 146--..................-.............................

>MF101758.1 Mycoplas 146--...A..............-.T..........AC..G.---------..

>KU746836.1 Mycoplas 146--...A..............-.TC.........................

>MH685445.1 Mycoplas 151ACT.GA.T..G.T...GG..A.....--..G.ATGAG.C----------

>MG564233.1 Mycoplas 146--.............------------------------------

>MG564232.1 Mycoplas 144---

>MG564231.1 Mycoplas 146--...........-------------------------------

>MG564230.1 Mycoplas 146--..................-..............-------------

>JQ859817.1 Mycoplas 151ACT.GA.T..G.T...GG..A.....AT.CC.T.TGAA.CGGTGAAA...

>JQ820403.1 Mycoplas 146--..................-..A.........................

>JN543264.1 Mycoplas 146--..................-.............................

>HM635904.1 Mycoplas 146--..................-.............................

>GQ409971.1 Mycoplas 146--..................-.............................

>NR_041743.1 Mycopla 146--..................-.............................

>HQ661827.1 Mycoplas 146--..................-.............................

>HQ661828.1 Mycoplas 146--..................-.............................

>JN935883.1 Mycoplas 146--..................-.............................

>JQ903578.1 Mycoplas 146--..................-.............................

>JQ903580.1 Mycoplas 146--..................-.............................


```
      210    220    230    240    250
      ....|....|....|....|....|....|....|....|....|
```

>MK271641 Mycoplasm 194 ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTTGGTCAAGTCCTGCAACG

>MK271638 Mycoplasm 192 ....

Fig. 2. (*continued*)

>MK271639 Mycoplasm 192 ....

>MK271640 Mycoplasm 193 ....

>KP972459.1 Mycoplas 194...

>KP972458.1 Mycoplas 194...

>MF101758.1 Mycoplas 185C..A.C.G..C.CCA--------------------------------

>KU746836.1 Mycoplas 194...

>MH685445.1 Mycoplas 187--

>MG564233.1 Mycoplas 160--

>MG564232.1 Mycoplas 144--

>MG564231.1 Mycoplas 158--

>MG564230.1 Mycoplas 179--

>JQ859817.1 Mycoplas 201G.A.A.A.ATGG.AGAACACCAAA.GCGAAGGCA.CTT.C.GGGT.T.TA

>JQ820403.1 Mycoplas 194.............T...A........T.....................C

>JN543264.1 Mycoplas 194...

>HM635904.1 Mycoplas 194...

>GQ409971.1 Mycoplas 194...

>NR_041743.1 Mycopla 194...

>HQ661827.1 Mycoplas 194...

>HQ661828.1 Mycoplas 194...

>JN935883.1 Mycoplas 194...

>JQ903578.1 Mycoplas 194...

>JQ903580.1 Mycoplas 194...

         260   270   280   290   300

           ....|....|....|....|....|....|....|....|....|....|

>MK271641 Mycoplasm 244 AGCGCAACCCCTATCTTTAGTTACTAACGAGTCATGTCGAGGATTCTAGA

>MK271638 Mycoplasm 242 ....

>MK271639 Mycoplasm 242 ....C.....

>MK271640 Mycoplasm 243 ....

>KP972459.1 Mycoplas 244...C......

>KP972458.1 Mycoplas 244...C......

>MF101758.1 Mycoplas 199---

>KU746836.1 Mycoplas 244...C......

>MH685445.1 Mycoplas 187--

>MG564233.1 Mycoplas 160--

>MG564232.1 Mycoplas 144--

>MG564231.1 Mycoplas 158--

>MG564230.1 Mycoplas 179--

Fig. 2. (*continued*)

>JQ859817.1 Mycoplas 251CTGA.GCTGAGGGA.GAA..CGTGGGGA.CAAACA.GAT----------

>JQ820403.1 Mycoplas 244.C...C...........C.T......................C......

>JN543264.1 Mycoplas 244...C......

>HM635904.1 Mycoplas 244...C......

>GQ409971.1 Mycoplas 244...C......

>NR_041743.1 Mycopla 244...C......

>HQ661827.1 Mycoplas 244...C......

>HQ661828.1 Mycoplas 244...C......

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

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

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

                      ....|

>MK271641 Mycoplasm 294 GATAC

>MK271638 Mycoplasm 292 .....

>MK271639 Mycoplasm 292 .....

>MK271640 Mycoplasm 293 .....

>KP972459.1 Mycoplas 294.....

>KP972458.1 Mycoplas 294.....

>MF101758.1 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.....

>HM635904.1 Mycoplas 294.....

>GQ409971.1 Mycoplas 294.....

>NR_041743.1 Mycopla 294.....

>HQ661827.1 Mycoplas 294.....

>HQ661828.1 Mycoplas 294.....

>JN935883.1 Mycoplas 294.....

>JQ903578.1 Mycoplas 294.....

>JQ903580.1 Mycoplas 294.....

Fig. 2. (*continued*)

Seq->	>MK271641 WA 5.Egypt	>MK271638 WA 1.Egypt	>MK271639 WA 2.Egypt	>MK271640 WA 4.Egypt	>KP972459.1 Dak-2/M.arg/EG014.Egypt	>KP972458.1 Dak-1/M.arg/EG014.Egypt	>MF101758.1 Egy2.Egypt	>KU746836.1 DUK-EG013.Egypt	>MH685445.1 cu1.Egypt	>MG564233.1 SHEB-HS.Egypt	>MG564232.1 KETAW-HS.Egypt	>MG564231.1 SIDIKRIR-HS.Egypt	>MG564230.1 AGAMI-HS.Egypt	>JQ859817.1 Egy-Cam1.Egypt	>JQ820403.1 Egy-K-She-12.Egypt	>JN543264.1 C2-Ass-11.Egypt	>HM635904.1 C1-Beh-10.Egypt	>GQ409971.1 284F08.England	>NR_041743.1 G230.Sweden	>HQ661827.1 E2.5.South Africa	>HQ661828.1 4X.South Africa	>JN935883.1 ATCC 23243.USA	>JQ903578.1 CBER2012BHK clone 1.USA	>JQ903580.1 CBER2012BHK clone 3.USA
>MK271641 Mycoplasma arginini strain WA 5.Egypt	ID	99%	99%	100%	100%	100%	58%	98%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	100%	100%	100%	100%
>MK271638 Mycoplasma arginini strain WA 1.Egypt	99%	ID	100%	100%	99%	99%	58%	98%	24%	53%	48%	52%	59%	31%	93%	99%	99%	99%	99%	99%	99%	99%	99%	99%
>MK271639 Mycoplasma arginini strain WA 2.Egypt	99%	100%	ID	99%	99%	99%	58%	97%	24%	53%	48%	52%	59%	31%	93%	99%	99%	99%	99%	99%	99%	99%	99%	99%
>MK271640 Mycoplasma arginini strain WA 4.Egypt	100%	100%	99%	ID	99%	99%	58%	98%	24%	53%	48%	53%	60%	31%	94%	99%	99%	99%	99%	99%	99%	99%	99%	99%
>KP972459.1 Mycoplasma arginini strain Dak-2/M.arg/EG014 .Egypt	100%	99%	99%	99%	ID	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	100%	100%	100%	100%
>KP972458.1 Mycoplasma arginini strain Dak-1/M.arg/EG014 .Egypt	100%	99%	99%	99%	100%	ID	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	100%	100%	100%	100%
>MF101758.1 Mycoplasma arginini isolate Egy2 .Egypt	58%	58%	58%	58%	58%	58%	ID	59%	39%	74%	67%	73%	82%	27%	56%	58%	58%	58%	58%	58%	58%	58%	58%	58%
>KU746836.1 Mycoplasma arginini strain DUK-EG013.Egypt	98%	98%	97%	98%	99%	99%	59%	ID	24%	54%	48%	53%	59%	31%	93%	99%	99%	99%	99%	99%	99%	99%	99%	99%
>MH685445.1 Mycoplasma arginini strain cu1 .Egypt	24%	24%	24%	24%	24%	24%	39%	24%	ID	34%	29%	33%	38%	62%	24%	24%	24%	24%	24%	24%	24%	24%	24%	24%
>MG564233.1 Mycoplasma arginini isolate SHEB-HS .Egypt	54%	53%	53%	53%	54%	54%	74%	54%	34%	ID	90%	99%	89%	22%	51%	54%	54%	54%	54%	54%	54%	54%	54%	54%
>MG564232.1 Mycoplasma arginini isolate KETAW-HS .Egypt	48%	48%	48%	48%	48%	48%	67%	48%	29%	90%	ID	91%	80%	19%	46%	48%	48%	48%	48%	48%	48%	48%	48%	48%
>MG564231.1 Mycoplasma arginini isolate SIDIKRIR-HS .Egypt	53%	52%	52%	53%	53%	53%	73%	53%	33%	99%	91%	ID	88%	21%	50%	53%	53%	53%	53%	53%	53%	53%	53%	53%
>MG564230.1 Mycoplasma arginini isolate AGAMI-HS.Egypt	60%	59%	59%	60%	60%	60%	82%	59%	38%	89%	80%	88%	ID	25%	57%	60%	60%	60%	60%	60%	60%	60%	60%	60%
>JQ859817.1 Mycoplasma arginini strain Egy-Cam1 .Egypt	31%	31%	31%	31%	31%	31%	27%	31%	62%	22%	19%	21%	25%	ID	30%	31%	31%	31%	31%	31%	31%	31%	31%	31%
>JQ820403.1 Mycoplasma arginini strain Egy-K-She-12 .Egypt	94%	93%	93%	94%	94%	94%	56%	93%	24%	51%	46%	50%	57%	30%	ID	94%	94%	94%	94%	94%	94%	94%	94%	94%
>JN543264.1 Mycoplasma arginini strain C2-Ass-11 .Egypt	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	ID	100%	100%	100%	100%	100%	100%	100%	100%
>HM635904.1 Mycoplasma arginini strain C1-Beh-10 .Egypt	100%	98%	99%	100%	100%	100%	58%	99%	24%	53%	48%	53%	60%	31%	94%	100%	ID	100%	100%	100%	100%	100%	100%	100%
>GQ409971.1 Mycoplasma arginini strain 284F08.England	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	ID	100%	100%	100%	100%	100%	100%
>NR_041743.1 Mycoplasma arginini strain G230.Sweden	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	ID	100%	100%	100%	100%	100%
>HQ661827.1 Mycoplasma arginini strain E2.5.South Africa	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	ID	100%	100%	100%	100%
>HQ661828.1 Mycoplasma arginini strain 4X.South Africa	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	ID	100%	100%	100%
>JN935883.1 Mycoplasma arginini strain ATCC 23243.USA	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	100%	ID	100%	100%
>JQ903578.1 Mycoplasma arginini strain CBER2012BHK clone 1.US!	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	100%	100%	ID	100%
>JQ903580.1 Mycoplasma arginini strain CBER2012BHK clone 3.US!	100%	99%	99%	99%	100%	100%	58%	99%	24%	54%	48%	53%	60%	31%	94%	100%	100%	100%	100%	100%	100%	100%	100%	ID

Fig. 3. Identity % of 16s rRNA gene of *M. arginini* isolates.
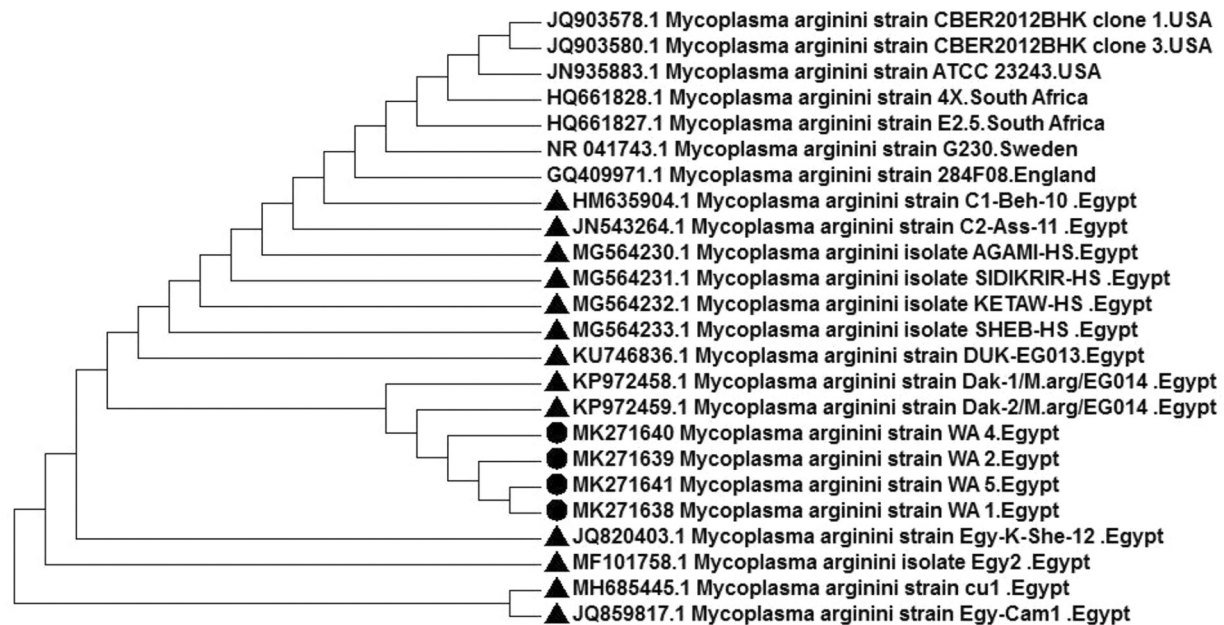
▲Egypt. Previous isolates

●Our isolated strains

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

promote host colonization (Jeqchlinger et al., 2004). The *uvr*C 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 *uvr*C gene failed to be evident in addition to the other two virulence genes, *vsp* and *gap*A. 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 *gyr*A and *par*C 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 H_2S and investigating other potential virulence factors found in this organism, more can be understood about *M. arginini*. Ultimately, the goal of this research and future research is to fully understand pathogens in possible hosts; this type of information can lead to novel, more effective treatment, control, and preventive measures and improve vaccine development.

CRediT authorship contribution statement

Walaa Mohammed Abdelazeem: Data curation, Investigation. **Tara Rava Zolnikov:** Writing - review & editing. **Zeinab Roshdy Mohammed:** Supervision. **Alaa Saad:** Data curation, Investigation. **Kamelia M Osman:** Supervision, Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest

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