Phenotypic and genotypic antimicrobial susceptibility patterns of the emerging human respiratory pathogen *Mycoplasma amphoriforme* isolated from the United Kingdom and Denmark

@mike_beeton

Day, J.,[1] Afshar, B.,[1] Rowlands R. S.,[2] Umer, T. S.,[2] Windsor H.,[3] Paukner S.,[4] Jensen., J.S.,[5] Spiller O.B.,[1,6] Chalker, V. J,[1] and Beeton M. L.[1,2]

[1.]United Kingdom Health Security Agency, Colindale, London, UK. [2.] Microbiology and Infection Research Group, Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, UK. [3.]Mycoplasma Experience, Brewer Street Dairy Business Park, Bletchingley, Surrey, UK. [4.] Nabriva Therapeutics GmbH, 1110 Vienna, Austria, [5] Research Unit for Reproductive Microbiology, Statens Serum Institut, Copenhagen, DK-2300, Denmark, [6] Cardiff University, Division of Infection and Immunity, Department of Medical Microbiology, University Hospital of Wales, Cardiff, UK.

Background

- *Mycoplasma amphoriforme* was first isolated in 1999 from a common variable immune deficiency patient with recurrent RTI of unknown cause.(1)
- Studies have identified *M. amphoriforme* among respiratory samples from 20% of immune deficient patients and 5-6% of immunocompetent patients.(2,3)
- Similar to other mycoplasmas, *M. amphoriforme* is intrinsically resistant to many antibiotics.
- Very little is known regarding acquired resistance among these bacteria.

Aims:

1. Determine the antimicrobial susceptibility of *M. amphoriforme* isolated in the UK and Denmark.
2. Characterise the molecular mechanisms of resistance.

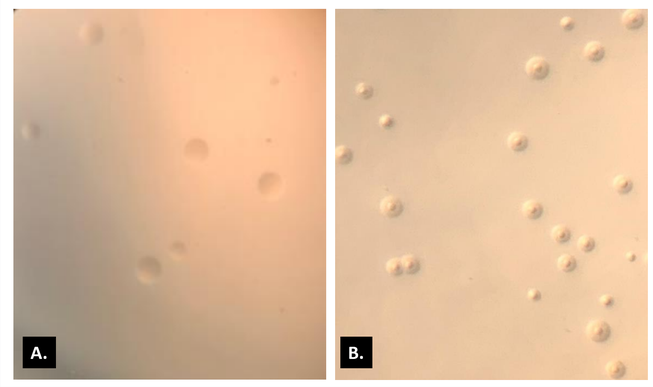

Figure 1. Comparison between *M. amphoriforme* and *M. pneumoniae* colonies. *M. amphoriforme* A39 grown on Mycoplasma agar (Mycoplasma Experience, UK) with 'ground glass' appearance (A.) in comparison with *M. pneumoniae* which exhibit classic 'fried egg' colony morphology (B.). Agar plates observed under x40 magnification.

Methods

Seven isolates of *M. amphoriforme* from the UK (6) and Denmark (1) were examined for antimicrobial susceptibility to seven antibiotics using the microbroth dilution assay in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines for mycoplasmas. Due to the lack of interpretive criteria for minimum inhibitory concentration (MIC) values for *M. amphoriforme*, resistance was determined by the presence of an elevated MIC value ≥ 8-fold increase relative to base-line values. Each isolate was additionally subjected to Illumina whole-genome sequencing to identify mutations associated with resistance to macrolide and fluoroquinolones. Based on the consensus sequences from the genomic data, PCR primers were designed, and tested, for the amplification of the quinolone resistance determining region within the *parC* gene.

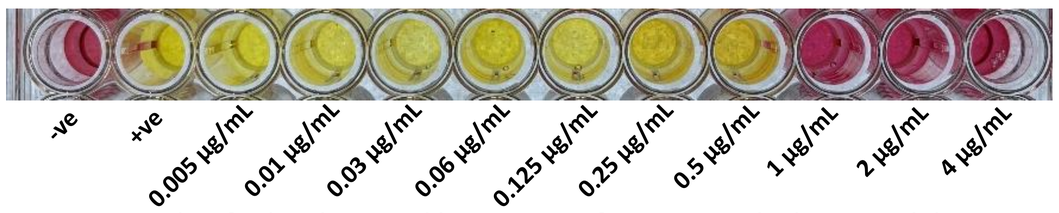

Figure 2. Example of a broth microdilution assay for antimicrobial susceptibility testing of *M. amphoriforme*. Growth is indicated by the presence of a colour change in the media from red to yellow as a result of a drop in pH following the formation of acid from glucose.

Results

	Culture collection number	Genome prediction		Minimum inhibitory concentration (µg/mL)						
		Macrolide	Fluoroquinolone	Azithromycin	Erythromycin	Tetracycline	Moxifloxacin	Levofloxacin	Clindamycin	Lefamulin
M. amphoriforme										
A39	NCTC 11740	Susceptible	Resistant	0.03	0.06	0.06	0.5	2	0.5	0.001
A55	NCTC submitted	Susceptible	Resistant	0.001	0.125	0.06	0.5	2	0.5	0.001
A70	NCTC submitted	Susceptible	Susceptible	0.001	0.06	0.06	0.03	0.06	0.25	0.001
A84	NCTC submitted	Susceptible	Resistant	0.0005	0.06	0.06	1	1	0.125	0.001
H04	NCTC 14399	Susceptible	Susceptible	0.001	0.125	0.06	0.06	0.06	0.25	0.001
H29	NCTC 14400	Resistant	Resistant	64	128	0.06	0.5	2	64	0.004
M5572	NCTC submitted	Susceptible	Susceptible	0.001	0.125	0.06	0.03	0.06	0.25	0.001
M. pneumoniae										
M129	ATCC 29342	Susceptible	Susceptible	0.001	0.03	0.25	0.06	0.5	0.5	0.001

Table1. Genotypic prediction of antibiotic susceptibility and accompanying phenotypic minimum inhibitory concentration values for *Mycoplasma amphoriforme* isolates. Isolates which were deemed resistant are indicated by low-lighting in grey.

Isolate	Partial ParC sequence
M. amphoriforme A39	^{84}HPHGD**F**SIYEAL^{95}
M. amphoriforme A55	^{84}HPHGD**F**SIYEAL^{95}
M. amphoriforme A70	^{84}HPHGDSSIYEAL^{95}
M. amphoriforme A84	^{84}HPHGD**F**SIYEAL^{95}
M. amphoriforme H04	^{84}HPHGDSSIYEAL^{95}
M. amphoriforme H29	^{84}HPHGD**F**SIYEAL^{95}
M. amphoriforme M5572	^{84}HPHGDSSIYEAL^{95}

Figure 3. Partial amino acid alignments of the quinolone-resistance determining region of the ParC protein from seven *M. amphoriforme* isolates. Amino acids in bold and underlined are predicted to account for fluoroquinolone resistance phenotype in isolates A39, A55, A84 and H29.

	23S rRNA nucleotide position																	
	218	277	650	1231	1570	1602	1607	1610	1869	1885	2046	2059	2185	2201	2224	2721	2855	2869
A39	G	T	A	A	C	C	A	A	C	A	T	A	C	C	G	C	C	A
A55	G	T	A	A	C	C	A	A	C	A	T	A	C	C	G	C	C	A
A70	G	T	A	A	C	C	A	A	C	A	T	A	C	C	G	T	C	A
A84	G	T	A	A	C	C	A	A	C	A	T	A	C	C	A	T	C	A
H04	A	T	A	A	C	T	A	A	C	A	T	A	C	C	G	T	C	A
H29	G	T	A	A	C	C	A	A	C	A	T	**G**	C	T	A	T	C	A
M5572	G	C	G	G	T	C	G	G	T	G	C	A	T	C	G	T	T	G

Table 2. Single nucleotide polymorphisms within the 23S rRNA gene of seven *M. amphoriforme* isolates. Differences between isolates are indicated by low-lighting in grey. The nucleotide in bold and underlined is predicted to account for macrolide resistance phenotype in isolate H29 corresponding to 2058 in *Escherichia coli* numbering.

Key findings

- 57% of isolates were resistant to at least one antibiotic.
- Isolate H29 was resistant to five of the seven antibiotics examined.
- An S89F substitution within ParC protein was associated with fluoroquinolone resistance.
- An A2059G mutation within the 23S rRNA was associated with macrolide resistance.
- Antimicrobial resistance among *M. amphoriforme* isolates is an area of concern.

Future direction

- Continue to examine clinical samples from high-risk patients for the presence of *M. amphoriforme.*
- Increase the number of available isolates for phenotypic monitoring of resistance.
- Develop guidelines for MIC interpretive criteria for *M. amphoriforme*.

(1)Webster *et al.*, 2003. Chronic bronchitis in immunocompromised patients: Association with a novel *Mycoplasma* species. *Eur J Clin Microbiol Infect Dis.* (2)Ling *et al.,* 2014. Tools for detection of *Mycoplasma amphoriforme*: A primary respiratory pathogen? *J. Clin. Microbiol.* (3)Rehman *et al*., 2021. Molecular exploration for *Mycoplasma amphoriforme*, *Mycoplasma fermentans* and *Ureaplasma* spp. in patient samples previously investigated for *Mycoplasma pneumoniae* infection. *Clin. Microbiol. Infect.*