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Phylogenetic variants of *Mycoplasma hominis* from pregnant women and women presenting with infertility in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

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Abstract:

Background: Much controversies have been associated with the pathogenicity of *Mycoplasma hominis* but little has been done to unravel the mystery behind the different views. This study aimed at investigating the genetic variants abounding within *M. hominis* and the distribution of the virulent genes among the variants.

Methodology: Twenty (20) *M. hominis* isolates from high vaginal swabs of women (11 from pregnant women and 9 from women presenting with infertility) attending the Obstetrics and Gynaecology clinics of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria, were sequenced using 16S rRNA universal gene target for the purpose of phylogenetic analysis and epidemiological typing. The isolates were also screened for the presence of *M. hominis* variable adherence antigen (*vaa*) and p120 virulent genes using primer constructs from the respective genes in a conventional PCR protocol.

Results: Of the 20 *M. hominis* vaginal isolates, 4 phylogenetic strains were detected; strain MHS43 constituted 10/20 (50.0%) [2/9 (22.2%) from infertile women and 8/11 (72.7%) from pregnant women]; strain MHBS constituted 3/20 (15%) [3/9 (33.3%) from infertile women and 0/11 (0%) from pregnant women]; strain MHSWP2 constituted 4/20 (20.0%) [3/9 (33.3%) from infertile women and 1/11 (9.1%) from pregnant women]; while strain MHKC87 constituted 3/20 (15%) [1/9 (11.1%) from infertile women and 2/11 (18.2%) from pregnant women]. Each of *vaa* and p120 genes was detected in 14 of 20 isolates, while 6 isolates did not carry the genes. A 2-way ANOVA test showed that none of the genes was significantly associated with a particular strain ($p=0.8641$).

Conclusions: The different views regarding the pathogenicity of *M. hominis* may be linked to the heterogeneity within the species and lack of homogeneity in the virulent genes as witnessed both in the intra species and intra strain levels.

Keywords: *Mycoplasma hominis*, pathogenicity, virulence, strains, heterogeneity

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Variantes phylogénétiques de *Mycoplasma hominis* chez les femmes enceintes et les femmes présentant une infertilité au Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

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Résumé:

Contexte: De nombreuses controverses ont été associées à la pathogénicité de *Mycoplasma hominis*, mais peu a été fait pour percer le mystère derrière les différents points de vue. Cette étude visait à étudier les variantes génétiques abondantes au sein de *M. hominis* et la distribution des gènes virulents parmi les variantes.

Méthodologie: Vingt (20) isolats de *M. hominis* provenant d'écouvillonnages vaginaux élevés de femmes (11 de femmes enceintes et 9 de femmes présentant une infertilité) fréquentant les cliniques d'Obstétrique et de Gynécologie du Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria, ont été séquencé à l'aide du gène cible universel de l'ARNr 16S à des fins d'analyse phylogénétique et de typage épidémiologique. Les isolats ont également été criblés pour la présence de l'antigène d'adhérence variable de *M. hominis* (*vaa*) et des gènes virulents p120 en utilisant des constructions d'amorces à partir des gènes respectifs dans un protocole de PCR conventionnel.

Résultats: Sur les 20 isolats vaginaux de *M. hominis*, 4 souches phylogénétiques ont été détectées; la souche MHS43 constituait 10/20 (50,0%) [2/9 (22,2%) de femmes infertiles et 8/11 (72,7%) de femmes enceintes]; la souche MHBS constituait 3/20 (15,0%) [3/9 (33,3%) de femmes infertiles et 0/11 (0%) de femmes enceintes]; la souche MHSWP2 constituait 4/20 (20,0%) [3/9 (33,3%) de femmes infertiles et 1/11 (9,1%) de femmes enceintes]; tandis que la souche MHKC87 constituait 3/20 (15,0%) [1/9 (11,1%) de femmes infertiles et 2/11 (18,2%) de femmes enceintes]. Chacun des gènes *vaa* et p120 a été détecté dans 14 des 20 isolats, tandis que 6 isolats ne portaient pas les gènes. Un test ANOVA à 2 voies a montré qu'aucun des gènes n'était significativement associé à une souche particulière ($p=0,8641$).

Conclusions: Les différents points de vue concernant la pathogénicité de *M. hominis* peuvent être liés à l'hétérogénéité au sein de l'espèce et au manque d'homogénéité des gènes virulents, comme en témoignent les niveaux intra-espèce et intra-souche.

Mots clés: *Mycoplasma hominis*, pathogénicité, virulence, souches, hétérogénéité

Introduction:

Mycoplasma hominis is an opportunist human pathogen capable of colonizing epithelial cells and causing urogenital and extragenital pathologies (1,2). At present, it remains unresolved which factors lead to virulence; however, as a first step of invasion, adherence to the epithelium must occur. As the cell wall is absent, this is most likely mediated by structures in the mycoplasma cell membrane. A characteristic feature of the mycoplasmas is the presence of variable surface proteins which may play an important role in the adaptation of the cell-wall less organisms to their host environments (3,4).

The variable adherence antigen (*vaa*) has been shown to be a major adhesin of *M. hominis* and displays a prominent mutational variation in size as well as sequence and antigenic variations (5,6). Variability of *vaa* cytoadhesin has been postulated to be responsible for *Mycoplasma* persistence in various individuals. These play important roles in the pathogenesis of *M. hominis* infections by providing altered structures for escape from immune responses and protein structures that enhance cell and tissue colonization and penetration of the mucosal barrier (7).

Much controversies have been associated with the virulence of *M. hominis* but less has been done to unravel the mystery behind the differences in opinions. *Mycoplasma hominis* have long resisted detailed analysis due to high levels of heterogeneity within the specie (3,8) and although some studies have been conducted in Nigeria to isolate these pathogens, the isolates have not been molecularly typed (1,9,10). The aim of this study is to determine the variability within *M. hominis* isolates from pregnant women and women presenting with infertility using primers targeted at the universal 16SrRNA, *vaa* and p120 genes, in order to detect genetic polymorphisms within the species.

Materials and method:

Study design, setting and participants:

The design was quantitative cross-sectional observational study of pregnant women and women with infertility attending the Obstetrics and Gynaecology clinics of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, southeast Nigeria from January 2021 to August 2022.

Ethical approval and consent to participate:

This study was performed in line with

the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi with approval number NAUTH/CS/66/VOL/9/31/2017/026. Informed consent was obtained from all individual participants included in the study.

Isolation *M. hominis* from participants

Mycoplasma hominis were isolated as described in previous studies (9,10), from high vaginal swab samples collected by a consultant gynaecologist using a plastic sterile disposable speculum and a commercial sterile cotton tipped swabs. All samples were collected before initiation of any antimicrobial therapy. Mycoplasma agar base (CM0401), Mycoplasma broth base (CM 0403), and Mycoplasma supplement G (SR 0059) from Oxoid Ltd, UK, were reconstituted and used according to the manufacturer's instruction.

All inoculated broths were incubated aerobically for up to 7 days and examined periodically for color change. Bottles showing color change were sub-cultured onto agar plates (55 mm) prepared with Mycoplasma agar base CM0401, Mycoplasma supplement-G (SR0059) and L-arginine, for further examination. Plates were incubated in moist chambers aerobically, anaerobically and in 10% CO₂ atmosphere. The agar surfaces were examined for characteristic colonial appearances after 3 to 7 days' incubation with a dissecting microscope at 60x magnification using obliquely transmitted light. A characteristic colony with the center of the colony embedded beneath the surface, giving a 'fried-egg' appearance indicated presumptive isolation of *M. hominis*.

Purification of the suspected *M. hominis* isolates was achieved by subculturing a single colony three times to achieve a pure colony before subjecting the isolates to molecular identification. This was carried out by removing a plug of agar containing a colony from the plate and using it to inoculate further plates of medium and finally-sub cultured into Mycoplasma broth medium and stored at -80°C until needed for molecular identification.

Extraction of DNA from *M. hominis* isolates:

The molecular analysis of the isolates was carried out at Iykenson Medical and Diagnostics Co. Ltd; Road 2 Mofor Estate Unizik Temporary site, Awka, and Inqaba Biotec West Africa Ltd PMB 5320, Oyo Road, Ibadan 200001 Oyo State, Nigeria. Pure isolates of *M. hominis* in the Mycoplasma broth kept frozen at -80°C were recovered by thawing. The isolates were heat-inactivated by incubating at 95°C for 30

minutes. Total genomic DNA was extracted from the bacterial colonies using the Quick DNATM Miniprep Plus Kit (Catalog nos. D4068 & D4069) which was set up according to manufacturer's instructions.

To release the bound DNA on the silica membrane spin column, 50 µl of DNA Elution Buffer (10 mM Tris, pH 8.5, 0.1 mM EDTA) were added to the extracted DNA, incubated for 5 minutes, and then centrifuged for 1 min at full speed and the DNA extracts were collected into a clean microcentrifuge tube. The DNA concentration was determined by measuring the absorbance of the sample at 260 nm.

PCR amplification of *M. hominis* isolates:

Conventional polymerase chain reaction was performed on the DNA extract of the *M. hominis* isolates using primers as listed in Table 1 and in accordance with previous studies (11,12,13,14). All primers were synthesized by Inqaba Biotec West Africa Ltd. PCR amplifications were performed in a thermal cycler (Tecne Prime; Stone, UK), using 50µl reaction mix containing Quick load One Taq One Step PCR Master Mix (2x) 25µl, forward primer (10 µM) 5µl, reverse primer (10µM) 5µl, template DNA 1-10µl and making up to 50µl with nuclease free water. PCR reactions consisted of an initial denaturation step of 3 minutes at 94°C, followed by 35 cycles of 60 seconds at 94°C. A final extension step was maintained for 10 minutes at 72°C. Water blank was included in every PCR run.

Agarose gel electrophoresis of PCR products:

Ten microliters of the PCR products were analyzed on 1% agarose gel electrophoresis, stained with ethidium bromide for visualization. Ten microliters of ready to use DNA ladder (100-1500bp) mixed with loading dye was loaded in the first well of every run. It was set at 120volts for 20 mins for genomic DNA and was viewed under gel documentation system with UV transilluminator.

PCR amplification of *M. hominis* isolates with bacterial universal 16SrRNA gene target:

The 16SrRNA gene target-typing of *M. hominis* included a nested step, where the PCR product from first amplification round was used as the template DNA in the second round. PCR amplifications were performed as above on the DNA extracts using 16SrRNA gene target universal to all bacteria. Agarose gel electrophoresis and visualization were also performed as above. Bacterial amplicons of approximately 750bp in size were expected

Table 1: Forward and Reverse Oligonucleotide Primers Used

Sequence target	Used for	Primer information	Reference
16SrRNA Universal bacterial gene	Sequencing	F-5'-GTGCCAGCAGCCGCGTAA-3' Barcode: S3B 1E; Length: 19 bases, 886bp. For 100uM stock solution, add 285.97ul buffer R-5'-AGACCCGGGAACGTATTCAC -3' Barcode: S3B 1F; Length: 20 bases, 886bp. For 100uM stock solution, add 490.85ul buffer	11
Open-reading frame encoded by the <i>vaa</i> gene	Amplification of <i>vaa</i> gene	F-5'-CCCCGGAGATTATTAAGTCTC-3' R-5'-GTGCCATTAGTAGCACTATTTTTTG-3'	12
Hypervariable domain of p120 gene	Amplification of p120 gene	F-5'-GCTATTGTTGAAATTCC-3' R-5'-CTTTGATTGTTGAGTAGG-3'	13
16SrRNA gene of <i>Mycoplasma hominis</i>	Amplification of 16SrRNA gene	F-5'-CAATGGCTAATGCCGGATACGC-3' R-5'-GGTACCGTCAGTCTGCAAT-3'	14

PCR products cleaning

The PCR products were cleaned using Exo-SAP IT kit. The Exo-SAP IT Master mix was prepared by adding 50.0 µl Exonuclease I (NEB M0293) 20 U/ul and 200.0 µl Shrimp Alkaline Phosphatase (NEB M0371) 1U/ul in a 0.6 ml microcentrifuge tube. Exo-SAP IT Mix of 2.5 µl was then added to 10.0 µl of PCR mixture, mixed properly and incubated at 37°C for 30 minutes after which the reaction was stopped by heating at 95°C for 5 minutes.

Phylogenetic analysis of *M. hominis* with bacterial universal 16SrRNA sequencing:

The ultra-pure DNA was sequenced with ABI3500XL analyzer with a 50cm array, using POP7. Sequence data generated were analyzed with Geneious version 9.0.5 and phylogenetic tree were constructed using neighbor joining method based on the nucleotide sequences of the 16SrRNA gene target. Neighbor-joining trees were constructed for each individual locus. Genes of interest were identified in scaffolds using NCBI Blast (<http://blast.ncbi.nlm.nih.gov/>) with the setting; 'align two sequences'. The gene sequence from the reference genome was used to identify the same/similar sequence in the scaffolds.

Statistical analysis of data:

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 20 and Graph Pad Prism version 6.0. Descriptive statistics were used to describe the study participants in relation to relevant variables. Chi-square and Fisher's exact test were employed to compare variables. P value < 0.05 was considered statistically significant.

Results:

The 16SrRNA universal gene sequencing revealed four strains (clonal or phylogenetic) of *M. hominis* (Table 2). The Fisher's exact test showed no significant difference in occurrence of the strain (clonal) types between pregnant and infertile women ($p>0.05$). Although strain MHS43 (72.7% v 22.0%) and MHKC87 (18.2% v 11.1%) appeared to be more frequently associated with pregnant than infertile women, strain MHBS (33.3% v 0%) and MHSWP2 (33.3% v 9.1%) appeared to be more frequently associated with infertile compared to pregnant women. However, the differences in frequency of occurrence were not statistically significant for all strains between both groups ($p>0.05$).

Table 3 showed distribution of virulent genes among the four phylogenetic types of *M. hominis* in the pregnant and infertile women, and with the Fisher's exact test, neither *vaa* nor p120 gene was significantly associated with frequency of occurrence of any strain type with respect to source ($p>0.05$). The 3 MHBS strains were all exclusive to infertile women and all possessed virulent genes.

Table 4 showed the strain types and how the virulent genes (*vaa* and p120) were distributed among them. A two-way ANOVA analysis showed none of the gene was significantly associated with a particular strain ($p=0.8641$). All strains appear to possess different genes in different capacity with no particular pattern apart from MHBS which showed strain homology in pattern.

Plates 1 and 2 showed the gel electrophoresis picture of *vaa* and p120 genes respectively while Figs 3.1 to 3.4 represent the phylogenetic variants (strain types) of *M. hominis*.

Table 2: Phylogenetic variants (strain) of *Mycoplasma hominis* and distribution among study participants

Clonal type	Number (%)	Infertile women (%)	Pregnant women (%)	p-value	OR (95% CI)
MHS43	10 (50.0)	2 (22.2)	8 (72.7)	0.06	0.1071 (0.014 - 0.838)
MHBS	3 (15.0)	3 (33.3)	0	0.07	12.385 (0.549 - 279.4)
MHSWP2	4 (20.0)	3 (33.3)	1 (9.1)	0.28	5.00 (0.419 - 59.7)
MHKC87	3 (15.0)	1 (11.1)	2 (18.2)	1.00	0.5625 (0.043 - 7.45)
Total	20	9	11		

OR: Odds ratio; CI: Confidence interval

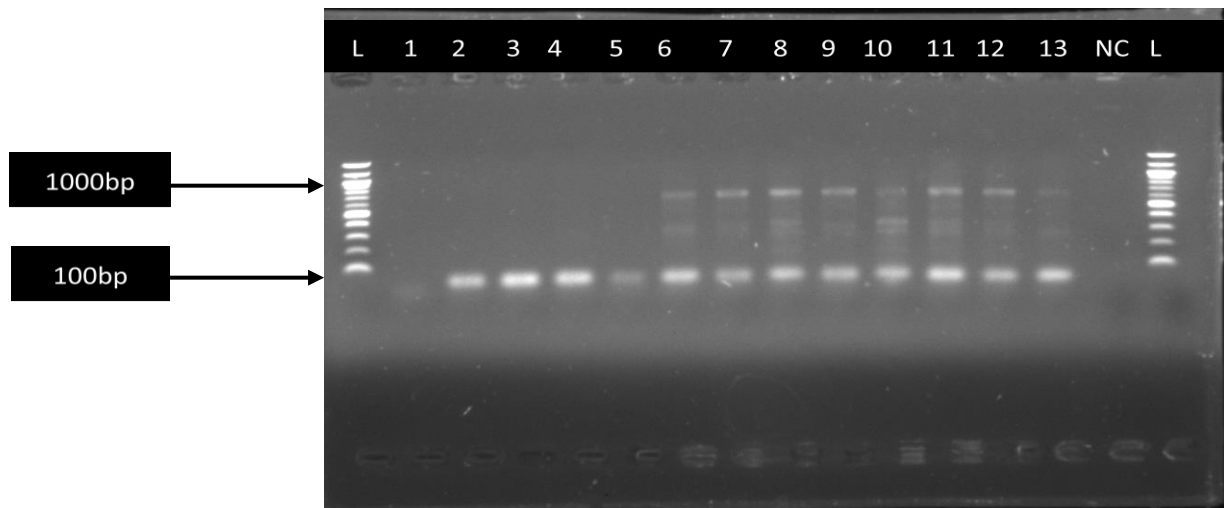
Table 3: Distribution of virulent genes among the four variants of *Mycoplasma hominis* isolates with respect to source

Clonal type	Vaa genes					P120 genes				
	Infertile women		Pregnant women		p value	Infertile women		Pregnant women		p value
	Freq	Pos	Freq	Pos		Freq	Pos	Freq	Pos	
MHS43 (n=10)	2	2	8	4	0.47	2	1	8	5	1.00
MHSWP2 (n=4)	3	1	1	1	1.00	3	2	1	1	1.00
MHBS (n=3)	3	3	0	0	1.00	3	3	0	0	1.00
MHKC87 (n=3)	1	1	2	2	1.00	1	0	2	2	0.33
Total (n=20)	9	7	11	7		9	6	11	8	

Freq =Frequency of occurrence; Pos=Number positive

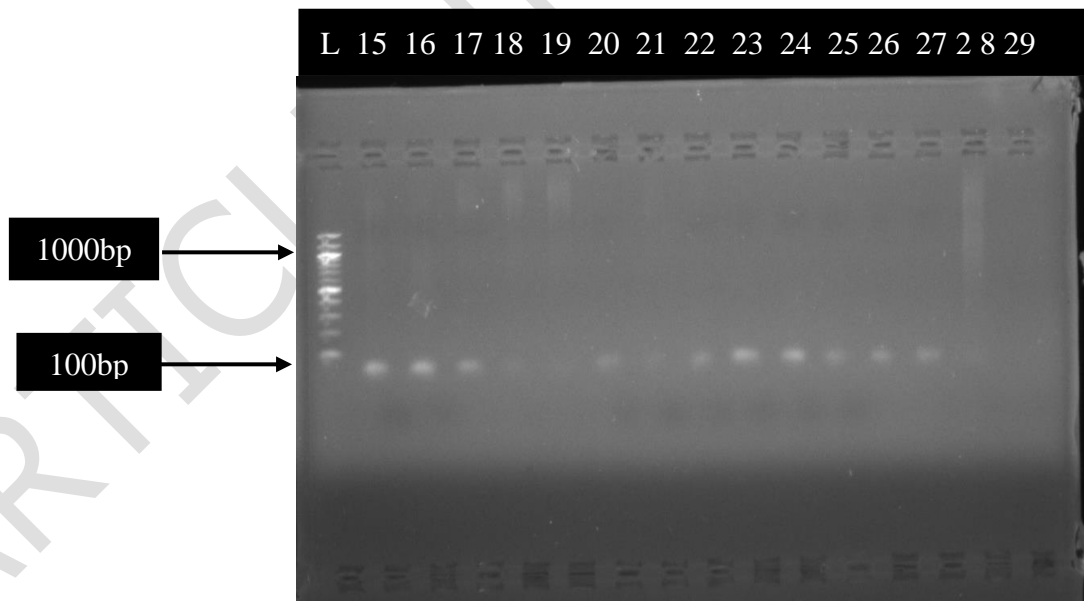
Table 4: Virulent genes carriage among the four *Mycoplasma hominis* phylogenetic groups

Strains/Virulent genes	Total vaa gene	Total p120 gene	vaa gene only	p120-gene only	vaa + p120
MHS43 (10)	6	6	3	3	3
MHBS (3)	3	3	0	0	3
MHSWP2 (4)	2	3	1	2	1
MHKC87 (3)	3	2	1	0	2
Total	14	14	5	5	9



L is a 100bp-1000bp DNA ladder (molecular marker). Samples 2, 3, 4, 5,6,7, 8, 9, 10,11, 12 and 13 are positive bands for the expressed *vaa* genes at 100 and 600bp. Sample 1 is a negative band. NC is a No template control

Plate 1: Gel electrophoresis picture of PCR amplicon of *vaa* genes



L is a 100bp-1000bp DNA ladder (molecular marker). Samples 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 and 28 are positive bands. Sample 29 is a negative band

Plate 2: Gel electrophoresis picture of PCR amplicon of *p120* gene

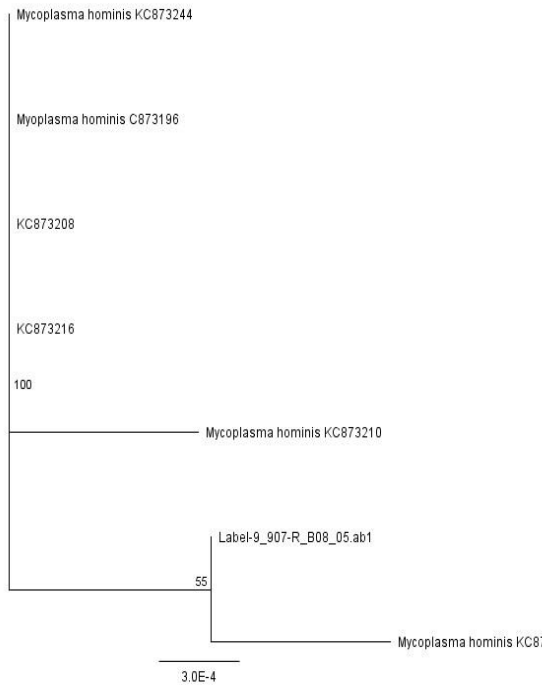


Fig. 3.1: Phylogenetic tree for *Mycoplasma hominis* strain KC87

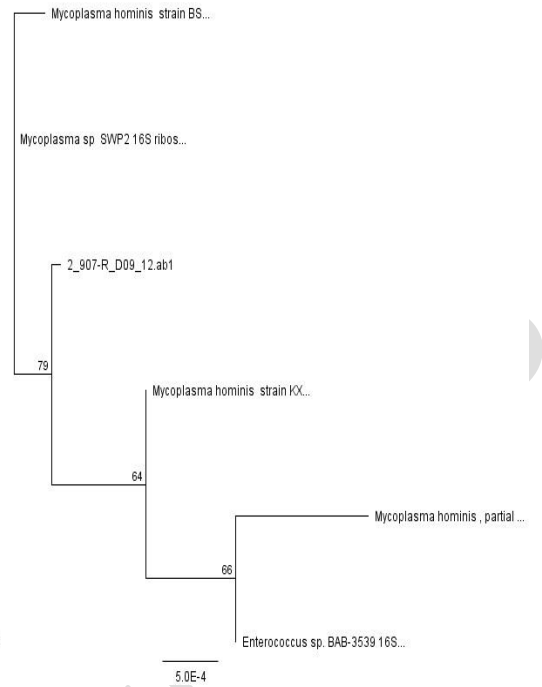


Fig. 3.2: Phylogenetic tree for *Mycoplasma hominis* strain BS

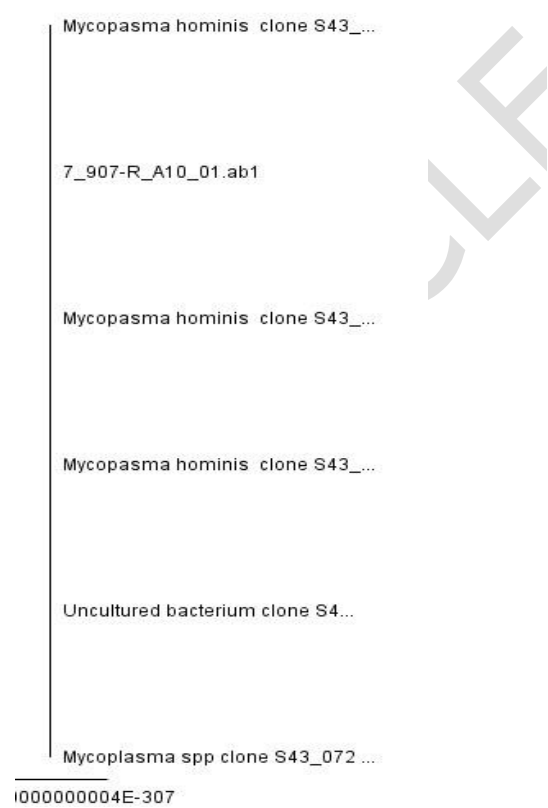


Fig. 3.3: Phylogenetic tree for *Mycoplasma hominis* strain S43



Fig. 3.4: Phylogenetic tree for *Mycoplasma hominis* strain SWP2

Discussion:

The 20 *M. hominis* isolates yielded four phylogenetic variants. This finding is in agreement with other studies that made use of molecular typing methods. Using RFLP, Jironkin et al., (15) reported high levels of genetic heterogeneity among *M. hominis* and Sogaard et al., (16) examined six-house-keeping gene sequences to investigate evidence of genomic recombination in *M. hominis* to show a high degree of variability between these genes. Ferandon et al., (17) used multiple locus variable number tandem repeat (VNTR) analysis (MLVA) molecular typing system for the discrimination of 210 French *M. hominis* clinical isolates. In their study, they described 40 MLVA types, revealing a high genetic heterogeneity among this specie. Their result showed that a high level of intragenic and intergenic recombination occurred in *M. hominis* and these recombination levels are presumably important for the adaptation potential of this specie (15).

Although in our study, strains MHS43 and MHKC87 appeared to be more frequently associated with pregnant than infertile women, and strain MHBS and MHSWP2 appeared to be more frequently associated with infertile than pregnant women, the difference in frequency of occurrence was not significant ($p > 0.05$). This implies that none of the *M. hominis* variant is uniquely associated with infertility, which could be an indication that pathogenicity of each strain is host dependent. According to Christiansen (18), comparisons of 12 genital isolates with other strains from different origins demonstrated that the genital isolates varied as much from each other as strains of non-genital origin. This agrees with the findings in this study although it was originally suggested that strains isolated from the same anatomical region should reveal a higher degree of similarity than strains isolated from another anatomical sites (19).

While 70% (14/20) of the *M. hominis* isolates was found to carry p120 virulent gene in our study, 30% (6/20) did not possess this gene. This shows that p120 gene, though more conserved and unique among *M. hominis* than *vaa* gene, was not present in all the isolates. Nyvold et al., (13) classified the p120 gene of *M. hominis* into 4 classes based on restriction endonuclease cleavage patterns of the hyper-variable domains. The different classes of p120 observed by Nyvold et al., (13) may well explain why 30% of *M. hominis* were found to be without p120 gene in our study. Mardassi et al., (20), in their study of patients with urogenital infections, discovered that the p120

protein undergoes substantial level of genetic variability at its surface exposed region. Part of the reason for the absence of p120 gene in a few isolates could be due to the use of primers constructed from the hyper-variable regions which are highly discriminatory unlike primers from the conserved regions which have the capacity to amplify p120 gene from all isolates. Mardassi et al., (20) reported that in a larger sample of *M. hominis* recovered from patients with urogenital infections, p120' protein, a variant of p120, also undergoes substantial level of genetic variability at its surface exposed region. Orville et al., (21) reported that the highly antigenic p120 gene displays a hyper-variable region due to accumulation of mutations while the *vaa* gene product displays both size variation and frameshift mutation to create variant products.

Thirty percent (30%) of the *M. hominis* isolates screened lacked *vaa* gene. For the sake of evolution and genome down-sizing, and possibly through gene truncation and activities of mobile genetic element (MGE), this is very possible and some of these isolates might have lost theirs to the environment. The detection of *vaa* gene in only 70% of *M. hominis* in our study disagrees with the assertion of Henrich et al., (22) that *vaa* genes are carried by all *M. hominis* species. The *vaa* locus has previously been described as a highly dynamic region of the *M. hominis* genome (3). It has been proposed that the mechanism underlying variation in the *vaa* gene is intra-species recombination whereby variable regions of *vaa* are exchanged resulting in a variable and dynamic 'hot-spot' in *M. hominis* genome (23). Based on Southern blot analysis and sequencing of the *vaa* gene, Henrich et al., (22) also proved the mechanism of variability to be based on specific truncations of the *vaa* gene. In addition to truncation, a coincidental duplication of some gene segments was also detected. Their work provided evidence for the genetic basis of a further variation in the *M. hominis vaa* adhesin

Vladislav et al., (24) identified 15 *M. hominis* isolates containing various versions of the *vaa* genes characterized by different amounts of homologous replaceable cassettes. They also determined a hyper-variable region of the *vaa* gene connected with an area encoding the immuno-significant part of the *Mycoplasma vaa* protein. This region is essential for immune recognition, persistence of *Mycoplasma* in humans, and colonizing host cells. It is noteworthy that presence of *vaa* gene, whether truncated or not, does not necessarily mean it is expressed. Hence, presence of *vaa* gene does not correlate with cytoadherence any-

more. Evidence abound that *M. hominis* can revert to other surface proteins such as p120, in instances where *vaa* gene is no longer functional (22).

Our study provides evidence that primers from *vaa* gene may no longer be suitable in screening for *M. hominis*. The shortfall in using *vaa* gene as a target for screening for *M. hominis* include omission of *vaa*-negative *M. hominis* giving rise to false negative, and lastly, the possibility of evading detection even in isolates that possess *vaa* gene, since other researches have proven that more genetic variability occur in *vaa* gene. The presence of *vaa* gene have been previously reported to be a prerequisite for virulence of *M. hominis* but the finding of our study not only indicated that it is no longer common to all *M. hominis* as speculated, but also not exclusive to perceived virulent strains. This was confirmed by the presence of *vaa* gene among strains (strain MHS43) that showed no apparent pathogenicity due to source of isolation, although this association was found to be non-significant, which implies that pathogenicity of *M. hominis* has to do with more than just possessing *vaa* gene, which means that issue of gene expression is of utmost importance. However, it is also important to note that variation or absence of this surface-exposed antigen may prevent the eradication of *M. hominis* by the host immune response (25), which is an added pathogenicity potential.

There is no significant variability among the virulent genes with regards to source, which confirms the findings of Rakhmatulina et al., (26) who demonstrated a potential effect of the *vaa* surface protein on the virulence of opportunistic *M. hominis* pathogen. They opined that certain variants of *vaa* are more frequently associated with clinical manifestations of inflammatory diseases while others are associated with clinically healthy people. Hence, pathogenicity may not just be about presence of *vaa* gene but of the *vaa* variant and gene expression. This implies that the strains from different sources do not vary significantly with respect to virulent genes of interest. This by implication means that any difference in the pathogenicity of these variants does not lie in the presence or absence of virulent genes of study. On the other hand, 100% (2/2) of strain MHKC87 from pregnant women had *vaa* and p120 indicating pathogenicity to be host-dependent. However, there is no conclusive evidence to this assertion yet because no further screening was carried out on the pregnant women because pregnancy was generally taken as evidence of fertility, but cases abound where pregnant

women may have one inflamed or blocked fallopian tubes and may have conceived through one tube (27). However, there was no such record and pregnancy does not annul the possibility of sequelae of reproductive tract infections. Thus, pregnancy may mask any underlying impact of the organism in the individual.

Other strains displayed inconsistent pattern both within and among strains, apart from strain MHBS which showed strain homology in pattern both in the source of isolation and how the virulent genes were carried. MHBS appeared not to have lost any of its adherence capability and this could have a profound influence on its pathogenicity potential. It could mean that strain MHBS is the most virulent among the four identified strain and that this strain is less subject to mutations and variabilities. Just like it is easy to shed of genes not serving it, it is just as well easy and possible for any *Mycoplasma* strain to pick up a gene from its environment to fortify itself in a new environment due to lack of cell wall, and this singular factor appeared to be the greatest weapon exploited by the species in promoting its pathogenicity potentials. Allen-Daniels et al., (27) identified a gene in *M. hominis* that was significantly associated with bacterial burden in the amniotic fluids suggesting that it could play a role in survival or fitness in this niche. The limitation of our study includes the small sample (isolate) size and the absence of adequate clinical information of the study population where the participants were selected from.

Conclusion:

The differences in pathogenicity of *M. hominis* may be linked to heterogeneity within the specie and lack of homogeneity in the virulence factors as witnessed both in the intra specie and intra strain levels. This finding has become a foundational ground for re-examining the scientific fact that *vaa* gene is exclusive to *M. hominis* and has by extension, call for review of the use of primer construct from *vaa* gene as biomarkers for direct molecular screening of samples for *M. hominis*.

For more effective and meaningful progress and assertions in the determination of *M. hominis* strains and its pathogenicity, there should be a globally accepted standardized method of strain characterization. This will enable a uniformity of approach and outcome across the globe. Hitherto, there are just a conglomeration of different methodologies which hinders streamlined outcome and comparison.

Contribution of authors:

CCP was involved in material preparation, data collection, analysis, and writing of the first draft of the manuscript. Other authors participated in review of the manuscript draft. All authors read and approved the final manuscript.

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References:

- Agbakoba, N. R., Adetosoye, A. I., and Adewole, I. F. Presence of *Mycoplasma* and *Ureaplasma* species in the vagina of women of reproductive age. *West Afr J Med*. 2007; 26 (1): 28-31. doi: 10.4314/wajm.v26i1.28299.
- Chukwuka, C. P., Agbakoba, N. R., Emele, F. E., et al. Prevalence of Genital Mycoplasmas in the Vaginal Tract of Adolescents in Nnewi South-Eastern Nigeria. *World J Med Sci*. 2013; 9 (4): 248. doi: 10.5829/idosi.wjms.2013.9.4.81122.
- Brown, R. J., Chalker, V. J., and Spiller, O. B. *Mycoplasma hominis* Variable Adherence-Associated Antigen: A Major Adhesin and Highly Variable Surface Membrane Protein. *Adv Microbiol*. 2014; 4: 736-746. doi: 10.4236/aim.2014.411080.
- Chen, Y., Wu, Y., Qin, L., Zhu, C., and You, X. Infection strategies of Mycoplasmas: Unravelling the panoply of virulence factors. *Virulence*. 2021; 12 (1): 788-817. doi: 10.1080/21505594.2021.1889813
- Pereyre, S., Renaudin, H., Charon, A., Bebear, C., and Bebear, C. M. Emergence of a 23S rRNA mutations in *Mycoplasma hominis* associated with a loss of the intrinsic resistance to erythromycin and azithromycin. *J Antimicrob Chemother*. 2006; 57: 753-756. doi: 10.1093/jac/dkl026.
- Citti, C., Nouvel, L. X., and Baranowski, E. Phase and antigenic variation in mycoplasmas. *Future Microbiol*. 2010; 5: 1073-1085. doi: 10.2217/fmb.10.71
- Rottem, S. Interaction of Mycoplasmas with Host Cells. *Physiol Rev*. 2003; 83 (2): 417-432. doi: 10.1152/physrev.00030.2002
- Bebear, C. M., Renaudin, H., Charron, A., Clerc, M., Pereyre, S., and Bebear, C. DNA Gyrase and topoisomerase IV mutations in Clinical Isolates of *Ureaplasma spp.* and *Mycoplasma hominis* resistant to fluoroquinolone. *Antimicrob Agents Chemother*. 2003; 47: 3323-3325. doi: 10.1128/AAC.47.10.3323-3325.2003
- Agbakoba, N. R., Adetosoye, A. I., and Adewole, I. F. Biochemical and serological characterization of *Mycoplasma* strains isolated from the genital tracts of humans in Nigeria. *Afr J Med Sci*. 2006; 35 (2): 125-129.
- Agbakoba, N. R., Adetosoye, A. I., Adewole, I. F., Obi, C. M., and Enemu, S. C. Prevalence of Genital Mycoplasmas in Women of Various Socio-Economic Statuses. *Int J Microbiol Res*. 2011; 2 (2): 162-166
- Chikkala, R., Ch, S., Dvyakolu, S., Ratnakar, K. S., and Sritharan, V. A simple sample processing protocol and multiplex PCR for direct detection of MRSA from uncultured clinical samples - a pilot study. *Adv Infect Dis*. 2019; 9: 25-38. doi: 10.4236/aid.2019.91003
- Chernov, V. M., Gorshkov, O. V., Chernova, O. A., Baranova, N. B., Akopian, T. A., and Trushin, M. V. Variability of the vaa cytoadhesin genes in clinical isolates of *Mycoplasma hominis*. *The New Microbiologica*. 2005; 28: 373-376.
- Nyvold, C., Birkelund, S., and Christiansen, G. The *Mycoplasma hominis* P120 membrane protein contain a 216 amino acid hypervariable domain that is recognized by the human humoral immune responses. *Microbiology*. 1997; 143: 675 - 688. doi: 10.1099/00221287-143-2-675
- Keskin, F., Ciftci, S., Keceli, S. A., et al. Comparison of culture and real-time polymerase chain reaction methods for detection of *Mycoplasma hominis* in amniotic fluids samples. *Nig J Clin Pract*. 2018; 21 (9): 1127-1131. doi: 10.4103/njcp.njcp.230.17
- Jironkin, A., Brown, R. J., Underwood, A., Chalker, V. J., and Spiller, O. O. Genomic determination of minimum multilocus sequence typing schemas to represent the genomic phylogeny of *Mycoplasma hominis*. *BMC Genomics*. 2016; 17: 964.
- Sogaard, I. Z., Boesen, T., Mygind, T., et al. Recombination in *Mycoplasma hominis*. *Infect Genet Evol*. 2002; 1(4): 277-285. doi: 10.1016/s1567-1348(02)00036-9
- Ferandon, C., Peuchant, O., Renaudin, H., and Bebear, C. Diversity of *Mycoplasma hominis* clinical isolates from Bordeaux, France, as assessed by multiple locus variable number tandem repeat analysis. *BMC Microbiol*. 2013; 13: 120.
- Christiansen, G. Genetic variation in natural populations. In: Maniloff, J., McElhaney, R. N., Finch, L. R., and Baseman, J. B (eds.). *Mycoplasmas Molecular biology and pathogenesis*. American Society for Microbiology, Washington DC. 1992: 561-574
- Barile, M. F., Grabowski, M. W., Stephens, E. B., et al. *Mycoplasma hominis*-tissue cell interactions: a review with new observations on phenotypic and genotypic properties. *Sex Transm Dis*. 1983; 10: 345-354.
- Mardassi, B. B. A., Ayari, H., Bejaoui-Khiari, A., Miik, B., Moalla, I., and Amouna, F. Genetic variability of the P120 surface protein gene of *Mycoplasma hominis* isolates recovered from Tunisian patients with urogenital and infertility disorders. *BMC Infect Dis*. 2007; 7: 142.
- Orville, R., Karen, E., and Bidyut, R. Virulence and molecular adaptation of human urogenital mycoplasmas: a review. *J Biotechnol Equipment*. 2019; 33 (1): 689-698. doi: 10.1080/13102818.2019.1607556
- Henrich, B., Klaudia, L., Annette, K., Colin, M., and Ulrich, H. Truncation as a novel form of variation of the p50 gene in *Mycoplasma hominis*. *Microbiology*. 1998; 144: 2979-2985. doi: 10.1099/00221287-144-11-2979
- Kong, Y., Ma, J. H., Warren, K., et al. Homologous recombination drives both sequence diversity and gene content variation in *Neisseria meningitidis*. *Genome Biol Evol*. 2013; 5: 1611-1627. doi: 10.1093/gbe/evt116
- Vladislav, M., Chernov, O. V., Gorshkov, O. A., Chernova, N. B., Baranova, T. A., and Trushin, A. M. V. Variability of the vaa cytoadhesin genes in clinical isolates of *Mycoplasma hominis*. *The New Microbiologica*. 2005; 28: 373-376.
- Capoccia, R., Greub, G., and Baud, D. *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse

- pregnant outcomes. *Current Opin Infect Dis.* 2013; 26 (3): 231-240.
[doi: 10.1097/QCO.0b013e328360db58](https://doi.org/10.1097/QCO.0b013e328360db58)
26. Rakhmatulina, M. R., Plakhova, K. I., and Kubanov A. A. Genetic variants of genital mycoplasmas and their relation to the clinical course of inflammatory diseases of the urogenital system in women. *Vestnik Dermatologii Venerologii.* 2014; 6: 107-113
27. Allen-Daniels, M. J., Myrna, G., Serrano, L. P., et al. Identification of a gene in *Mycoplasma hominis* associated with preterm birth and microbial burden in intra-amniotic infection. *Am J Obstet Gynaecol.* 2015; 212 (6): 779.e1-779.e13
[doi: 10.1016/j.ajog.2015.01.032](https://doi.org/10.1016/j.ajog.2015.01.032)

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