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Specificity of Cultural and Molecular Diagnosis for Identification of Mycoplasma Hominis

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Abstract— **A. Background**

Mycoplasma hominis is increasingly being associated with tubal factor infertility; increased risk of pregnancy complications, such as premature membrane rupture, vaginitis and preterm birth. The study was carried out to test the specificity of culture and molecular diagnosis for identification of M. hominis.

B. Methods:

A cross sectional study was conducted and demographic variables collected using a structured questionnaire. High vaginal swab (HVS) samples were collected from 200 women (100 from women presenting with infertility and 100 from pregnant women) and cultured for Mycoplasma hominis. Identification of organism was based on laboratory cultural characteristics of M. hominis and Polymerase Chain Reaction (PCR) of the presumptively identified isolates using 16S target gene.

C. Result:

Of the 200 HVS samples analyzed for M. hominis by laboratory culture method, 35 samples (19 from infertile) and (16 from pregnant women) were positive. Of the 35 presumptive isolates of M. hominis, 20 isolates (9 from infertile) and (11 from pregnant) were confirmed M. hominis by the PCR of the 16SrRNAtarget gene specific to M. hominis.

D. Conclusion:

There is need for modification of culture medium used in the isolation of M. hominis to curb the proliferation of other urogenital organisms in medium specific to M. hominis. However, basing diagnosis only on PCR equally poses a challenge due to the high genetic variability within the specie. Hence, combination of methods appears to be a plausible solution to minimize erroneous results.

Index Terms— **M. hominis, specificity, Molecular diagnosis, culture, identification..**

I. INTRODUCTION

We acknowledge the Management of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State for granting permission to recruit its patients and utilization of its laboratory equipments in this study. Much thanks also to MLS Nduka Arinze, of Microbiology Dept., NAUTH, Nnewi for his scientific input.

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There is no conflict of interest among the authors and no association might pose a conflict of interest

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II. INTRODUCTION

Sexually Transmitted Infections (STIs) are an important public health problem and among the five most frequent causes for seeking health care services. The World Health Organization (WHO) estimates that about 340 million new cases of STIs occur each year [1]. Mollicutes are included in STIs, but they are also found in healthy individuals. However, five decades ago some mollicutes were considered infectious agents of the human urogenital tract [2].

Ureaplasmaurealyticum and *Mycoplasma hominis*, also known as genital mycoplasmas, are commensals that can be detected in the lower genitourinary tract of sexually active women, resulting in colonization of the genitalia by sexual contact. Vaginal colonization of these bacteria mainly causes vaginosis, postpartum fever, pelvic inflammatory disease, infertility, postpartum septicemia, preterm labor, premature rupture of the membranes, systemic neonatal infections, and preterm birth [3], [4].

M. hominis has also been associated with urinary tract infection, sterile pyuria, non-gonococcal urethritis (NGU) and rarely with bacteremia, arthritis, peritonitis, and meningitis [5]. They decrease the sperm count mobility and increase the percentage of abnormal sperms [6]. In women, an increase of vaginal discharge may be reported as well as dysuria, bacterial vaginosis, but the infection is mostly asymptomatic [7]. The exact prevalence and epidemiology of *M. hominis* is still unknown among infertile and pregnant females in Nigeria. Many studies had been conducted in Nigeria and elsewhere to detect and isolate this pathogen [8],[9]and PCR developed to better understand its incidence and distribution [10]; however, the specificity of the culture or PCR method has not been verified.

Therefore, the study was aimed not only to determine the prevalence of this organism in the study groups but also the specificity of cultural and molecular method in identification of *M. hominis* using 16SrRNA target gene.

III. METHODOLOGY

A. Study Design

It is a hospital based cross sectional study conducted in a tertiary health referral institution in Nigeria that offers antenatal and gynecology care among other specialties.

B. Study Population and Sampling Technique

Ethical approval was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethical Boardfollowing the Declaration of Helsinki (NAUTH/CS/66/VOL.9/31/2017/026) and demographic variables were collected using a structured questionnaire. Two high vaginal swab (HVS) samples were collected from 200 women each (21-49 years) who consented and have not received antimicrobial treatment in the preceding two weeks; and cultured for *Mycoplasma hominis* isolation (100 presenting with infertility (subjects) and 100 apparently healthy pregnant women (control). Analysis of the samples was based on laboratory cultural characteristics of *M. hominis* using Oxoid products (Mycoplasma agar base (CM0401), Mycoplasma broth base (CM 0403), Mycoplasma supplement G (SR 0059)) and Polymerase Chain Reaction (PCR) of the presumptively identified isolates using 16SrRNA target gene specific to *Mycoplasma hominis (Table 1)*. Also, other routine vaginal pathogens were screened for following conventional microbiology methods.

Table I: The Oligonucleotide Primers Used for Amplification of 16SrRNA gene of the Mycoplasma hominis

Target Gene	Primer Sequence (5' - 3')	PCR Productbp	Ref.
16SrRNA gene of the M. hominis	F-CAA TGG CTA ATG CCG GAT ACG C R- GGT ACC GTC AGT CTG CAA T	344	[11]

C. Data Analysis

Data was analyzed using the Statistical package for Social Sciences (SPSS) version 20. Descriptive statistics were used to analyze the study participants in relation to relevant variables. Chi-square and Fisher's exact test were employed to compare variables at 95% confidence limit ($p<0.05$).

IV. RESULTS

Culture method based on laboratory diagnostic characteristics of *M. hominis* (Fig.1) revealed 17.5% presumptive isolates of *Mycoplasma hominis* from the clinical specimen; 19% from infertile and 16% from pregnant women. Of the 35 presumptive isolates of *M. hominis*, 20(57.1%) isolates were confirmed *M. hominis* by the conventional PCR of the 16SrRNA target gene specific to *M. hominis*(plates 1); 9(47.4%) from infertile and 11(68.8%) from pregnant women. The results for the culture and PCR are shown in Table II. From the PCR result, the overall prevalence rate of *M. hominis* isolation from the general population was 10.0% (20/200) of which 9/100 (9%) were from the women presenting with infertility and 11/100 (11%) from the control group. Table III shows the rate of isolation of *M. hominis* and other common vaginal pathogens and the most commonly encountered vaginal infections were aerobic bacteria 65/200(32.5%) and candidiasis 58/200 (29%). The proportion of aerobic bacteria was higher among the pregnant women 38/100 (38%) than in women presenting with infertility 27/100 (27%). Only *T. vaginalis* appeared higher among the infertile group (3/100 (3%)). There was no significant relationship between isolated microorganisms, prevalence and categories of women sampled ($P=0.4658$; $\alpha \leq 0.05$ and $P=0.637352$; $\alpha \leq 0.05$ respectively)

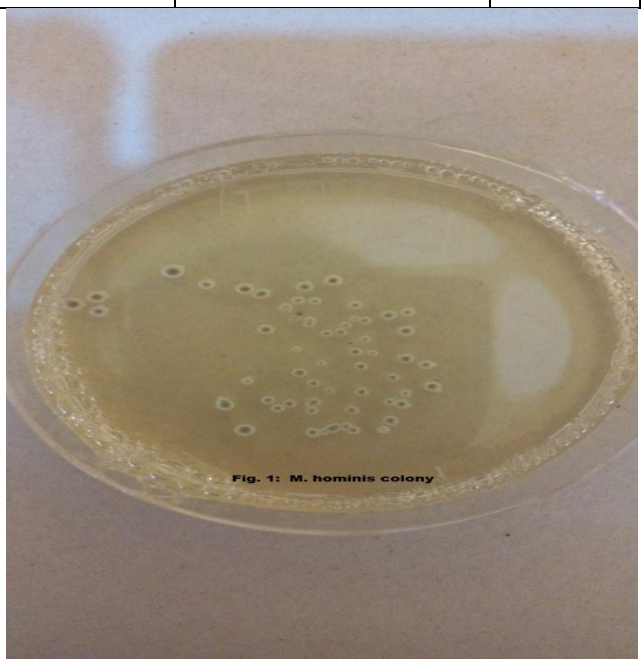

Fig. 1: Typical *Mycoplasma hominis* colonies growing on Mycoplasma solid medium.

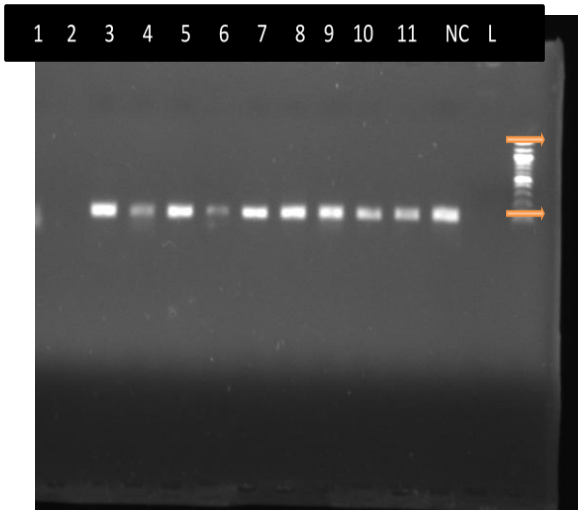

Plate 1: PCR results for *Mycoplasma hominis* (using specific primer) analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a

100bp-1000bp DNA ladder (molecular marker). Samples 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 are positive bands at100bp. Sample 1 is a negative band. NC is a No template control.

1000bp

100bp

Table II: Comparison of Specificity of PCR and Culture for *M. hominis* identification.

Organism(M.hominis)	Total cases	Infertile	Pregnant
Tested cases by culture	200(100%)	100(100%)	100(100%)
Positive cases by culture	35(17.5%)	19/100(19%)	16/100(16%)
Negative cases by culture	165(82.5%)	81/100(81%)	84/100(84%)
Tested cases by PCR	35(100%)	19(100%)	16(100%)
Positive cases by PCR	20(57.1%)	9/19 (47.4%)	11/16(68.8%)
Negative cases by PCR	15(42.9%)	10/19(52.6%	5/16(31.2%

Table III: Percentage of encountered Isolates from the study population

Microorganism	No. of Positives(n=200)	Women presenting with infertility (n= 100)	Pregnant Women (Controls) (n= 100)
M. hominis	20(10%)	9 (9%)	11 (11%)
G. vaginalis	40(20%)	16 (16%)	24 (24%)
***Candida* spp**	58(29%)	19 (19%)	39 (39%)
T. vaginalis	4(1%)	3 (3%)	1 (1%)
Common aerobic bacteria	65(32.5%)	27 (27%)	38 (38%)

Table IV Risk factors to *Mycoplasma hominis* infection

M. hominis co-infections	Infertile women (subject)	Pregnant women (Control)
M. hominis + *G. vaginalis*	3	1
M. hominis + *Candida*spp	1	3
M. hominis + *T. vaginalis*	1	0
M. hominis + aerobic bacteria	2	3
Fishers' Exact Test: P value = 0.5176		
M. hominis alone	2	4
M. hominis co-infections	7	7
Fishers' Exact Test P value = 0.6424		
Groups	***M. hominis* alone**	***M. hominis* + co-infections**
Infertile	2	7
Fertile	4	7
Fishers' Exact Test P value = 0.6424		

Table IV revealed the impact of monomicrobial and polymicrobial existence on prevalence of *M. hominis* between the two study groups. Comparing the rate of occurrence of *M. hominis* coexisting with different organism in infertile and pregnant women, the Fishers' Exact Test (P value = 0.5176; $\alpha \leq 0.05$) showed no significant association. This shows that none of the organisms isolated as coexisting with *M. hominis* had special impact on prevalence of *M. hominis* infection. Also, an overall assessment of polymicrobial influence on prevalence of *M. hominis*, Fishers' Exact Test (P value = 0.6424; $\alpha \leq 0.05$) revealed no significant difference, indicating that polymicrobial etiology is not a risk factor for infection of *M. hominis* in both categories of women. The differences between the single and polymicrobial conditions across the two categories were not statistically significant (p=0.6424). Comparing occurrence in both categories (infertile and pregnant (control); showed no statistically significant association (p=0.6424; $\alpha \leq 0.05$)

V. DISCUSSION

Culture is considered the gold standard for detection of *M. hominis* because; it is required to identify active infection.

However, in this era of high genetic variation, diagnostic focus is gradually shifting towards nucleic acid amplification or combination of two methods.

The high discrepancy observed in this work can be attributed to the variation in the specie - specific genes caused by high rates of mutation [12],[13]. often witnessed in the species which may have given rise to false negative results. Equally, the activities of plasmids conferring genes [14], hitherto not present in certain species giving rise to characteristics previously associated only to a Mycoplasmatale may also have played a role. The discrepancy may also be due to increase in resistance strains among non-*M. hominis* isolates giving rise to false positive result in the isolation rate.

Discrepancies between culture and PCR results was also documented for *M. hominis* by Al-Ghizawiand colleague [15]. Out of 200 patients, 13 (6.5%) tested positive for *M. hominis* using culture method, and when subjected to molecular confirmation, only 6/13 (46.2%) were confirmed positive for *M. hominis*. Again, in the same study carried out on *Ureaplasma* specie, they showed that out of 73 isolates of *Ureaplasma* from culture, only 35/73 (48%) isolates of *U. urealyticum* were positive with PCR [15].

Discrepancies between culture and PCR results have been said to be influenced by multiple factors such as media, personnel and type of target gene used. It can also be due to antibiotics used, type of sample, microbial load and method of sampling [16],[17]. Hence, it appeared that sensitivity varies between culture and molecular technique depending on the gene target and molecular method used. However, assays utilizing the gap gene as a target for *M. hominis* detection showed 100% agreement between culture and PCR results [18].

The use of selective and differential media for the isolation of genital mycoplasma including *M. hominis* is a very efficient traditional way of identifying mycoplasma [6], however, with the rise in genetic variations and gene transfers existing among species and other bacteria existing within the same niche; it is fast becoming unreliable. This finding might be an indication that there is need for mycoplasma medium review and upgrade or probably that some of the organisms were actually *M. hominis* which may have lost some vital amino acids in the sequences used for primer construction, hence failure to be detected.

Overall, a 10% prevalence rate of *M. hominis* in infertile and fertile (pregnant) females was recorded which is much higher than 3.14% positivity rate observed among the Iranian infertile and pregnant women as recordedbyMehri *et al.*, [19]. The prevalence of the bacteria was higher among pregnant (11%) than infertile women (9%) but there was no significant statistical difference between both categories (P-value = 0.637352; $\alpha \leq 0.05$). This is in consonance with the work of Bayraktar and colleagues[17], who reported a 10% prevalence rate among symptomatic women but contradicts the report by Mehri [19], who stated that prevalence was higher in infertile women than in pregnant control.

A prevalence of 35.7% was recorded among women of reproductive age in Ibadan, Nigeria[6] while a prevalence rate of 20% genital mycoplasma was recorded among the asymptomatic adolescent girls screened in South-Eastern Nigeria, out of which 4% of the isolate was *Mycoplasma hominis*[20].*Mycoplasma hominis* and *Ureaplasmaurealyticum*, was also found in infertile men and women (48% and 40% respectively) [21] while a prevalence rate of 8.6% for *M. hominis* was reported in women of reproductive age in an Italian study [22].

Other microorganisms other than *Mycoplasma hominis* were also encountered in this study. The most commonly identified vaginal infections were common aerobic bacteria 65/200 (33%); 27/100 (27%) in infertile women and 38/100 (38%) among the pregnant women, followed by *Candida* spp. 58/200 (29%); 19/100 (19%) from the infertile group and 39/100 (39%) among the pregnant ones while the least common was *Trichomonas vaginalis* 4/200 (2%); 3(3%) among the infertile and 1(1%) among the pregnant ones. Other researchers have varying organisms as the most prevalent ones. In a study conducted in Vietnam [23], Bangladesh [24], Ethiopia [25] and Nepal [26]. Candidiasis followed by Bacterial Vaginosis were the most prevalent. Elsewhere, in a study done in India [27], trichomoniasis was the most prevalent. Yet again, findings from studies done in Shandong [28], BV was found to be the most prevalent. The varying reports could be attributed to geographical location, season of sampling, nature of specimen, experimental methods, the population under study, variations in socio economic conditions as well as personal behavioral patterns like number of sexual partners, hygiene standard etc.

Some factors likely to be associated with *M. hominis* infection were analyzed in Table 4. The impact of mode of existence on infection with *M. hominis* between the two study groups were analyzed and there was no significant difference(P value =0.6424; $\alpha \leq 0.05$) in association between mono existence and polymicrobial existence of *M. hominis* and none of the organisms isolated as coexisting with *M. hominis* appeared to have any special influence on prevalence of *M. hominis* infection too (P value 0.5176; $\alpha \leq 0.05$), although a mutual relationship between *Trichomonas vaginalis* and virulent *Mycoplasma hominis* in the transmission of the infection had previously been established [29]. We also found that neither pregnancy nor infertility predisposes women to *M. hominis* infection in any special way ($p = 0.6424$; $\alpha \leq 0.05$). This shows both groups are equally disposed to infection with *M. hominis*. This may also indicate that *M. hominis* is only an incriminatory factor in infertility but not a causal agent or may be limited to opportunistic mode of pathogenicity. According to Campos, change in vaginal pH (bleeding in pregnancy, sexual intercourse or vaginal douching) may predispose any lady to an over growth of potential pathogens irrespective of their fertility status [30].

VI. CONCLUSION

Culture method remains the gold standard for identification of Mycoplasmas, yet great need arises for a more assured result. There is need for modification of the culture medium used in the isolation of *M. hominis* to enhance the selectiveness of the medium.This will make culture method moreconfirmatoryfor the organism. However, until this is done, specificity can only be enhanced by the combination of the two techniques to ensure a reliable diagnosis of this organism known for its high rate of genetic heterogeneity which apparently, could influence the

molecular studies giving rise to false negative result.

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