Highlighting the clinical need for diagnosing *Mycoplasma genitalium* infection

Ison CA[1], Fifer H[2], Gwynn S[3], Horner P[4, 5, 6], Muir P[6,7], Nicholls J[5], Radcliffe K[8], Ross. J[8], Taylor-Robinson D[9], White J[10]

1. Formerly of Public Health England, London UK
2. Public Health England, London UK
3. SG Market Access Ltd, UK
4. School of Social and Community Medicine, University of Bristol, UK
5. Bristol Sexual Health Centre, University Hospitals Bristol NHS Trust, Bristol, UK
6. National Institute for Health Research Health Protection Research Unit (NIHR HPRU)
7. Public Health England, Bristol, UK
8. University Hospitals Birmingham NHS Foundation Trust, Birmingham. UK
9. Imperial College, London, UK
10. Guy's and St Thomas' NHS Foundation Trust, UK

Address correspondence to: Professor Catherine Ison; Formerly of Public Health England, London UK; ison.catherine@gmail.com

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ABSTRACT

Introduction Despite *Mycoplasma genitalium* (MG) being increasingly recognised as a genital pathogen in men and women, awareness and utility of commercially available MG-

testing may be low. The opinion of UK sexual health clinicians and allied professionals was sought on how MG-testing should be used.

Methodology Thirty-two consensus statements were developed by an expert group and circulated to clinicians and laboratory staff, who were asked to evaluate their level of agreement with each statement; 75% agreement was set as the threshold for defining consensus for each statement. A modified Delphi approach was used and high levels of agreement obviated the need to test the original statement set further.

Results and Discussion Of 201 individuals who received questionnaires, 60 responded, most (48) being sexual health consultants, more than 10% of the total in the UK. Twenty-seven (84.4%) of the statements exceeded the 75% threshold. Respondents strongly supported MG-testing of patients with urethritis, pelvic inflammatory disease, or unexplained persistent vaginal discharge, or post-coital bleeding. Fewer favoured testing patients with proctitis and support was divided for routinely testing chlamydia-positive patients. Testing of current sexual contacts of MG-positive patients was supported, as was a test of cure for MG-positive patients, although agreement fell below the 75% threshold. Respondents agreed that all consultant or specialist-led services should have access to testing for MG (98.3%).
Conclusion There was strong agreement for having MG-testing available for specific patient groups, which may reflect concern over antibiotic resistance and the desire to comply with clinical guidelines that recommend MG-testing in sexual health clinic settings.

Abstract 250 words

INTRODUCTION

Despite being a recognised genito-urinary (GU) pathogen for two decades in men [1] and more recently in women, [2] diagnostic testing has been limited. Molecular assays, both in house and CE approved have been available but, until recently, there was no commercial assay fully approved by the Food and Drug Administration (FDA). [3, 4] Commercial availability of MG-testing presents an opportunity for improving the diagnostic and management pathways of several clinical syndromes associated with MG infection, including

urethritis in men and cervicitis and pelvic inflammatory disease (PID) in women. However the process has become complicated because of the rapid emergence of antimicrobial-resistant MG. [5, 6, 7] Diminishing the risk of increasing macrolide and quinolone resistance in MG requires a robust diagnostic and treatment pathway.

The 2016 European MG guidelines [7] recommended testing for MG in: men with symptoms or signs of urethritis or acute epididymo-orchitis if aged <50 years ; and in women with mucopurulent cervicitis, cervical or vaginal discharge with risk factor for sexually transmitted infection (STI), and those with intermenstrual or post coital bleeding or acute pelvic pain and/or PID. [7] These symptoms and signs are also commonly associated with *Chlamydia trachomatis* and patients when treated syndromically are likely to be prescribed azithromycin 1g , which was a recommended first line therapy for chlamydial infection until recently. [8-11]

There is, therefore, a clear need to identify MG infection [5] and treat correctly to avoid inappropriate or sub-optimal treatment, which is associated with the development of resistance. [5]

The worldwide focus on antimicrobial stewardship must be considered against the background of extreme financial pressure which impacts healthcare provision in every country. [12] In the United Kingdom, Local Authorities are the funding source for all screening and treatment related to sexual health and they must balance the pressure for short-term cost savings against the long-term cost impacts of undiagnosed or untreated conditions.

The only methodology appropriate for MG diagnosis is a nucleic acid amplification test (NAAT). [3,4, 7] Although the optimal treatment for MG is unclear, treatment with azithromycin 1g, which is associated with the development of macrolide resistance, is not recommended. [6-8, 13] This study assesses the attitudes of clinical professionals involved in the diagnosis and management of conditions associated with MG infection in order to make appropriate recommendations for best practice.

METHODOLOGY

A group of experts in GU medicine and microbiology (the authors of this paper) met in order to examine issues facing clinicians in the diagnosis and management of MG infection. All of the group are either currently or were previously involved in MG diagnostic research. Discussion identified 6 themes which were Rationale for MG-testing, Selection of Patients, Diagnostic Strategy, Cost Effectiveness, Antimicrobial Resistance / Antimicrobial Stewardship and Availability of MG-testing. These were then expanded to develop a series of 32 consensus statements (Table 1).

Table 1. Consensus Statements by Theme (n = number of responders, score = percentage of respondents in agreement with each statement)

Ref:	Topic:	Statement:	n:	Score (%)
1	Rationale for M. genitalium testing	M. genitalium is a proven pathogen for urethritis in men	59	98.3
2		M. genitalium may cause symptoms such as urethral discharge, dysuria and urethral discomfort in men	60	100
3		Effective targeted treatment for M. genitalium is available and may differ from standard urethritis treatment	60	93.3
4		M. genitalium is a proven pathogen for cervicitis and may be associated with post coital bleeding	59	89.8
5		M. genitalium is a proven pathogen for PID in women	59	83.1

6		M. genitalium is a proven but uncommon pathogen for vaginal discharge and irritation in women	60	56.7
7		M. genitalium is a proven pathogen for proctitis in MSM	57	56.1
8	Selection of Patients	Men with symptoms suggestive of urethritis should be tested for chlamydia, gonorrhoea and M. genitalium	60	85
9		Asymptomatic patients should not be tested for M. genitalium	60	76.7
10		Current sexual contacts of those with confirmed M. genitalium should be tested regardless of symptoms	60	81.7
11		Women with a clinical diagnosis of PID should be tested for chlamydia, gonorrhoea and M. genitalium	60	78.3
12		MSM presenting with proctitis should be tested using NAAT for chlamydia, gonorrhoea, herpes and M. genitalium	60	65
13		Testing for M. genitalium should be considered in women with unexplained, persistent vaginal discharge	59	79.7
14		Patients who are chlamydia positive should be tested for M. genitalium	57	50.9
15	Diagnostic Strategy	Where possible all men with symptoms of urethritis should be assessed with urethral microscopy	60	95
16		Where possible all men with symptoms of urethritis should be tested for chlamydia, gonorrhoea and M. genitalium concurrently	59	84.7
17		Where possible all men with confirmed urethritis on microscopy should be tested for chlamydia, gonorrhoea and M. genitalium concurrently	60	88.3

18	Cost Effectiveness	It is likely that targeted testing of symptomatic patients for M. genitalium will be cost effective	59	81.4
19		It is unlikely that testing asymptomatic patients for M. genitalium will be cost effective	58	82.8
20		More evidence is needed regarding the cost effectiveness of M. genitalium screening	59	88.1
21	Antimicrobial Resistance / Antimicrobial Stewardship	Individuals identified as M. genitalium positive should have a test of cure performed to confirm clearance	60	68.3
22		Confirmed M. genitalium should be treated specifically according to the result of any resistance testing or the knowledge of local resistance patterns	60	98.3
23		Detection of any macrolide resistance will assist with the selection of prescribed therapy and likelihood of clearance	60	98.3
24		Treatment of M. genitalium should proceed irrespective of the availability of resistance testing	60	78.3
25		Antimicrobial stewardship is important and best informed by resistance testing	60	98.3
26		Detection of quinolone resistance may assist with the specificity of prescribed therapy and likelihood of clearance	60	90
27		Moxifloxacin should be reserved for those with a confirmed diagnosis of M. genitalium	59	93.2
28		M. genitalium infection (where antibiotic resistance status is not known) should be treated with azithromycin 500mgs then 250mgs for 4 days	58	93.1

29		Men with NGU found to be M. genitalium positive with persistent urethritis following doxycycline treatment should be treated with a combination of azithromycin 500mgs then 250mgs for 4 days and metronidazole 400mgs bd 5 days.	58	77.6
30		Use of azithromycin 1g stat is likely to lead to high levels of macrolide resistance in M. genitalium and should be avoided where possible	59	94.9
31	Availability of M. genitalium testing	All level 3 GUM clinics in the UK should have access to M. genitalium testing	59	98.3
32		All level 3 GUM clinics in the UK should have access to M. genitalium resistance testing	59	96.6

While the author group developed the initial consensus statements, the involvement of a wider audience reflecting the views of key stakeholders across the UK was sought in order to develop a more robust and representative consensus view. To achieve this, the statements were circulated by questionnaire to the following groups: Clinical and laboratory colleagues of the author group (41 in number), Bacterial Special Interest Group of the British Association of Sexual Health & HIV (BASHH) mailing list (19, who forwarded it to a further 28), all consultants in sexual health in South West England (23), Laboratory personnel by Hologic user network (36 laboratories; 32% of laboratories in the UK) and UK Clinical Virology Network members (54 individuals viewed the questionnaire from 48 laboratories). Thus it is calculated that 201 individual workers received a questionnaire.

In order to define consensus, a modified Delphi methodology was used. [14] This approach works through written feedback, in order to measure consensus or agreement where differing opinions may exist. The level of individual agreement with each statement was measured using a 4-point Likert scale, which allows delegates to record levels of agreement or disagreement with each statement. Whilst the four point Likert scale allowed respondents to vary their level of agreement or disagreement (strongly or weakly), responses were interpreted in a binary fashion as either agreement or disagreement according to the Delphi

process. Following review of the responses as numbers increase, the process allows the modification of each statement if necessary in order to increase the level of agreement and therefore represent the majority view of respondents. In order to ensure that feedback was reviewed, the questionnaires were assessed as they were received. The levels of agreement with each statement were monitored by the group, who concluded that due to the high levels of agreement (84.4%) achieved by most statements, the requirement to modify the statements was obviated.

Reflecting best practice, an agreement threshold of 75% was considered for each statement as demonstrating consensus. [15, 16]

RESULTS

Of 201 individual recipients, sixty respondents from various UK localities returned completed questionnaires – a response rate of 29.8%. Overall, 27 (84.4%) of the statements achieved greater than 75% agreement with 19 (59.4%) achieving greater than 90% agreement across the respondent group. Five of the statements (15.6%) failed to meet the 75% agreement threshold (Table 1). Of the 60 completed questionnaires received back from respondents, the majority (48) were from sexual health consultants. The remainder were from consultants in Microbiology (2), Gynaecology (1), and Urology (1), in addition to responses from Laboratory personnel (2), Specialist Nurses (3), other disciplines (1) and two being of unknown discipline.This sample of sexual health consultants represents approximately 12% of the population in the UK, estimated to be 400 in number. [18]

Respondents indicated strong support for identification and effective targeted treatment of MG as detailed below and in Table 1.

Rationale for MG-testing

Respondents indicated strong consensus regarding the role of MG as a pathogen causing urethritis in men. There was less agreement regarding MG as a cause of discharge in women and proctitis in MSM, which may indicate a need for further evidence.

Selection of Patients

Strong agreement with all but two of the statements in this section of the questionnaire reflects the current evidence base. Respondents agreed that women with unexplained

persistent vaginal discharge should be tested for MG, but did not appear as confident that MG is a proven pathogen in this respect.

Diagnostic Strategy

Respondents strongly supported all three statements in this section. There was agreement by 84.7% of respondents that all men presenting with symptoms of urethritis or in whom urethritis is confirmed by microscopy should be tested for MG, *C. trachomatis* and *Neisseria gonorrhoeae* concurrently.

Cost Effectiveness

Most respondents believed targeted testing of symptomatic patients to be cost effective. Equally, respondents believed that asymptomatic screening may not be cost effective.

Antimicrobial Resistance / Antimicrobial Stewardship

Respondents strongly supported most of the statements within this section of the questionnaire. Only the assertion that patients tested as MG-positive should have a test of cure performed to confirm clearance failed to meet the 75% consensus threshold.

Availability of MG-testing

The statements that MG-testing and resistance testing should be available to all level 3 clinics were strongly supported by respondents.

DISCUSSION

A series of statements regarding MG- testing and treatment have been identified and were strongly supported by respondents to the questionnaire indicating the increasing importance of *M. genitalium* as a pathogen.

However, a limitation of this study is that there was a dominance of sexual health consultants among the respondents which is initially surprising as questionnaires were offered to a broad range of associated specialties including gynaecology and urology. This could suggest that the existence of MG is not well known outside sexual health clinical practice and as a consequence, non- sexual health consultants may be unfamiliar with the topic. Microbiologists, laboratory managers, senior scientific personnel and laboratory staff were

also invited to return questionnaires, but in most cases did not do so. The reasons for this are unknown. The results are therefore biased in favour of those who felt able to engage with the questionnaire and suggest that expert knowledge of MG is limited outside the sexual health specialty.

The high level of agreement with the majority of the statements (84.4%) indicates that respondents views echo those of the author group**;** 59.4% of the statements achieved agreement scores >90%. The remaining statements achieved varying levels of agreement.

Although MG is associated with cervicitis [1,2] and can, therefore, potentially cause a vaginal discharge, three studies of women with vaginal discharge, two in a community setting and one among high risk sexual dyads, did not provide any evidence of an association with the mycoplasma. [18-20] This may explain why in "selection of patients" there was a consensus that women with unexplained, persistent vaginal discharge should be tested. Although it is biologically plausible that MG causes proctitis**,** there is only one large study, published in 2016, that demonstrates an association of MG with proctitis. [22] Further evidence of the association of MG with vaginal discharge, particularly in those in whom this persists despite standard treatment, and in men and women with proctitis, is therefore needed.

With regard to the selection of patients for MG testing, early research indicated that only 2-5% of asymptomatic individuals with *C. trachomatis* in the general population were co-infected.[23-27] However, a more recent study from London suggested that co-infection might be as high as 9.7% in 15-19 year-old women infected by chlamydiae. [28] The earlier work may explain why respondents did not necessarily associate the two infections and this possibly reflects the low consensus for MG-testing of patients with chlamydial infection (50.9%). Respondents agreed that asymptomatic patients should not be tested**,** which may also explain the low consensus for MG-testing of patients with a chlamydial infection. Given that asymptomatic chlamydial infection in the community is often treated with azithromycin 1g **,** which is associated with the development of macrolide resistance in MG, the former explanation is the most likely. [9, 22]

There is currently no evidence to demonstrate whether screening does more good than harm at a reasonable cost. An important consideration when undertaking cost-effectiveness studies is the potential harm associated with development of anti-microbial resistance and its cost implications as a result of sub-optimal therapy in patients with MG who remain undetected .[8]

High levels of antimicrobial resistance are reported and specific treatment for MG is therefore important. [5] To this end, test of cure is advisable irrespective of the resolution of symptoms, as appropriate testing to ensure that the infection has cleared in response to treatment is an important tool in the management of antimicrobial resistance. [7] Surprisingly, while responses were positive (68.3% agreement), consensus was not reached supporting MG-test of cure, despite this being strongly recommended in the European guideline. [7] Perhaps this may be because respondents disagree with routine testing of cure, feeling that it is unnecessary when symptoms have abated as is currently recommended for men with NGU following treatment in the UK and USA. [29, 30] However, patients may become asymptomatic but remain persistently infected. [7, 31] It is reassuring to see that respondents are against unwarranted use of moxifloxacin, with strong consensus (statement 27**:** 93.2% agreement) reflecting concern over presumptive use, although moxifloxacin may be used specifically for PID according to a strong evidence base whether or not MG is present. [32] Almost all respondents agreed that use of 1g azithromycin should be avoided in the treatment of MG in order to reduce the risk of macrolide resistance. [5, 22] Currently, azithromycin 500mgs and then 250mgs od for 4 days is recommended to treat MG infection. [7, 33] While previous studies indicated that development of macrolide resistance was uncommon following treatment with this extended azithromycin regimen [34-36]**,** a more recent retrospective study from Australia indicated a similar rate to that observed historically with azithromycin 1g. [13] The reason for this apparent discrepancy is unclear and further prospective studies are therefore indicated. This reinforces the importance of undertaking a test of cure following treatment. Moxifloxacin can be used in those patients who fail azithromycin and/or in those patients with proven MG-macrolide resistance. In view of the concern regarding antimicrobial resistance there was strong support for resistance testing which is not yet routinely available.

Despite strong levels of agreement with the need for MG testing as advised by BASHH, it does not happen universally. This may be due to limitations in resources and capacity and the lack of awareness of the commercial availability of a NAAT for MG detection. Future guidelines from BASHH may strengthen the case that sexual health clinicians can expect laboratories to provide MG-testing.

Respondents clearly identified the following groups as having a high priority for MG-testing: men with symptoms of urethritis, women with symptoms of PID, women with unexplained persistent vaginal discharge or post-coital bleeding and current sexual contacts of MG-positive patients.

This consensus approach has two methodological limitations; firstly, the exact number of clinicians invited to complete the questionnaire is unclear and thus the response rate cannot be calculated with any accuracy, although we estimate it to be about 30%. Secondly, the responders are likely to show bias in their knowledge of the management of MG infection as they were clearly sufficiently interested to share their opinions. However, the strong representation of sexual health consultants offers a useful insight to the attitudes of this group of professionals with regard to testing for and management of MG infection.

RECOMMENDATIONS AND CONCLUSIONS

The following six recommendations are offered on the basis of this work:

- The following groups should be regarded as having a high priority for MG-testing:
    - Men with symptoms of urethritis
    - Women with symptoms of PID
    - Women with unexplained persistent vaginal discharge or post-coital bleeding
    - Current sexual contacts of MG-positive patients

- More evidence is needed for MG as a cause of unexplained and persistent discharge in women
- More evidence is needed for MG as a cause of proctitis in MSM and in women
- A UK MG guideline is likely to facilitate the introduction of targeted MG-testing and AMR-testing in clinical practice. Such a policy would be expected to be cost effective, but this remains to be demonstrated.
- Until a UK MG guideline becomes available, closer adherence to the European guidance is needed
- Test of cure is required following the use of azithromycin, and moxifloxacin used for further treatment if required

KEY MESSAGES

- Commercial availability of MG-testing presents an opportunity for improving the diagnostic and management pathways of several clinical syndromes associated with MG infection.
- Clinicians should ensure that their local laboratories meet the requirements of accepted guidelines for MG-testing
- Test of cure is advisable irrespective of the resolution of symptoms, to ensure that the infection has cleared in response to treatment
- 1g azithromycin should be avoided in the treatment of MG infection in order to reduce the risk of macrolide resistance

CONTRIBUTORS

SG convened the panel, facilitated the meetings and prepared the first draft of the manuscript. CAI, HF, PH, PM, JN, KR, JR, DTR and JW were members of the expert panel and commented on all drafts and approved the final manuscript.

COMPETING INTERESTS

CAI, SG, PH, PM, JN, KR, JR, DTR and JW were in receipt of a paid consultancy for this study. CAI is in receipt of a patent for the *Mycoplasma genitalium* detection assay based on MG219 gene (PCT/GB2007/001913 and foreign equivalents). HF is a member of the Scientific Advisory Board for Discuva Ltd. PH had non-financial support from Hologic, during the conduct of the study; personal fees from Crown Prosecution service, personal fees from British Association for Sexual Health and HIV, grants from Mast Group Ltd, non-financial support from Hologic, outside the submitted work. In addition, PH has a patent A sialidase spot test to diagnose bacterial vaginosis issued to University of Bristol. PM has been in receipt of grants, conference expenses and consultancy fees from Hologic Inc and grants from Elitech UK Ltd. JN has received fees from Hologic for conference presentations. KR is a consultant to Hologic. JR has been in receipt of a paid consultancy for GSK pharma and for Hologic. DTR has no other competing interests. JW has previously received research and educational support and hospitality from BD Diagnostics, Genprobe/Hologic, Abbott Diagnostics, Alere, Viiv, Gilead, Boerhinger Ingelheim, Abbott Pharmaceuticals, Diagnostics Development Unit/DRW, University of Cambridge, and is Editor in Chief of Int J STDs & AIDS, SAGE publishing.

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