

# Laboratory detection of bacterial pathogens and clinical and laboratory response of syndromic management in patients with cervical discharge: A retrospective study

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## Abstract

**Background:** Cervical discharge as part of cervicitis and pelvic inflammatory disease is a cause of significant morbidity in sexually active women worldwide. Non-gonococcal and non-chlamydial bacterial pathogens are becoming more prevalent.

**Aims:** This study aims to determine bacterial pathogens causing cervical discharge using culture and/or polymerase chain reaction and assess the clinical and laboratory response to the conventional syndromic kit regimen established by the World Health Organisation.

**Methods:** A retrospective review of records of women with cervical discharge over one year period. Culture and/or polymerase chain reaction results of endocervical swabs of various bacterial pathogens at baseline and after four weeks of treatment with syndromic kit regimen were recorded.

**Results:** A total of 70 case records were reviewed for clinical details, out of which results of bacterial culture and polymerase chain reaction were available for 67 cases. Infectious aetiology was found in 30 (44.7%) patients with *Ureaplasma* species being the most common organism isolated on culture (18, 26.8%) and polymerase chain reaction (25, 37.3%), respectively. Polymerase chain reaction for *Chlamydia trachomatis* and *Mycoplasma hominis* was positive in ten (14.9%) and four (6%) cases, respectively. None of the patients showed positive culture for *Neisseria gonorrhoeae*. Coinfection was seen in eight (11.9%) patients with the majority showing *Chlamydia trachomatis* and *Ureaplasma* spp. coinfection (five patients). Forty one cases (58.5%) received tab. cefixime 400 mg and tab. azithromycin one gram stat (kit 1), while 29 cases (43.3%) received tab. cefixime 400 mg stat, tab. metronidazole 400 mg and cap. doxycycline 100 mg, both twice daily for 14 days (kit 6). Minimal to no clinical improvement with treatment was seen in 14 out of 32 cases (44%) at the end of four weeks with the conventional kit regimen. Post-treatment culture and/or polymerase chain reaction were positive in nine out of 28 cases (32.1%) with *Ureaplasma* spp. being the most common.

**Limitations:** Retrospective study design, small sample size and fewer cases with follow-up data were the main limitations.

**Conclusion:** *Ureaplasma* spp. was the most common infectious cause of cervical discharge in our patients. Treatment given as part of syndromic management led to a clinical and microbiological response in around half and two-third cases, respectively.

**Keywords:** Cervical discharge, cervicitis, pelvic inflammatory disease, syndromic management, genital mycoplasma

## Plain Language Summary

Cervical discharge is a common cause of morbidity in sexually active females worldwide. It can be either due to inflammation of cervix alone (cervicitis) or involvement of upper genital tract (pelvic inflammatory disease). It is either infectious or non-infectious in nature. Infectious causes include various bacteria, viruses and protozoa. Among bacteria, *Neisseria gonorrhoeae*

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and *Chlamydia trachomatis* are commonly implicated. However, other bacteria, especially genital mycoplasma (*Ureaplasma* spp., *Mycoplasma genitalium* and *Mycoplasma hominis*), are becoming more common. In India, cervical discharge is treated with a set of drugs (kit 1 or kit 6) as part of syndromic management under National Aids Control Organisation. The present study reviewed the bacterial causes of cervical discharge using culture and molecular methods and the response to treatment with syndromic kit regimen. We found an infectious cause in 30 out of 67 cases. *Ureaplasma* spp. was the most common agent isolated on culture in 18 cases and by molecular methods in 25 cases. Infection with *Chlamydia trachomatis* and *Mycoplasma hominis* was found in ten and four cases, respectively. None of the cases showed infection with *Neisseria gonorrhoeae*. Coinfection with two or more organisms was seen in eight cases. Forty one cases received kit 1, while 29 cases took kit 6. No clinical improvement was seen in 14 out of 32 cases after one month of treatment. *Ureaplasma* spp. was the most common organism isolated at follow-up.

## Introduction

Cervical discharge as part of cervicitis (CS) or pelvic inflammatory disease is a significant cause of morbidity in sexually active women worldwide. Syndromic management forms an essential cost-effective strategy to tackle sexually transmitted infections in developing countries. It involves identifying a particular sexually transmitted infection syndrome based on the constellation of signs and symptoms followed by its treatment with antimicrobial agents covering major pathogens responsible for the syndrome. In India, the National Aids Control Organisation attends to the sexually transmitted infection needs of the population based on syndromic management.<sup>1</sup> Under National Aids Control Organisation, cervical discharge and lower abdominal pain are two such syndromes representative of cervicitis and pelvic inflammatory disease, respectively. While cervical discharge syndrome is treated with tablet cefixime 400 mg and tablet azithromycin 1 gram stat (kit 1), lower abdominal pain syndrome is treated with tablet cefixime 400 mg stat, tablet metronidazole 400 mg and capsule doxycycline 100 mg, both twice daily for 14 days (kit 6).

Cervicitis is caused mainly by bacterial pathogens although viruses such as herpes simplex virus, adenovirus, cytomegalovirus and protozoa (*Trichomonas vaginalis*) can be responsible in few cases. Among the bacterial pathogens, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are commonly implicated organisms. Nevertheless, non-gonococcal, non-chlamydia cervicitis caused by organisms such as *Ureaplasma* spp., *Mycoplasma hominis* and *Mycoplasma genitalium* is becoming common. The importance of isolating these newer pathogens lies in the fact that some of these are resistant to usual antibiotics used for the treatment of cervical discharge, especially in the context of syndromic management. If left untreated, these organisms may severely impact maternal and reproductive health and additionally lead to increased acquisition and transmission of human immunodeficiency virus.

Similarly, the primary organisms causing pelvic inflammatory disease include *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, although anaerobes have also been implicated as secondary organisms.

Here, we present a retrospective study conducted to determine the bacterial pathogens causing cervicitis or pelvic inflammatory disease and to assess the clinical and laboratory response to the conventional kit regimen established by National Aids Control Organisation.

## Methods

This was a retrospective review of records of cases of cervical discharge/lower abdominal pain (either cervicitis or pelvic inflammatory disease cases) who visited the sexually transmitted disease clinic of All India Institute of Medical Sciences, New Delhi, between December 2016 and November 2017. Records of women with complaints of vaginal discharge and/or lower abdominal pain who were subsequently found to have cervical discharge on per speculum examination and had >30 polymorpho-leucocytes per high-power field on microscopy of endocervical swab were included in the study. Cervicitis was defined as the presence of purulent or mucopurulent discharge at the cervical os and/or sustained endocervical bleeding easily induced by gentle manipulation. Those patients who, in addition to cervical discharge, had lower abdominal pain, cervical motion tenderness or adnexal tenderness on pelvic examination were classified as pelvic inflammatory disease. The records of these cases were searched for details of history, examination (especially concerning sexually transmitted infections) and treatment received (kit 1 or 6). All patients were advised abstinence till the follow-up visit and partner treatment was given, wherever indicated, as part of routine care. The results of the culture and polymerase chain reaction of endocervical smears were collected. *Neisseria gonorrhoeae* was cultured on modified Thayer-Martin medium containing vancomycin, colistin, nystatin, trimethoprim inhibitors and chocolate agar (Columbia agar base plus sheep blood). Oxidase superoxol test and rapid carbohydrate utilisation test were used for confirmation of isolates. Pleuropneumonia-like organisms medium broth (Difco, USA) containing urea and arginine was used for the culture of *Ureaplasma* spp. and *Mycoplasma hominis*, respectively. For polymerase chain reaction, DNA was extracted using the QIAamp Mini Kit (QIAGEN, Hilden, Germany) and stored at  $-20^{\circ}\text{C}$  till further use. A multiplex polymerase chain reaction targeting the urease gene of *Ureaplasma* spp. and 16Sr DNA of *Mycoplasma hominis* was performed. For *Chlamydia trachomatis*, a DNA polymerase

chain reaction was performed targeting the cryptic plasmid using primers KL-1 and KL-2 and confirmed by a second polymerase chain reaction targeting the ompA gene. Cases with incomplete clinical, treatment and microbiological records, as also those with sexually transmitted diseases other than cervicitis or pelvic inflammatory disease were excluded from the study. Clinical and microbiological records were reviewed after four weeks of initial treatment for the assessment of treatment response. Descriptive statistics were used for the analysis of the data collected.

## Results

A total of 70 case records were reviewed with a mean age of 33.2 years (19–57 years). The majority of the women had non-foul-smelling discharge (59, 84.3%) in a moderate amount (33, 47.1%) with white colour (37, 52.9%) being most common followed by yellowish (17, 24.3%) colour. Cervical erosion and tenderness were seen in 12 (17.4%) and 17 (24.3%) cases, respectively [Table 1].

Bacterial culture and polymerase chain reaction results of 67 cases (cervical discharge: 41 cases and pelvic inflammatory disease: 26 cases) were available for final analysis. Out of 67 cases, infectious aetiology was found in 30 (44.7%) patients. *Ureaplasma* spp. was the most common organism isolated on culture (18, 26.8%) followed by *Mycoplasma hominis* (3, 4.5%). Similarly, polymerase chain reaction for *Ureaplasma* spp., *Mycoplasma hominis* and *Chlamydia trachomatis* was positive in 25 (37.3%), 4 (6%) and 10 (14.9%) patients, respectively. None of the cases showed positive culture for *Neisseria gonorrhoeae* [Table 2]. Coinfection was seen in eight cases (11.9%) with the majority showing *Chlamydia trachomatis* and *Ureaplasma* spp. coinfection (five patients). One patient each showed coinfection with *Chlamydia trachomatis* and *Mycoplasma hominis*; *Ureaplasma* spp. and *Mycoplasma hominis*; *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma* spp., respectively.

Syndromic treatment was given to all 70 patients, 41 cases (58.5%) received kit 1 and the remaining 29 cases (41.5%) received kit 6. The record for clinical response after four weeks of treatment was available in 32 patients, of which 15 (46.9%) patients showed complete improvement while 14 (43.8%) patients showed minimal to no clinical improvement [Table 1].

Repeat investigations for test of cure were sent in 28 patients at the end of four weeks, of which an infectious aetiology was found in nine (32.1%). Post-treatment culture for *Ureaplasma* spp. and *Mycoplasma hominis* was positive in six (21.4%) and two (7.1%) patients, respectively. Polymerase chain reaction for *Ureaplasma* spp., *Mycoplasma hominis* and *Chlamydia trachomatis* was positive in eight (28.5%), two (7.1%) and one (3.6%) patients, respectively [Table 3]. Few of these cases showed positivity at follow-up, while being negative at baseline (two cases: *Mycoplasma*

**Table 1: Demographic and clinical profile of cases and controls**

Feature	Cases, n=70 (%)
Mean age (range)	33.2 years (19–57)
Heterosexual exposure	70 (100)
Women having sex with women	0 (0)
Extramarital exposure	13 (19.4)
Premarital exposure	5 (7.1)
Penovaginal sexual contact	70 (100)
Peno-oral sexual contact	5 (7.1)
Penoanal sexual contact	1 (1.4)
Barrier protection use	42 (60)
Total no. of partners (average)	1.3
Total no. of partners (range)	1–6
History of the past sexually transmitted infections	17 (24.3)
Family type	
Nuclear	38 (54.3)
Joint	32 (45.7)
History of abortion	36 (51.4)
History of infertility	2 (2.9)
History of intrauterine death	10 (14.3)
History of use of intrauterine contraceptive device	16 (22.9)
History of dyspareunia	28 (40)
History of dysuria	21 (30)
Lower abdominal pain	29 (41.4)
Fever	9 (12.9)
Substance abuse	3 (4.3)
History of chronic illness	20 (28.6)
Colour of cervical discharge	
Whitish	37 (52.9)
Yellow	17 (24.3)
Slightly yellow	14 (20)
Greyish	2 (2.9)
Smell of discharge	
Non-foul	59 (84.3)
Foul	11 (15.7)
Amount of discharge	
Mild	22 (31.4)
Moderate	33 (47.1)
Profuse	15 (21.4)
Cervical erosion	12 (17.4)
Cervical tenderness	17 (24.3)
Treatment received	KIT 1: 41 (58.5) KIT 6: 29 (41.4)
Clinical improvement	
Complete	15/32 (46.9)
Moderate	3/32 (9.4)
Minimal to none	14/32 (43.8)

*hominis* culture and polymerase chain reaction; two cases: *Ureaplasma* species polymerase chain reaction and one case: *Chlamydia trachomatis* polymerase chain reaction). Two patients showed coinfection, one each with *Mycoplasma*

**Table 2: Pre-treatment results of culture and molecular tests of endocervical swabs**

	Total cases, n = 67 (%)	Cervicitis cases, n = 41 (%)	Pelvic inflammatory disease cases, n = 26 (%)
Infectious aetiology	30 (44.7)	15 (36.5)	15 (57.6)
<i>Mycoplasma hominis</i> (culture)	3 (4.5)	1 (2.4)	2 (7.6)
<i>Mycoplasma hominis</i> (PCR <sup>#</sup> )	4 (6)	2 (4.9)	2 (7.6)
<i>Ureaplasma</i> species (culture)	18 (26.8)	9 (21.9)	9 (34.6)
<i>Ureaplasma</i> species (PCR <sup>#</sup> )	25 (37.3)	12 (29.3)	13 (50)
<i>Chlamydia trachomatis</i> (PCR <sup>#</sup> )	10 (14.9)	5 (12.2)	5 (19.2)
<i>Neisseria gonorrhoeae</i> (culture)	0 (0)	0 (0)	0 (0)

<sup>#</sup>PCR: Polymerase chain reaction

**Table 3: Post-treatment results of culture and molecular tests of endocervical swabs**

	Total cases, n=28 (%)	Cervicitis cases, n=13 (%)	Pelvic inflammatory disease cases, n=15 (%)
Infectious aetiology	9 (32.1)	5 (38.4)	4 (26.6)
<i>Mycoplasma hominis</i> (culture)	2 (7.1)	1 (7.6)	1 (6.6)
<i>Mycoplasma hominis</i> (PCR*)	2 (7.1)	1 (7.6)	1 (6.6)
<i>Ureaplasma</i> species (culture)	6 (21.4)	4 (30.7)	2 (13.3)
<i>Ureaplasma</i> species (PCR*)	8 (28.5)	5 (38.4)	3 (20)
<i>Chlamydia trachomatis</i> (PCR*)	1 (3.6)	1 (7.6)	0 (0)
<i>Neisseria gonorrhoeae</i> (culture)	0 (0)	0 (0)	0 (0)

\*PCR: Polymerase chain reaction

*hominis* and *Ureaplasma* spp. and *Chlamydia trachomatis* and *Ureaplasma* spp., respectively.

## Discussion

Cervical discharge seen in CS and pelvic inflammatory disease is a significant cause of morbidity in sexually active women worldwide. Among the demographic data, the common risk factors found were non-usage of barrier protection (28 cases, 40%), history of past sexually transmitted infections (17 cases, 24.3%) and extramarital exposure (13 cases, 19%). Infectious aetiology could not be established in a large proportion of patients primarily due to lack of laboratory facilities. In our study, an infectious cause was found in 46% of cases, which is higher than in previous studies, 39% and 20%, respectively.<sup>2,3</sup> A plausible explanation could be the availability and use of highly sensitive nuclear amplification methods, as our centre is a tertiary care hospital. Over the years, more and more studies have detected non-gonococcal, non-chlamydial causes of cervical discharge.<sup>3-5</sup> We found *Ureaplasma* spp. as the most common isolate detected in around 37% of cases followed by *Chlamydia trachomatis* in 15% and *Mycoplasma hominis* in 6%. McIver *et al.* found genital mycoplasma using multiplex polymerase chain reaction in 135 out of 175 (77%) women attending sexually transmitted infection clinics. *Ureaplasma parvum* was the most common organism (93/175, 53%) followed by *Mycoplasma hominis* (13/175, 7.4%).<sup>4</sup> Similar results were obtained by Leli *et al.* in 1761 symptomatic women (*Ureaplasma parvum*: 38.3%, *Ureaplasma urealyticum*: 9%, *Mycoplasma hominis*:

8.6% and *Mycoplasma genitalium*: 0.6%).<sup>5</sup> In a recent Chinese study, *Ureaplasma urealyticum* was found in 62% of patients and *Chlamydia trachomatis* in 10%, using real-time polymerase chain reaction.<sup>6</sup> However, lower rates of genital mycoplasma were noted in an Indian study by Arif *et al.* Among 218 endocervical swabs, *Ureaplasma* spp., *Mycoplasma hominis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were found in 5.6%, 2.6%, 8% and 3%, respectively.<sup>3</sup> Notably, culture for *N. gonorrhoea* was negative in all patients. Bhargava *et al.* found low positivity rate (<0.5%) for detecting *N. gonorrhoea* by culture and/or microscopy in women with vaginal or cervical discharge.<sup>7</sup> In another study from India, *Neisseria gonorrhoeae* was not detected using culture and/or microscopy in 319 women with vaginal discharge syndrome.<sup>8</sup> Such low positivity rates using two modalities (culture and microscopy) once again raises the issue of over-treatment with syndromic management, even though low positivity rates could be improved by using molecular methods like polymerase chain reaction. Regarding coinfection, we noted it in 12% of cases, with coinfection by *Ureaplasma* spp. and *Chlamydia trachomatis* being the most common. Arif *et al.* found coinfection in 2% with *Ureaplasma* and *Chlamydia trachomatis* being the most common organisms.<sup>3</sup> Cai *et al.* found *Ureaplasma* and *Chlamydia trachomatis* coinfection in 7.3% of cases.<sup>6</sup> The coinfection of *Chlamydia trachomatis* with *Ureaplasma* has survival advantage as indicated in a few studies.<sup>9</sup>

Besides establishing the bacterial aetiology of cervical discharge, our study also looked at the clinical and laboratory

response to syndromic management. Clinically around half of the patients showed minimal improvement. Bacterial pathogens were identified after four weeks of treatment in up to one-third of the tested cases. The positivity rates were similar in culture and polymerase chain reaction, ruling out false-positive polymerase chain reaction results due to the persistence of dead pathogen remnants. However, follow-up data of only one-third of cases were available in our study. Nonetheless, it points towards the poor efficacy of syndromic management in clearing cervical infection. This indirectly raises the issue of increasing antibiotic resistance. Both *Mycoplasma hominis* and *Ureaplasma* have been reported to have resistance to azithromycin (used in kit 1) with the former being intrinsically resistant. Zhu *et al.* found resistance to azithromycin in 85.9% and 15.2% isolates of *Mycoplasma hominis* and *Ureaplasma*, respectively.<sup>9</sup> Skiljevic *et al.* noted resistance to azithromycin in 50% and 32% isolates of *Mycoplasma hominis* and *Ureaplasma*, respectively.<sup>10</sup> Resistance to doxycycline was, however, less than 15% in both these studies.<sup>10,11</sup> Given the intrinsic resistance of *Mycoplasma hominis* to azithromycin and increased level of resistance for azithromycin for *Ureaplasma* infection, doxycycline should be preferred whenever possible. Oral doxycycline 100 mg twice daily for seven days is the first-line regimen for the management of cervicitis according to latest sexually transmitted infections treatment guidelines.<sup>12</sup> Other agents found effective includes fluoroquinolones and macrolides. Other reasons for lack of response to treatment in our study could be infection with other bacterial and non-bacterial agents such as *Mycoplasma genitalium*, herpes simplex virus and *Trichomonas vaginalis*; reinfection from an untreated partner or a new partner and lack of compliance to treatment. Genital mycoplasma has been implicated in various complications in women such as adverse pregnancy outcomes, infertility and increased human immunodeficiency virus transmission. Hence, appropriate treatment with correct antibiotics is of utmost importance, more so in empirical therapy as part of syndromic management.

### Limitations

A small sample size and the retrospective nature of the study are the major limiting factors. Follow-up was available for only one-third of cases. Biovar typing of *Ureaplasma* spp., polymerase chain reaction for *Mycoplasma genitalium* and antibiotic resistance testing were not performed. The lack of a control group was another limitation.

### Conclusion

*Ureaplasma* spp. was found to be the most common infectious cause of cervical discharge in our patients. This shows the changing trend of cervicitis toward the non-gonococcal, non-chlamydia cause. Treatment given as part of syndromic management led to a clinical and microbiological response in around half and two-third cases, respectively. The external validity of our results needs to be established in future larger multicentre prospective studies.

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### Declaration of patient consent

The patient's consent is not required as the patients' identities are not disclosed or compromised.

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### Conflicts of interest

There are no conflicts of interest.

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