

Novel Techniques in Microbiology to Discover Unsuspected Microbes Causing Tubo-Ovarian Abscesses

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ABSTRACT

Background: Next-Generation Sequencing (NGS) is a relatively unknown diagnostic technique. The applications and clinical value of NGS in detecting pathogens is growing and the technique is superior to conventional culture. It is capable to detect bacteria at very low DNA levels (high sensitivity), has a very high specificity, it can detect mixed infections and fastidious micro-organisms.

Cases: In this article we present two women with tubo-ovarian abscesses and NGS helped guide antibiotic treatment indirectly. Although NGS did not provide information on antimicrobial susceptibility of the individual bacterial strains, identification of micro-organisms predicted antimicrobial sensitivity based on average epidemiology.

Conclusion: The advantage of this technique is that antibiotics can be used more specifically. As a result, there is less resistance, side effects, costs and better outcomes.

Keywords: Microbiology; Pathogens; Tubo-ovarian abscesses

LIST OF ABBREVIATIONS

IUD:	Intra Uterine Device
Cm:	Centimetre
CRP:	C-Reactive Protein
PCR:	Polymerase Chain Reaction
PID:	Pelvic Inflammatory Disease
Ct:	Cycle threshold

Two teaching points:

1. Antibiotic treatment in most PID cases is important and must provide broad spectrum coverage of possible pathogens such as *N. gonorrhoeae*, *C. trachomatis* and enteric flora (Enterobacterales and anaerobes).
2. Next-Generation Sequencing is a relative new technique that should be performed if conventional culture is negative in women with tubo-ovarian abscesses.

INTRODUCTION

Next-Generation Sequencing (NGS) is, to many clinicians, still a relatively unknown diagnostic technique. The applications and clinical value of NGS in detecting pathogens is growing and the technique is superior to conventional culture. NGS is capable to detect bacteria at very low DNA levels (high sensitivity), has a very high specificity, it can detect mixed infections and fastidious micro-organisms. We present two women with tubo-ovarian abscesses and NGS helped guide antibiotic treatment.

Case A

A 21-year-old female patient visited the emergency room with abdominal pain and fever (38.5°C). For two weeks she had progressive lower abdominal pain. Her medical history was unremarkable except for an appendectomy 13 years before. One month before, an Intra Uterine Device (IUD) was inserted. Prior to the placing of the IUD the patient was tested negative for sexual transmitted diseases. Ultrasound sonography after insertion of the IUD showed a normal position of the IUD inside the uterus.

At presentation she reported no complaints of nausea, vomiting, dysuria, or abnormal defecation. Examination of the abdomen was painful and suggestive for local peritonitis. There was no purulent vaginal discharge. Transvaginal ultrasonography of the uterus was unremarkable. The left ovary contained a simple cyst of 2 centimetre (cm). The right ovary showed two abnormalities of 8x8 cm and 4x4 cm, containing fluid; suggestive for abscesses or endometriomas (Figure 1).



Figure 1: The right ovary containing two abnormalities suggestive for abscesses.

Laboratory tests showed normal leucocytes and an elevated level of the C-reactive protein (CRP) of 178 mg/L. Pelvic inflammatory disease was suspected with tubo-ovarian abscesses (TOA). She was admitted to the gynaecological ward and intravenous treatment with tobramycin, ofloxacin and cefazolin was started after taking blood cultures and cultures of the cervix.

After three days later there was no clinical improvement, CRP had increased to 277mg/L and she remained febrile. It was decided to drain the suspected abscesses. Through a transabdominal puncture 1.5 liter of pus was drained from the ovarian abscesses and sent microbiological culture.

Microbiological cultures of the pus performed on conventional media for aerobic and anaerobic bacteria, yeast and Actinomyces all came up negative. PCR on *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and blood cultures were negative. However, next-generation sequencing (NGS)^[1] identifying bacteria by the specific DNA sequence of the 16S-23S rRNA gene region was performed directly on the pus specimen yielding a strong match with *Sneathia sanguinegens* (99.87% DNA ID match for 100% of the fraction, Ct 32).

Treatment with metronidazole, tobramycin, ofloxacin and cefazolin was continued for 14 days. The patient became asymptomatic and repeat ultrasonography after 3 months showed complete remission of the abscesses.

Case B

A 28-year-old female patient presented herself at the emergency room with complaints of abdominal pain. Blood pressure, pulse and temperature were normal. Her medical history included an appendectomy 17 years before. Examination of the abdomen was painful and no purulent vaginal discharge was seen. Transvaginal ultrasonography showed two cysts with sizes of 4.4x3.6 cm and 3.6x2.1 cm suspected to originate from the left ovary. Laboratory tests showed elevated blood leucocytes 15.8 10e9/L and CRP 142 mg/L.

Because of a high suspicion of a torsion of the left ovary a diagnostic laparoscopy was performed, which revealed a pyosalpinx on the left side. A therapeutic puncture of the left ovary was performed and was sent for microbiological examination. Intravenous antibiotic treatment was started: a single dose of ceftriaxone combined with metronidazole and levofloxacin for in total two weeks. On day 9 the patient returned to the hospital with recurrent abdominal pain. On ultrasonography a fluid collection of 8x8 cm (Figure 2) was demonstrated and subsequently drained.



Figure 2: Ultrasound image when patient returned on day 9.

Microbiological cultures came up negative and NGS was performed directly on the purulent fluid, yielding the DNA sequencing a *Campylobacter jejuni* (100.0% DNA ID match for 100% of the fraction, Ct 37). After the second drainage of the abscess the patient improved and recovered completely. Directed antimicrobial treatment against *C. jejuni* (azithromycin) was considered but abstained because of full clinical recovery.

DISCUSSION

In most cases of PID, conservative treatment by either oral or intravenous antibiotic treatment will suffice, however in complicated cases like the aforementioned abscess formation can be of such a degree that abscess drainage is necessary. Antibiotic treatment in most PID cases is empirical due to lack of intra-abdominal cultures and hence absence of antimicrobial susceptibility data. Therefore the antibiotic regime must provide broad spectrum coverage of possible pathogens such as *N. gonorrhoeae*, *C. trachomatis* and enteric flora (Enterobacterales and anaerobes).

In complicated PID cases cultures from the abscess fluid can help guide antibiotic treatment. However, as both cases demonstrate, conventional microbiological culture techniques can come up negative. In such cases NGS should be considered. Both *Sneathia* sp. and *Campylobacter* sp. require special growth media and are considered fastidious micro-organisms. *Sneathia* sp. are considered part of the normal vaginal microbiome; whether introduction of the IUD may have translocated *Sneathia* sp. and caused PID is debatable, however, it was the single micro-organism detected and *Sneathia* have been reported to ascent from the lower genital tract.^[3] *Campylobacter* sp. are known to cause a variety of abscesses and has also been detected in TOA before.^[4]

NGS (16-23S) is, to many clinicians, still a relatively unknown diagnostic technique. The applications and clinical value of NGS in detecting pathogens is growing and the technique is superior to conventional culture in many ways: it is capable to detect bacteria at very low DNA levels (high sensitivity), has a very high specificity, it can detect mixed infections and fastidious micro-organisms.^[1,2] Because of relative high costs of NGS, it should be considered

foremost in culture-negative complicated infections. NGS is not available in the average microbiology laboratory but samples can be sent to referral laboratories for analysis.

In both cases NGS helped guide antibiotic treatment indirectly: although NGS did not provide information on antimicrobial susceptibility of the individual bacterial strains, identification of micro-organisms did help predict antimicrobial sensitivity based on average epidemiology. Furthermore, in both cases an etiologic agent was demonstrated, which helps to determine prognosis and medical outcome. The advantage of this technique is that antibiotics can be used more specifically. As a result, there is less resistance, side effects and costs. A quicker and better effectiveness of the therapy is possible. Furthermore there will be no doubt about the diagnosis such as endometriosis or ovarian cancer. In this way, surgical intervention can be prevented. Future fertility will be impaired, due to the infection and ovarian abscesses, but with this technique and fast treatment maintained as much as possible. Moreover, NGS detects more micro-organisms in general and hence more non-sexually-transmitted-disease related micro-organisms, helping patients to overcome any prior psychological burden related to fear of having a sexual transmitted disease.

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