

Sexually transmitted infection (sti) case study



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Sexually Transmitted Infection (STI) Case Study 5

Case study 5

A male, 24 years of age presents to the STI clinic. He complains of a burning and sore sensation upon urination, along with discharge from his penis that has a mucopurulent consistency and is green-yellow in colour. In the preceding two weeks, he has had unprotected sex with numerous partners.

Laboratory tests

A penile/urethral swab is taken from the male and is inoculated onto NYC agar and chocolate agar. The plates are incubated at 37°C in CO₂ at the clinic and later that evening are transported to the microbiology laboratory. A Gram stain is carried out on a smear of the penile discharge. The patient is also screened for other STI's and is interviewed in relation to contact tracing his sexual partners.

Results

The following are the results obtained for the organisms growing on the chocolate agar and the organism growing on the NYC agar. Both agars were incubated in CO₂ at 37°C. Two organisms, A and B, were growing on the chocolate agar. Organism B was growing on both agars. This organism was identified as *Neisseria gonorrhoeae*. The preliminary identification of organism A was not obtained. *Neisseria gonorrhoeae* is the causative pathogen of gonorrhoeae, a sexually transmitted infection that is characterised by a pus filled infection of the surfaces of the mucous membranes of the throat, eye, vagina and urethra in males and females. This pathogen can be spread through direct sexual contact or through

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vertical transmission from mother to baby during birth. Symptoms of this bacteria in males include painful urination and urethral discharge, while females present with increased vaginal discharge. Usually females infected with this pathogen present as asymptomatic and are the biggest reservoir of this STI (Edwards and Apicella, 2004).

Table 1: Basic characterisation test results carried out for the chocolate agar plate and the NYC agar plate that were both incubated in CO₂ at 37°C for the 24 year old male patient in the STI Case Study 5. The preliminary identification of organism A was not obtained.

Plate name:	Chocolate agar plate incubated in CO ₂ at 37°C		NYC agar plate incubated in CO ₂ at 37°C		Control	
	Organism A	Organism B	Organism A	Organism B	Is: Positive	Negative
Colonial morphology	0.5mm, grey, smooth, circular, entire, no convex, odour.	0.5mm, light grey, smooth, circular, entire, no odour.	0.5mm, green, smooth, circular, entire, no odour.	0.5mm, /	/	/

odour.

Gram stain	Gram negative bacilli	Gram negative diplococci	Gram negative diplococci	/	/
Catalase	+	+	+	+	-
Oxidase	-	+	+	+	-
Preliminary identification	/	<i>N eisseria gonorrhoeae</i>	<i>N eisseria gonorrhoeae</i>	/	/

Legend:

Catalase: + = positive for the enzyme catalase- bubbles produced.

- = negative for the enzyme catalase -no bubbles produced

Oxidase: + = positive for the enzyme oxidase - purple colour formed

- = negative for the enzyme oxidase - no colour formed

Discussion

From the clinical details given in Case study 5 and from the basic characterisation tests, it is evident that the causative pathogen of the patient's dysuria and penile discharge and the organism that was growing as

organism B on chocolate agar and growing on the NYC agar is *Neisseria gonorrhoeae*.

There are numerous further tests that could be carried out to confirm this causative pathogen *Neisseria gonorrhoeae* that the patient in this case study is infected with. This pathogen should be confirmed using two different methods of detection such as biochemical such as the API NH strip for *Neisseria* and *Haemophilus* species and molecular and serological testing. Such tests include the Nucleic Acid Hybridization Test (NAAT) that utilises a DNA probe that is labelled with a chemiluminescent tag and is targeted to a region of the 16s rRNA of the *Neisseria gonorrhoeae* pathogen that is mixed with the patient's sample. This assay is based on the hybridization of nucleic acids. In the patient's sample if the pathogen is present, rRNA released from *Neisseria gonorrhoeae* will hybridize with the probe DNA. The probe that is not hybridized is removed. The DNA: RNA hybrids luminescence intensity is then measured. Samples used for this testing are endocervical and urethral swabs (Sood et al., 2014). According to the HPSC, NAAT testing is the standard test for the laboratory detection of *Neisseria gonorrhoeae*. The enzyme tube test, Gonocheck II can differentiate between the various *Neisseria* species such as *Neisseria meningitidis*, *Neisseria lactamica* and *Neisseria gonorrhoeae*. Specimens used for this test are well isolated colonies from either Modified Thayer Martin or chocolate agars. Enzymes produced by the bacteria act on colourless substrates to produce coloured end products. *Neisseria meningitidis* produces an end product that is yellow. *Neisseria gonorrhoeae* produces three enzymes – gammaglutamylaminopeptidase, hydroxyprolylaminopeptidase and

betagalactosidase and produce a red-pink coloured end product, confirming this pathogen (CDC, 2018). The GeneXpert CT/NG System by Cepheid is a real time PCR NAAT platform that allows sample preparation, amplification and detection of *Neisseria gonorrhoeae* from patient urine samples, male urethral swabs and female vaginal and endocervical swabs in 90 minutes (Gaydos et al., 2013). The Abbott RealTime CT/NG utilises RT-PCR and a fluorescent labelled oligonucleotide probe that allows for direct, real-time, fluorescent, qualitative detection of the genomic DNA of *Neisseria gonorrhoeae* and plasmid DNA of *Chlamydia trachomatis* from patient urine samples, male urethral swabs and female vaginal and endocervical swabs (Gaydos et al., 2010).

Neisseria gonorrhoeae possesses a wide abundance of virulence factors that enable it to efficiently establish infection and adapt to its host's environment, as it did in this patient in the case study. The entry site of this bacteria in males is the urethral cells of the penis and the vagina in females. This pathogen mainly infects the epithelia of the urogenital tract and infects areas such as the rectal mucosa, pharynx and conjunctiva less commonly.

Neisseria gonorrhoeae, with its repertoire of adhesion molecules attaches to the cuboidal and columnar epithelial cells present in the urethra, pharynx, endocervix and ano-rectal region. Such adherence molecules include pili, porin proteins – Opa and PI and type IV fimbriae. These adhesion molecules bind to host carcinoembryonic antigen cell adhesion molecules (CEACAM) receptors present on epithelial cells. Once attached to these receptors, the pathogen then rapidly proliferates and spreads up through the urethra in males and the cervix to the fallopian tubes in females where the infection

and healing processes causes fibrosis, blockage and damage to the tubes. These adhesion molecules are able to evade being removed by vaginal discharge or urine. The pili and fimbriae facilitate attachment to the mucosal epithelium and the pili protein genes possess hypervariable and constant regions that enable the pathogen to exhibit antigenic variation by recombination of its surface antigens. This proves difficult in developing a vaccine for this bacteria and also for the production of host antibodies that are only effective for a short duration and so, are not protective against this bacteria. Pili also enable twitching motility that allows the bacteria to ascend the mucous lined surfaces (Edwards and Apicella, 2004). Porin protein (PI) is responsible for forming pores in the host cell membrane and induces apoptosis in the epithelial cells causing the shed of epithelial cells and fallopian tube damage in females. However, in a study carried out by using Chang epithelial cells, an anti-apoptotic role of porin proteins was hypothesized. It was found that enhancing the survival of epithelial cells of the urethra could allow the bacteria to multiply within an intracellular environment that is protected and thus, enhance the colonization of *Neisseria gonorrhoeae*. PI also allows the bacteria to survive following apoptosis. *Neisseria gonorrhoeae* also possesses a lipo-oligosaccharide layer (LOS) that exhibits endotoxin activity by inducing inflammation. Pelvic inflammatory disease that can result in fallopian tube infection and infertility is caused by the shedding of the LOS that initiates local inflammatory injury (Chen and Seifert, 2013). The LOS is able to evade the activation of the complement cascade by concealing itself with host sialic acid, rendering it unrecognisable by the host immune system. Opa proteins present on the surface of *Neisseria gonorrhoeae*, bind to the CEACAM family of adhesion

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receptors present on neutrophils, epithelial cells and B and T lymphocytes, facilitating the activation of the adaptive and innate immune responses upon epithelial cell infection (Sadarangani et al., 2010). TNF- α , a cytokine released during the host innate immune response is pro-inflammatory and has a profound damaging effect on the host epithelial cells such as the fallopian tubes, This cytokine prompts the production of phospholipases and proteases, inducing excess inflammation and damage. *Neisseria gonorrhoeae* contains the enzyme IgA protease at its core. This is responsible for breaking down the host IgA1 antibodies found in mucosal membranes that have an immune function in protecting against infections in the mucous membranes. This bacteria also possesses a capsule that allows it to resist opsonisation and phagocytosis as it similar in composition to that of the connective tissue of the host. Thus, this enables the bacteria to multiply, survive and spread within the host to carry out further infection and damage (Edwards and Apicella, 2004). All of these virulence factors culminated to initiate infection in the male patient in this case study to cause his burning and sore sensation while urinating and his purulent penile discharge. If gonorrhoeae is not treated, disseminated Gonococcal Infection (DGI) occurs. This is due to *Neisseria gonorrhoeae* spreading systemically to other parts of the body via the bloodstream, causing joint pain and arthritis and lesions on the skin and endocarditis may also result from DGI but this is rare. DGI is more common in females due to them more frequently being asymptomatic (Russ and Wrenn, 2005).

Further investigation that could be carried out for this patient includes contact tracing all of his previous sexual partners in the past two weeks and

to notify them of his infection. The HPSC international guidelines for gonorrhoeae infections recommend that all male patients who have a urethral infection that is symptomatic must notify all of their sexual partners of the previous two weeks or if longer, their last partner. This ensures that his previous sexual partners are made aware of his infection as they too may be infected and may not be displaying symptoms (asymptomatic). His previous partners will also undergo a full STI screen to establish whether they are infected with gonorrhoeae or other STIs. Contact tracing reduces transmission of *Neisseria gonorrhoeae* and its reinfection, while also informing and aiding individuals and healthcare workers in the understanding of the patterns of transmission within communities. In Ireland, gonorrhoeae is a notifiable disease under the Infectious Disease Regulations as this pathogen can have consequences later in life such as infertility (HPSC, 2018).

In 2017, there were 2249 notified cases of gonorrhoeae in Ireland, causing it to become the second most commonly encountered STI in Ireland. This pathogen has an incidence rate in Ireland of 47.2 per 100,000 population. However, these figures are believed to be underestimated as 55% of males and 86% of females suffering with *gonorrhoeae* infections are asymptomatic and so, the actual figures are believed to be a lot higher (HPSC, 2018).

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Practical Risk Assessment Form

Practical title:

Practical description: Give a brief description of work to be undertaken and the nature of the materials and techniques to be used.

Working with *Neisseria gonorrhoeae*

Colonial morphology, Gram stains, catalase and oxidase tests will be carried out on the organism.

Hazard	High	Medium	Low	Current	Options for
				control measures for this hazard	improved controls
Biological:				Good aseptic techniques to control the exposure of this	Avoid touching the face or mouth with gloved hands to prevent becoming contaminated with the
<i>Neisseria gonorrhoeae</i>		X		infectious agent.	controls and
Oxidase positive and negative controls		X		Using gloves and laboratory coat to avoid	

becoming
contaminate
d with these
controls and
bacteria.

Using
disinfectant
to clean the
surface after
working with bacteria.
these
controls and
the bacteria.

Disposing
gloves, slides
and agar
plates when
they are
finished with.

Chemical:	X	Wearing	Closing
Gram stains	X	gloves and	laboratory
- Crystal		laboratory	coat
Violet,		coat to	properly.
Gram's		protect	
		hands,	

			clothes and skin from being stained with these chemical agents.
iodine, acetone and Carbol Fuschin Hydrogen peroxide			Wearing closed footwear rather than sandals or open-toed shoes. Avoid inhaling or ingesting the chemicals.
Electrical: Microscope Hot plate	X X	Correct handling of the microscope.	Not leaving them at the edge of the benchtop. Avoid tangling the leads and do

not have
them
hanging
down on the
floor.

Turning off
the
equipment
when they
are not in
use

Physical:	X	X	Proper	Not dropping
Glass slides			handling of	slides or
Chairs			slides.	disposing of
			Disposing of	them in the
			them in the	biohazard
			sharps bin	bins.
			when they	
			are finished	
			with.	
			Keeping	
			chairs	
			pushed in	
			under the	

workbenches

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