Urine tests on antibacterial activity

Urine sample U20 was allocated and the first thing done was to record the visual appearance of the urine. The urine was golden yellow with a pungent smell. At first sight, the urine did not appear to have any blood present in it. Further biochemical test using Ames Multistix Strips was done. The strip was dipped into the urine and at appropriate time intervals, results such as glucose, ketone and others were recorded. The pH of urine was found to be 6. 5 which is within the normal range. Urine results showed that there was trace of protein and also positive for nitrite. Presence of nitrite in urine is known as nitrituria and this indicates possible urinary tract infection (Medina-Bombardó and Jover-Palmer, 2011) caused by gram negative bacteria and E. coli is the most common bacteria responsible for it. An explanation for the presence of nitrite in urine of patients with urinary tract infection is that the pathogen convert dietary nitrates to nitrites (Hajar et al. 2011). Glucose was not present in the urine indicating that the patient is not diabetic. Leukocyte was found to be negative. Presence of leukocyte would result in the urine being cloudy. Moreover, according to Hooton (2012), presence of leukocytes in urine could indicate kidney infections, pelvis trauma as well as kidney stones. Other tests such as bilirubin, urobilinogen and specific gravity were all within the normal range.

On day 1 Week 1, streaking was done on agar plates. First, urine was streaked on Horse Blood Agar (HBA) plate using a calibrating loop. This was done by streaking one vertical line and then streaking horizontally several times to cover the whole plate and then incubated 24 hours at 37°C. The purpose of this was to determine whether the patient had infection or not. The following day, the HBA plate was examined and more than 100 dull grey

colonies (1. 5-2mm) were formed and no haemolysis (γ-haemolysis). As such it could be concluded that the patient was infected. Back to day one week one, urine sample was also streaked on Tryptone Soy Agar (TSA) and Mac Conkey Agar (MAC) plates. On day two week one, the plates were examined and it was found that there was growth on both plates. For TSA plate, small pale yellow round colonies of size 1-2 mm were formed. On the MAC plate, small round pink colonies of size 1. 5 – 2 mm were formed. Pink colonies on MAC plate indicates that the pathogen is lactose fermenting. MAConkey is a selective and differential media. It is selective since it contains bile salts and crystal violets which inhibit the growth of Gram positive bacteria while promoting the growth of Gram negative bacteria. Mac Conkey is differential since lactose fermenting bacteria appear as pink colonies while non lactose fermenting bacteria appear as beige colonies (Zvidzai et al. 2007). Gram stains were done from all the 3 plates namely HBA, MAC and TSA. The pathogen present was Gram negative rod and oxidase negative. As such, by elimination, *Staphylococcus spp, Streptococcus spp* , *Enterococcus spp* as well as *Pseudomonas aerogenes* can all be ruled out. From there, Microbact procedure was the next step so as to identify the remaining possible pathogens which were *Escherichia coli* , *Klebsiella pneumoniae* or *Enterobacter aerogenes* .

Test for antibacterial activity in urine was also done. This was performed by spreading 100µL of broth culture Escherichia coli on Mueller Hinton Agar (MHA) plate. Then a sterile disc dipped into the urine sample was placed in the middle. The plate was incubated 37°C for 24 hours. The next day, the

MHA plate was observed and no zone of inhibition was observed. As such, it could be concluded that there was no antibacterial activity in the urine.

On day one week two, colonies from HBA plate was restreaked on another fresh HBA plate and incubated 24hours at 37°C. The purpose behind this was to obtain fresh colonies for Microbact procedure. The next day, the colonies from the HBA was used for Microbact and the Microbact was incubated 37°C for 24 hours. From Microbact result, code generated was 6746 and this corresponds to the bacteria *Enterobacter aerogenes* . M27 urine ring was also used to test for Antibiotic Sensitivity Test. Zone of inhibition was measured for each antibiotic to know whether the pathogen is resistant or sensitive to the latter. It was found that *Enterobacter aerogenes* was sensitive to Carbenicillin (100µg) (8mm), Tetracycline (100µg) (7mm), Nalidixic acid (30µg) (8mm) and Cortimoxazole (25µg) (9mm) while being resistant to Ampicillin (25µg) (4 mm), Nitrofurantoin (50µg) (2 mm), Gentamicin (10µg) (6 mm) and Sulphamethizole (200µg) (5 mm).

Enterobacter aerogenes is a Gram negative rod bacteria from the family *Enterobacteriaceae* . It has been found to be the most common cause of infection in hospital hence nosocomial infection (Sanders and Sanders, 1997). *Enterobacter aerogenes* is found in the normal micro-flora of the gastro intestinal tract of the human body. The *Enterobacter aerogenes* usually live in the soil, water as well as dairy products. Infections from *Enterobacter aerogenes* usually arise due to the normal micro flora of the patient himself, or sometimes during cross infection when the doctors use medical devices for surgical operations (Sanders and Sanders, 1997). *Enterobacter aerogenes* is common for infection specially in individuals who

are immunocompromised, premature babies as well as babies who are born with a low weight. Also, *Enterobacter aerogenes* are commonly associated with urinary tract infections (UTI), skin infection, meningitis, gastrointestial infection and finally bacteraemia (Sanders and Sanders, 1997). Prevention methods consist of personal hygiene, control of antibiotics used and most importantly, aseptic measures ensured during medical intervention such as inserting catheters (Giamarellou, 2005). Therefore, from the results of M27 urine ring, Carbenicillin (100µg), Tetracycline (100µg), Nalidixic acid (30µg) and Cortimoxazole (25µg) can be prescribed to kill the bacteria. Moreover, a combination of two antibiotics can be prescribed for more effective results but this depends on specific cases.

Wound sample W34 was provided for analysis. Four streaking and one swab inoculation was done on the first day. First, streaking the wound sample on anaerobic HBA was done. Between the primary and secondary inoculums, a metronidazole disc was placed. The plate was incubated at 37°C for 48 hours anaerobically. This was done to detect the presence of anaerobes and *Streptococcus spp* and also, the disc was used since true anaerobes would be inhibited by the metronidazole. Then a wound swab was mixed with Cooked Meat Medium (CMM) and the tip broken and left into the CMM tube. The CMM tube was then incubated at 37°C for 48 hours. This was done to check for the presence of *Clostridium perfringens* . These two steps were done first and quick. Then the wound sample was streaked onto three aerobic plates, namely MAC, HBA and TSA. These 3 plates were then incubated aerobically for 24 hours at 37°C. The purpose of these 3 aerobic plates were to check for presence of any *Streptococcus spp, Staphylococcus*

spp and Gram negative bacteria. A Gram stain of the wound sample showed the presence of Gram positive rod shaped bacteria.

On day 2 week 1, the three aerobic plates were checked for growth. Growth was observed on all aerobic plates where a small yellow colonies were formed on TSA plate, pink colonies on MAC and dull grey colonies on HBA plate. Gram staining the colonies showed presence of Gram positive cocci. Furthermore, catalase test was done and the bacteria was catalase positive. This result was a bit confusing and was doubted to be due to cross contamination since Gram stain of the wound sample itself showed Gram positive rod. Results from the CMM and anaerobic HBA were therefore required before jumping to any conclusion. Hence, due to growth on aerobic plates and Gram positive cocci was present, the presence of *Staphylococcus aureus* was suspected. Hence antibiotic sensitivity testing (AST) was done using M43 ring and it was found that the bacteria which grew on the aerobic plates were sensitive to only Gentamicin (10µg) (7mm) while being resistant to the other antibiotics.

On week 2, day 1, the CMM and anerobic HBA plate was observed. In the CMM tube, the solution turned turbid, indicating possible presence of *Clostridium perfringens* . On the anaerobic HBA, white colonies and β-haemolysis was observed. There was a zone of inhibition around the metronidazole disc indicative the bacteria is sensitive to metronidazole. Gram staining the colonies and Gram stain from the CMM showed presence of Gram positive rod. Catalase test showed that the bacteria was catalase negative. Since on anaerobic and CMM plate, Gram positive rod was obtained and on the aerobic plates, Gram positive cocci was obtained,

Staphytech procedure and test for clostridium were both required to truly verify the pathogen. From the aerobic HBA, colonies were taken and restreaked on another HBA which was then incubated for 24 hours at 37°C. This was done to get fresh colonies for Staphytech procedure. As for the test for presence of *Clostridium perfringens* , a loop was dipped into the CMM and restreaked on HBA. A metronidazole disc was placed and then the plate was then incubated anaerobically for 48 hours at 37°C. Moreover, Mast ring M43 was used for AST where using swab method, culture from CMM was transferred onto HBA and then incubated for 48 hours. The next day, on the aerobic HBA for Staphytech procedure, white colonies were formed. Staphytech procedure gave a negative result where no agglutination was obtained. Hence *Staphylococcus aureus* could be ruled out. On the final day, the two plates for the *Clostridium perfringens* were obtained. On the HBA plate, pale yellow colonies with β-haemolysis were formed. No zone of inhibition was formed around the metronidazole disc. For the AST, it was found that *Clostridium perfringens* is sensitive to Penicillin (1 unit) (7mm), Tetracycline (10µg) (7mm) and Clindamycin (2µg) (8mm) while resistant to Trimethoprim (1. 25µg) (0mm), Sulphamethoxazole (25µg) (0mm), Gentamicin (10µg) (0mm), Fuscidic acid (10µg) (5 mm) and Erythromycin (25µg) (4mm).

As such, it could be concluded that the pathogen present in the wound sample was *Clostridium perfringens* and not *Staphylococcus aureus* . The presence of bacteria in the aerobic plates could be due to cross contamination. Moreover, it could be another bacteria other than *S. aureus*

which forms part of the normal microflora of the skin e. g. *Corynebacterium spp, Staphylococcus spp* (Chiller et al. 2001).

Clostridium perfringens is a Gram positive rod shaped bacteria. Clostridium perfringens is one of the bacterium which forms part of the normal flora of the human and animal gut. There are 5 types (A-E) of *Clostridium perfringens* and each of them produce toxins. These toxins are what make a person ill. Each type of *Clostridium perfringens* is responsible for different diseases such as food poisoning, PIG-BEL (fatal enterotoxaemia in humans) caused by type C *Clostridium perfringens* , Gas Gangrene (deep wound infection) caused by type A *Clostridium perfringens* and pulpy kidney disease in animals caused by type E *Clostridium perfringens* (Lindström et al. 2011). Here, in the project, *Clostridium perfringens* was associated with wound. Hence type A *Clostridium perfringens* is responsible. As such patients with infections from *Clostridium perfringens* will more likely develop gas gangrenev(Clostridial myonecrosis). This a deep wound infection and is caused due to the α-toxins produced by the type A *Clostridium perfringens* . Wound infected with *Clostridium perfringens* is distinguished by immediate inflammation at the infection site. Moreover, there is swelling as well as acute pain which will finally result in the necrosis of the infected wound (Chen et al. 2011). Infection cause the accumulation of gas in the infected wound. Presence of any material (even though sterile) which caused the wound can significantly increase the risk of infection. The infected area becomes red at first and eventually turns blackish green (Chen et al. 2011). Diagnosis of *Clostridium perfringens* is by the smell and inspection of the wound. The wound and its drainage possess a stinking smell. However,

confirmation of *Clostridium perfringens* is done by bacterial culture. Treatment is done by prescription of Penicillin and surgically removing debris from the wound. In severe cases, treatment involves amputation or excision of infected body part. Moreover, Hyperbaric oxygen therapy is also effective since it inhibits the growth of the obligate anaerobe *Clostridium perfringens* (Bakker, 2012).