MICROBIOLOGY PRACTICAL TEAMWORK 437

URINARY TRACT INFECTION

Objectives:

1- Know the important steps in specimen collection and transport to the lab.

2-How to process urine Specimens in the lab.
- Urine microbiological and biochemical analysis.
- Organisms culture and identification.
- Antibiotic susceptibility testing.
- Results interoperation.

3- Know the clinically important etiological Organisms associated with UTI, their identification and susceptibility testing.

Done by :
ABEER ALABDULJABBAR
NOURA ALOTHAIM
OMAR ALSUHAIBANI

Important aspects of Microbiologic Examination of UTI

SPECIMEN COLLECTION :

-Urine collection

SPECIMEN PROCESSING :

-Urine analysis

-Urine culture

-Interpretation of microbiology

-laboratory result

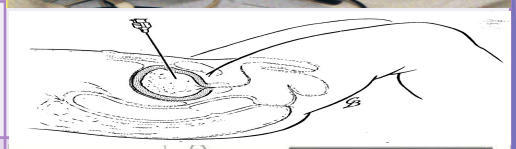
1- SPECIMEN COLLECTION

Type of specimens

Midstream urine (MSU): best for adult
The urine collected in a wide mouthed container from patients a mid stream specimen is the most ideal for processing
Female patients pass urine with a labia separated and mid stream sample is collected

Adhesive bag best for child

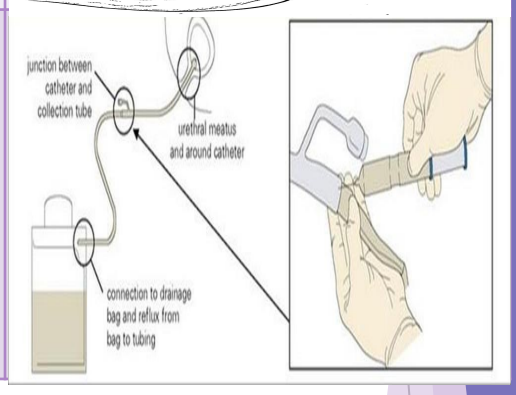
Suprapubic aspiration



Catheter sample: (used with elderly)

Urine specimens for laboratory investigations can be collected from catheterized patients as shown (left). The second port is for putting fluids into the bladder (right).

Urine from the drainage bag should not be tested because it may have been standing for several hours.



TRANSPORT MEDIA

-Sterile urine container

-Dip slide (one side is CLED media and the other is MacConkey agar or blood agar)

2- SPECIMEN PROCESSING

- A-Urine analysis
  - • Microbiological
    - Macroscopic
    - Microscopic
  - Biochemical
- B-Culture and identification (ID)
  - Culture media
  - Urine inoculation and reading of culture
  - Identification of cultured organisms
- C-Antimicrobial Susceptibility testing
  - Disk diffusion test
  - E test
- D-Results interpretation

A-Urine analysis

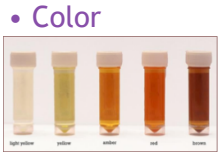
❖ **Biochemical : Biochemical Urine Examination (Dip stick)**

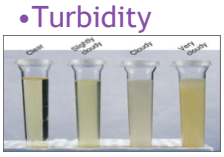
Testing for UTI : Midstream clean catch with dipstick analysis
Dipstick results may be affected by medications/dyes, i.e. Pyridium, nitrofurantoin, metronidazole, bilirubin, methylene blue, Vitamin B complex

- leukocyte esterase : + → indicates presence of white blood cells
- Nitrate test : + → for gram-negative bacteria which can convert nitrate to nitrite :
- PH
- Glucose
- Bilirubin
- Protein

❖ **Microbiological :**

- **Macroscopic :** • Color • Odor •Turbidity

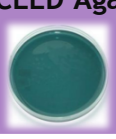
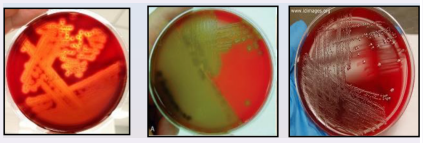




- **Microscopic:**
  - • Cell-counting (WBC, RBC)
  - • Parasite (Ovum, Trichomonas, yeast)
  - • Casts

B-Culture and identification (ID)

1- Culture media

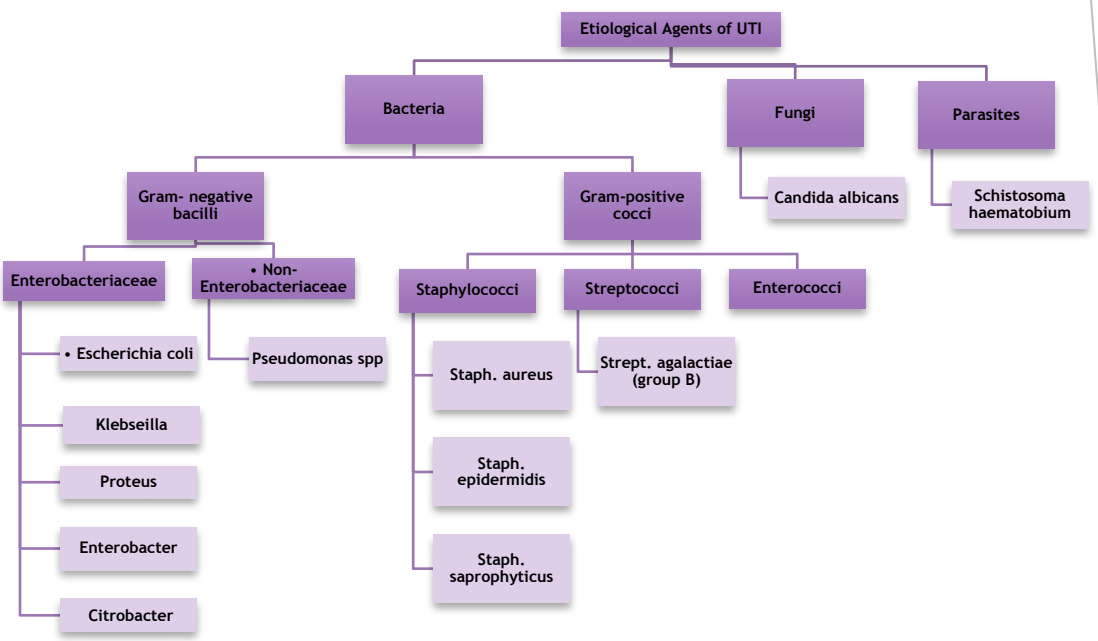
Blood Agar	MacConkey Agar	CLED Agar
 (Useful for gram positive)	 (Useful for gram negative)	 (Useful for gram negative)
Enriched culture medium, for culturing fastidious microorganism and observing the hemolytic reaction (Beta, Alpha, Gama).	Differential culture medium, showing both lactose and non-lactose fermenting colonies. LFC = Pink NLFC = Colorless or appear same as the medium	Selective culture medium, for detection and isolation of E. coli and coliform bacteria in urine.
		

2- Urine inoculation and reading of culture

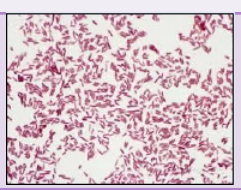
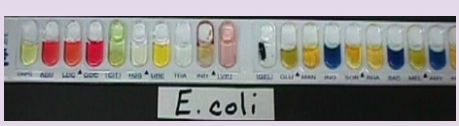
Urine Inoculation : -Quantitative (Colony counts):
-Semi-Quantitative Culture of Urine Sample

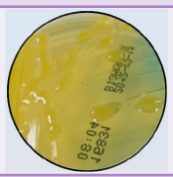
3- Identification of cultured organisms
-Biochemical tests.
-Type of hemolysis
-Serological tests

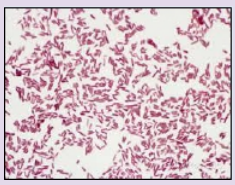
CLINICALLY IMPORTANT MICROORGANISMS CAUSING UTI



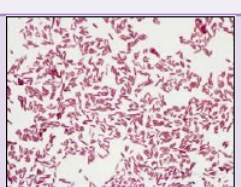
Gram Negative Bacilli : Enterobacteriaceae

	E. coli	
Morphology	Microscopic appearance Gram negative bacilli 	
Culture	MacConkey agar showing growth of Lactose fermenter Pink small and dry Colonies. LFC 	CLED agar Showing growth Of Lactose fermenter Yellow colonies LFC 
Identification	Indole Reactions Test: Positive 	APE 20 E test 

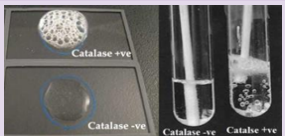
	Klebsiella pneumoniae spp	
Morphology	Microscopic appearance Gram negative bacilli 	
Culture	MacConkey agar showing growth of Lactose fermenter Mucoid Pink large Colonies LFC 	CLED agar Showing growth Of Lactose fermenter Mucoid Yellow colonies LFC 
Identification	Indole Reactions Test: Negative 	APE 20 E test 

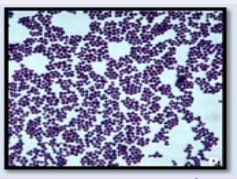
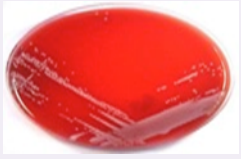
	Proteus spp		
Morphology	Microscopic appearance Gram negative bacilli 		
Culture	Blood culture Plate showing Swarming of Proteus. 	CLED [(Cystine-Lactose-Electrolyte-Deficient) -Inhibits The Proteus Swarming 	MacConkey agar showing growth of Non-lactose Fermenting (Yellow) 
Identification	Proteus is Urease positive Urease splits urea Into ammonia; and Alkalinizes the urine With production of crystals 	APE 20 E test 	

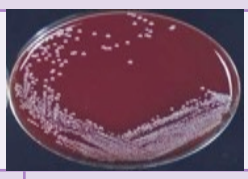
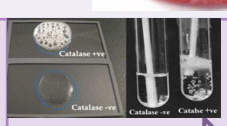
Gram Negative Bacilli : Non-Enterobacteriacaea

	Pseudomonas aeruginosa spp	
Morphology	Microscopic appearance Gram negative bacilli 	
Culture	MacConkey agar showing growth of Non-Lactose Fermenter Mucoid Pink colonies LFC 	Nutrient Agar showing growth of Pseudomonas pigmentation 
Identification	 Oxidase positive test	 APE 20 E test

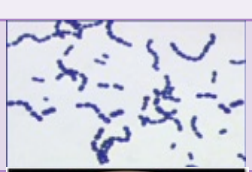
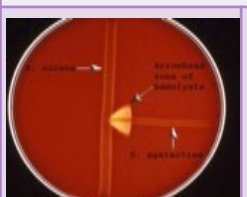
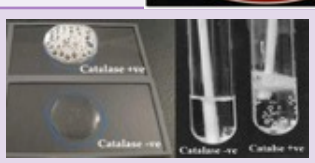
Gram Positive Cocci : Staphylococci

	Staph. aureus	
Morphology	Microscopic appearance: Gram positive cocci in clusters 	
Culture	Blood culture plate showing growth of golden yellow colonies	
Identification	 coagulase test = Positive	 2H2O2 Catalase O2 + 2H2O Catalase test = Positive Streptococci vs. Staphylococci

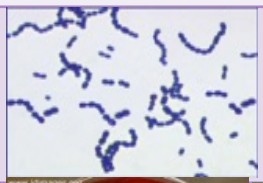
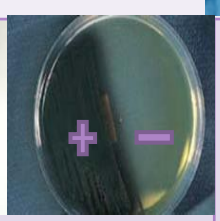
	Staph. epidermidis		
Morphology	Microscopic appearance: Gram positive cocci in clusters		
Culture	Blood culture plate showing growth of white non-hemolytic Dry colonies		
Identification	 Coagulase test Negative	 2H2O2 Catalase O2 + 2H2O Catalase test = Positive Streptococci vs. Staphylococci	 Staph.epidermidis vs. Staph. saprophyticus Novobiocin test Sensitive

	Staph. saprophyticus		
Morphology	Microscopic appearance: Gram positive cocci in clusters		
Culture	Blood culture plate showing growth of white non-hemolytic Dry colonies.		
Identification	 Coagulase test = Negative	 2H2O2 Catalase O2+2H2O Catalase test = Positive Streptococci vs.Staphylococci	 Novobiocin Test Resistant

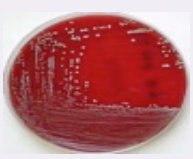
Gram Positive Cocci : Streptococcus (group B)

	Strept. agalactiae		
Morphology	Microscopic appearance: Gram positive cocci in chains		
Culture	Blood culture plate showing growth of Beta-haemolitic colonies		
Identification	 CAMP test Positive	 2H2O2 Catalase O2+2H2O Catalase test = Negative Streptococci vs.Staphylococci	  Streptics Mix bacterial colony with various group-specific antisera on a slide

Gram Positive Cocci : Enterococci

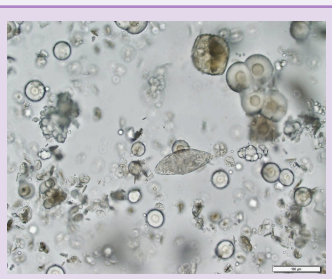
	Enterococci	
Morphology	Microscopic appearance: Gram positive cocci in chains	
Culture	Blood culture plate showing growth of Beta-haemolitic colonies	 
Identification	  Both Group D streptococci and enterococci produce a positive (left) bile Esculin hydrolysis test.	 $2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$ Catalase test = negative Streptococci vs. Staphylococci

Fungi: Candida albicans

	Candida albicans	
Morphology	Microscopic appearance: Gram positive cocci in chains (Yeast with Buding psuedohyphae)	
Culture	Small white Colonies in blood Agar. (Candida albicans on blood Agar) 	 Candida albicans on (SDA) Sabouraud's Dextrose Media
Identification	  Chlamydospore test Positive	  Germ tube test Positive

Schistosoma haematobium

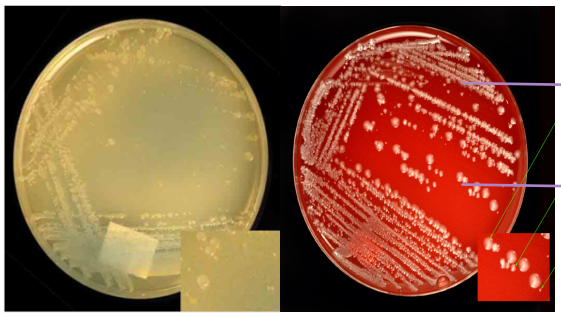
(urine; eggs 115-170 x 45-65 micrometers) (primates)



May cause Urinary bladder cancer

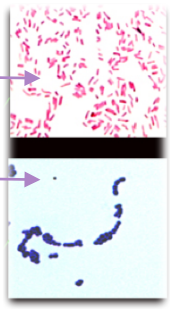
C- Methods of Antimicrobial Susceptibility Testing (AST) :

1- Disk diffusion test
2- E test



CLED plate

Blood agar



Gram stain

Case 1 :

The blood agar plate and CLED plate provided were inoculated with a sample of urine from a patient with a suspected urinary tract infection. Examine the plates and photographs provided.

Q:IdenIfy the colonies on the blood agar plates and photographs. The photographs show the results of the Gram stain of each colony type.

Answer:

- Large colonies are Gram Negative
- small colonies are Gram positive .

This case to show that UTI can be caused by 2 microorganism

Blood agar

CLED plate

Case 2 :

These Blood agar and CLED agar plates were inoculated with MSU from a 45 years old man suspected of having bladder stone and complaining of burning micturition. Moderate number of WBC and a PH of 8

Q1. What is the likely this pathogen? Proteus

Q2. How would you confirm the idenIty of this pathogen?

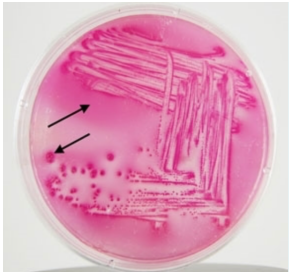
swarming + urease test

Q3.What is the role of this organism in forming stones?

Urease splits urea into ammonia; and alkalinizes the urine with production of crystals

Cases from female doctor

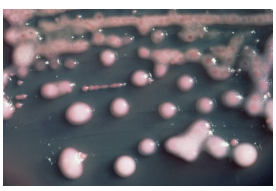
Answers



An 88 year old woman resides at nursing home. She has confirmed urinary tract infections 4-6 times yearly. One morning, her caregiver is unable to get her out of bed. She seems confused and disoriented. Vital signs reveal tachycardia in the 130's, respirations 22/min and hypotension 80/50. She is taken to an ER for evaluation. A chest x- ray and CT of the abdomen are normal. Catheter urine very positive for infection, elevated WBC count with a marked left shift, and marked confusion compared to her baseline. Sepsis secondary to urinary tract infection is suspected. Urine and blood cultures are subsequently positive for Lactose fermenter GNB, indol positive. A central line is placed, antibiotics and fluid replacement are instituted. After 10 hospital days, recovering but in a much weakened state, the patient is returned to nursery to complete 2 more weeks of antibiotics. The most likely organism is:

A- E coli
B- Proteus
C- Pseudomonas
D- Klebsiella

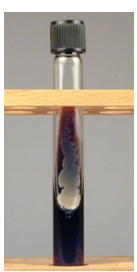
A- E coli



A 52 year old woman underwent surgery. She ha a Foley catheter. Five days later the patient called the office reporting low grade fevers, suprapubic and low back pain. Office visit revealed cloudy urine positive on dipstick for leukocytes, nitrites; culture revealed GNB lactose fermenter mucoid colonies. It was resistant to nitrofurantoin, sensitive to fluoroquinolone. The most likely organism is:

A- E coli
B- Proteus
C- Pseudomonas
D- Klebsiella

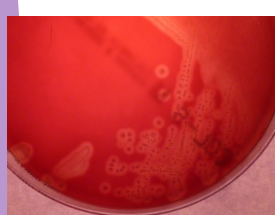
D- Klebsiella



A four-year-old girl presents with a 24-hour history of urinary frequency and dysuria. Examination is unremarkable apart from mildsuprapubic tenderness. A urine sample is positive for leucocyte esterase and nitrite on dipstick testing. Urine culture grew gram positive cocci, catalase negative and bile esculine positive. The likely organism is:

A- Enterococcus faecalis
B- Staphylococcus aureus
C- Streptococcus viridans
D- Streptococcus group B

A- Enterococcus faecalis



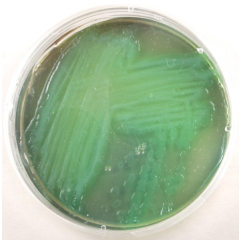
A 25 year old pregnant women presented with signs of lower UTI. Urine culture grew beta hemolytic gram positive cocci in chain that is catalase negative, bacitracin resistant. The likely organism is:

A- Group B streptococcus
B- Group A streptococcus
C- Staphylococcus aureus
D- Streptococcus viridans

A- Group B streptococcus

Cases from female doctor

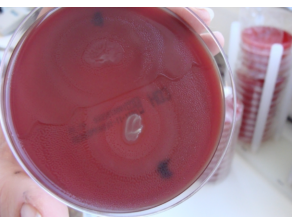
Answers



A 40 years old male patient having symptoms of urinary tract infection (UTI) like frequency of micturation for last 10 days and fever for 2 days of date of admission. Patient had history of renal calculus; He had operated 2 months back. He was with D J stenting. Appendisectomy was done. One month back, patient developed symptoms of urinary obstruction. Cystoscopy was done, which revealed stricture of urethane. Suprapubilc cystotomy (SPC) was done. Urine culture grew green pigment GNB, oxidase positive. The likely organism is :

A- Pseudomonas aeruginosa
B- Enterobacter spp
C- E coli
D- Klebsiella spp

A- Pseudomonas aeruginosa



The patient was a 19-year old female with a history of a urinary tract infection (UTI) 4 months prior to admission for which she was treated with oral ampicillin without complications. Five days prior to this admission she began to note nausea without vomiting. One day later she developed left flank pain, fevers, and chills and noted increased urinary frequency. She noted foul-smelling urine on the day prior to admission. She presented with a temperature of 38.8 °C, and physical examination showed left costovertebral angle tenderness. Urinalysis of a clean-catch urine sample was notable for >50 white blood cells per high-power filed, 3 to 10 red blood cells per high-power field and >3 bacteria. Urine culture was subsequently positive for > 105 CFU of an organism per ml. The bacteria were negative in the Gram-stain and had a rod shape. Growth of the isolate on McConkey agar showed NLF colonies. The organism was urease and swarming positive. The likely organism is :

A- E coli
B- Pseudomonas spp
C- Proteus spp
D- Klebsiella spp

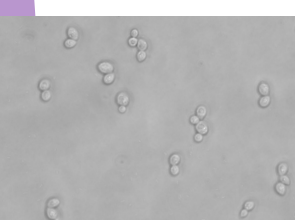
C- Proteus spp

A 58-year-old patient is admitted to the ED with GI bleed. Foley inserted.

• Day 2: Patient spikes temp of 38.6°C. Indwelling catheter remains in place.
• Day 3: Urine specimen is collected.
• Day 4: Culture results 100,000 CFU/ml Pseudomonas aeruginosa. Antibiotics started. All the following antibiotics can be used except :

A- Ampicillin
B- Gentamicin
C- Ceftazidime
D- Ciprofloxacine

A- Ampicillin



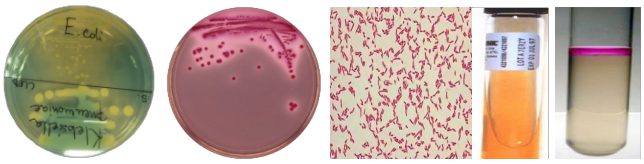
76- year-old woman is admitted for surgical debridement of sacral decubitus. Medical history notable for severe rheumatoid arthritis, CHF and atrial fibrillation. Routine admission U/A performed, positive for leukocyte esterase, and 3 WBC by HPF of spun urine. Patient afebrile, denies urinary urgency, frequency or pain. No suprapubic or CVA pain. Foley catheter present on admission. Foley draining cloudy urine. Urine microscopy showed budding yeast. The likely organism is

A- Staphylococcus
B- Candida albican
C- Streptococcus
D- Nocardia spp

B- Candida albican

Next cases are from team435 💜 , we highly recommend you reading it

Escherichia Coli



CLED Agar MacConkey's agar Gram stain Urease test Indole test

Q: Describe the microscopic appearance.
Gram stain showing gram -ve rods , *E.Coli* .

Q: What further investigations do we use to identify the microorganism in this case?
1. Culture on CLED agar
2. Culture on MacConkey's agar
3. Urease test
4. Indole test (to differentiate between it and klebsiella)

Q: Describe the organism's appearance on CLED agar.
Lactose positive yellow colonies.

Q: Describe the organism's appearance on MacConkey's agar.
Lactose fermenter (pink colonies).

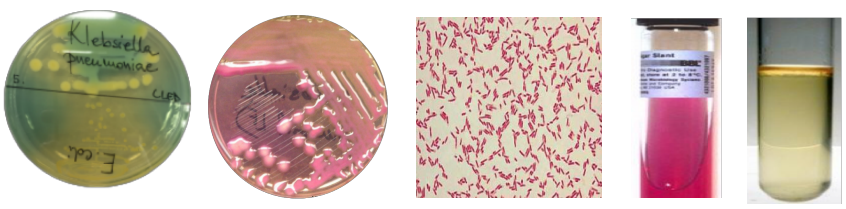
Q: Describe the organism's urease test.
Urease negative.

Q: Describe the organism's Indole test.
Indole positive.

Q: What is your differential diagnosis?
Fever → Acute pyelonephritis
No fever → Cystitis

Q: What is the best treatment used for this case?
1st line (Empiric): oral Trimoxazole for 3 days or oral Nitrofurantoin for 7-10 days.
If pregnant: oral Cefixime, intravenous Ceftazidime or intravenous Ceftriaxone

Klebsiella pneumoniae



CLED Agar MacConkey's agar Gram stain Urease test Indole test

Q: Describe the microscopic appearance.
Gram stain showing → gram -ve rods, klebsiella pneumoniae (similar to E .coli) .

Q: What further investigations do we use to identify the microorganism in this case?
1. Culture on CLED agar
2. Culture on MacConkey's agar
3. Urease test
4. Indole test

Q: Describe the organism's appearance on CLED agar.
Lactose positive (yellow mucoid colonies).

Q: Describe the organism's appearance on MacConkey's agar.
Lactose fermenter (pink mucoid colonies).

Q: Describe the organism's urease test.
Urease positive.

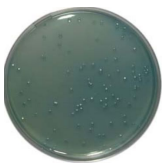
Q: Describe the organism's Indole test.
Indole Negative.

Q: What is your differential diagnosis?
Fever → Acute pyelonephritis
No fever → Cystitis

Q: What is the best treatment used for this case?
1st line (Empiric): oral Trimoxazole for 3 days or oral Nitrofurantoin for 7-10 days.
If pregnant: oral Cefixime, intravenous Ceftazidime or intravenous Ceftriaxone

Proteus


Blood agar

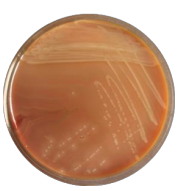

CLED Agar


MacConkey's agar

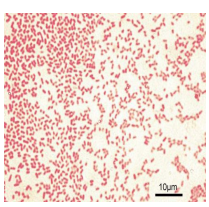

Urease test

Q: What further investigations do we use to identify the microorganism in this case?
1.Culture on Blood agar
2.Culture on CLED agar
3.Culture on MacConkey's agar
4.Urease test
5.Urine pH level test
Q: Describe the organism's appearance on blood agar. Swarming growth
Q: Describe the organism's appearance on CLED agar.
Swarming growth is inhibited , (Blue) Slightly elevated .
Q: Describe the organism's appearance on MacConkey's agar.
Non-lactose fermenter (pale colonies).
Q: Describe the organism's urease test.
Urease positive.
Q: What is the suspected urine pH level with the presence of such microorganism?
Alkaline urine, empowers the formation of urinary tract stones.
Q: What is your differential diagnosis?
Fever → Acute pyelonephritis
No fever → Cystitis
Q: What is the best treatment used for this case?
Gentamycin

Pseudomonas Aeruginosa


MacConkey's agar


Nutrient agar


Gram stain

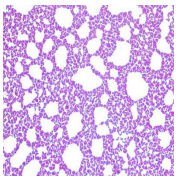

Oxidase test

Q: Describe the microscopic appearance.
Gram stain showing gram -ve rods, Pseudomonas Aeruginosa.
Q: What further investigations do we use to identify the microorganism in this case?
1) Culture on MacConkey's agar
2) Culture on Nutrient agar
3) Oxidase test
Q: Describe the organism's appearance on MacConkey's agar.
Non-lactose fermenter.
Q: Describe the organism's appearance on nutrient agar.
Produced pyocyanin pigment (blue green colonies).
Q: Describe the organism's oxidase test.
Oxidase positive
Q: What is your differential diagnosis?
Nosocomial, multidrug resistant complicated UTI
Q: What is the best treatment used for this case?
Ciprofloxacin.

Staphylococcus Saprophyticus


Novobiocin sensitivity

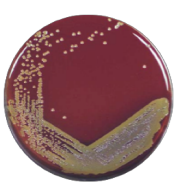

Blood agar

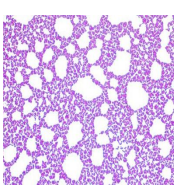

Gram stain


Coagulase test

Q: Describe the microscopic appearance.
Gram stain showing gram +ve cocci in clusters, Staphylococcus Saprophyticus.
Q: What further investigations do we use to identify the microorganism in this case?
1) Novobiocin sensitivity test
2) Culture on blood agar
3) Coagulase test
Q: Describe the organism's appearance on Novobiocin sensitivity test.
Shows resistance.
Q: Describe the organism's appearance on blood agar.
White colonies.
Q: Describe the organism's coagulase test.
Coagulase negative.
Q: What is your differential diagnosis?
Uncomplicated UTI.
Fever → A cute pyelonephritis
No fever → C ystitis
Q: What is the best treatment used for this case?
1 line (Empiric): oral Trimoxazole for 3 days or oral Nitrofurantoin for 7-10 days.
If pregnant: oral Cefixime, intravenous Ceftazidime or intravenous Ceftriaxone.

Staphylococcus Aureus


Blood agar

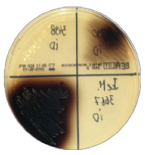

Gram stain


Coagulase test

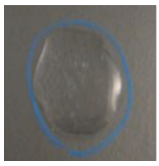
Q: Describe the microscopic appearance.
Gram stain showing gram +ve cocci in clusters, Staphylococcus Aureus.
Q: What further investigations do we use to identify the microorganism in this case?
1) Blood culture
2) Culture on blood agar
3) Coagulase test
Q: Describe the organism's appearance on blood agar.
Gold colonies.
Q: Describe the organism's Coagulase test.
Coagulase positive.
Q: What is your differential diagnosis?
Hematogenous spread acute pyelonephritis.

Enterococcus faecalis

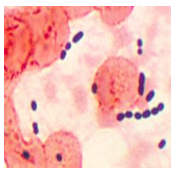
Blood agar



Bile Esculin Agar



Catalase test



Gram stain

Q: Describe the microscopic appearance.

Gram stain showing gram +ve cocci in short chains or pairs.

Q: What further investigations do we use to identify the microorganism in this case?

1) Culture on blood agar
2) Culture on Bile Esculin Agar (BEA) (To differentiate between Enterococcus and Streptococcus because both are -ve for catalase)
3) Catalase test

Q: Describe the organism's appearance on blood agar.

Non-hemolytic (gamma-hemolytic) colonies.

Q: Describe the organism's Catalase test.

Catalase negative.

Q: Describe the organism's Bile Esculin Agar.

Enterococcus are capable of growing and produce a black complex(Hydrolyzing esculin to glucose and esculetin. Esculetin combines with ferric ions to produce a black complex)

Q: What is your differential diagnosis?

Complicated UTI:

1-Hospital acquired.

2-Ascending Infection (or VUR reflux).

Q: What are the possible complications that associate this organism?

Bacteremia, Endocarditis and Meningitis.