<u> Unit VII – Problem 1 – Microbiology Lab</u>



- Differentiation between staphylococcus and streptococcus:

	Staphylococcus	Streptococcus
Catalase test	+	-
Gram stained smears	$\begin{array}{rcl} Gram &+ & cocci, & non-motile, \\ non-capsulated, & uniform size, \\ occur single or in pairs or \\ clusters, some strains are \\ \beta- \\ hemolytic on blood agar \end{array}$	Gram + cocci, non-motile, some are capsulated, forming long chains in fluid cultures, but found single or in pairs on forming short chains on solid media

- Catalase test:

- Used to differentiate between bacteria producing catalase enzyme (staphylococcus) and those which don't (streptococcus).
- A colony of the tested bacteria is added to 2-3ml of 3% hydrogen peroxide in a test tube. If the bacteria is catalase-positive, hydrogen peroxide will be broken down to water and oxygen (which will appear as bubbles in the solution).



- Differentiation between the species of staphylococcus:

Differentiation between the species of staphylococcus.				
Species	Coagulase test	DNAse test	MSA	
S.aureus	+	+	+ (mannitol utilized = yellow)	
S.epidermidis & species other than S.aureus	-	-	- (mannitol not utilized = pinkish)	

- Coagulase test:
 - Differentiating between coagulase positive staphylococcus such as S.aureus and CoNS such as S.epidermidis.
 - A drop of physiological saline is added to a slide → a colony of bacteria will be emulsified in it → a drop of undiluted human plasma is added → look for clumping within 10 sec.
- DNAse test (it confirms coagulase test):
 - Differentiating S.aureus from other species of Staphylococci which do not produce DNAse.
 - The organism which will be tested is culture on a medium containing DNA → weak HCl is added after overnight incubation → unhydrolized DNA will precipitate & DNAse producing colonies will be surrounded by clear areas.

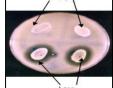
- Mannitol Salt Agar:

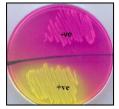
- Useful with fecal specimens when investigating staphylococcal food-poisoning.
- S.aureus colonies will grow and produce yellow zones around them.
- S.epidermidis colonies will grow and produce red or purple zones around them.

- <u>Staphylococcus aureus:</u>

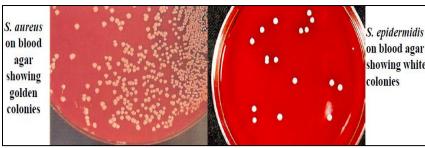
- Gram (+) cocci occurring mostly in clusters (grape-like groups) and has all the characteristics of staphylococcus genus mentioned in the first table above.
- Some strains produce a hight heat-stable protein toxin causing illness in humans.
- On blood or chocolate agars, they produce yellow creamy colonies (1-2mm in diameter).











- <u>Coagulase-negative staphylococcus (CoNS):</u>

- Constituent of normal human microbiota but cause infections with:
 - \checkmark Indwelling catheters.
 - ✓ Cardiac valves.
 - ✓ Cerebrospinal fluid shunts.
 - \checkmark Prosthetic devices.

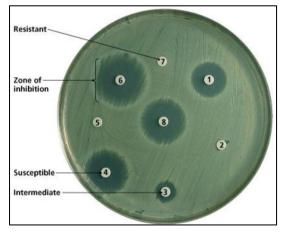
• They include:

They include.		
S.epidermidis	74%-92% of all hospital acquired CoNS infections	
S.hemolyticus	Relevant in endocarditis, sepsis, peritonitis, urinary tract	
	infections, osteomyelitis & wound infections	
S.saprophyticus	Urinary tract infection in young females	
S.lugdunensis	Native valve endocarditis	

• 50% of CoNS are resistant to methicillin (80% of S.epidermidis is resistant to it).

Antibiotic disk sensitivity test:

- Determines susceptibility of microorganisms to antimicrobial agents.
- For an organism which is sensitive to an antimicrobial agent, the colonies will be surrounded by zones of inhibition around the disc impregnated with the antimicrobial agent.
- Accurate measurement of zone diameter (using a ruler or a caliper) is necessary to properly interpret this test.
- Take a specimen from a culture using swab → spread it on Penassay Agar plate → let the plate dry at room temp. for several minutes → apply your antibiotic disks using a disk dispenser → incubate the plates for 1-2 days at 37



- <u>Mechanisms of resistance to beta-lactam antibiotics:</u>
 - Gram (+) bacteria have peptidoglycan which contains penicillin-binding proteins facilitating the entry of penicillins to the bacteria.

Resistance develops when beta-lactam antibiotics:

- \checkmark Fail to bind to altered penicillin-binding proteins.
- ✓ Or when they are hydrolyzed by beta-lactamases produced by the bacteria itself.

- <u>Clinical relevance:</u>

S.aureus	* Non-pyogenic infections (infections with no pus production)	
	* Pyogenic infections in many areas of the body: staphylococcal	
	skin scalded syndrome, toxic shock syndrome, food-poisoning	
	(enterotoxins)	
S.epidermidis	* In pace makers.	
	* Prosthetic devices.	
	* Central venous catheters.	
S.saprophyticus	* Urinary tract infections in females	