3-Gram Stain and Antibiotics Susceptibility Testing lab

Mohammad Al-Tamimi MD PhD
Second Year
Faculty of Medicine
The Hashemite University

Gram Stain

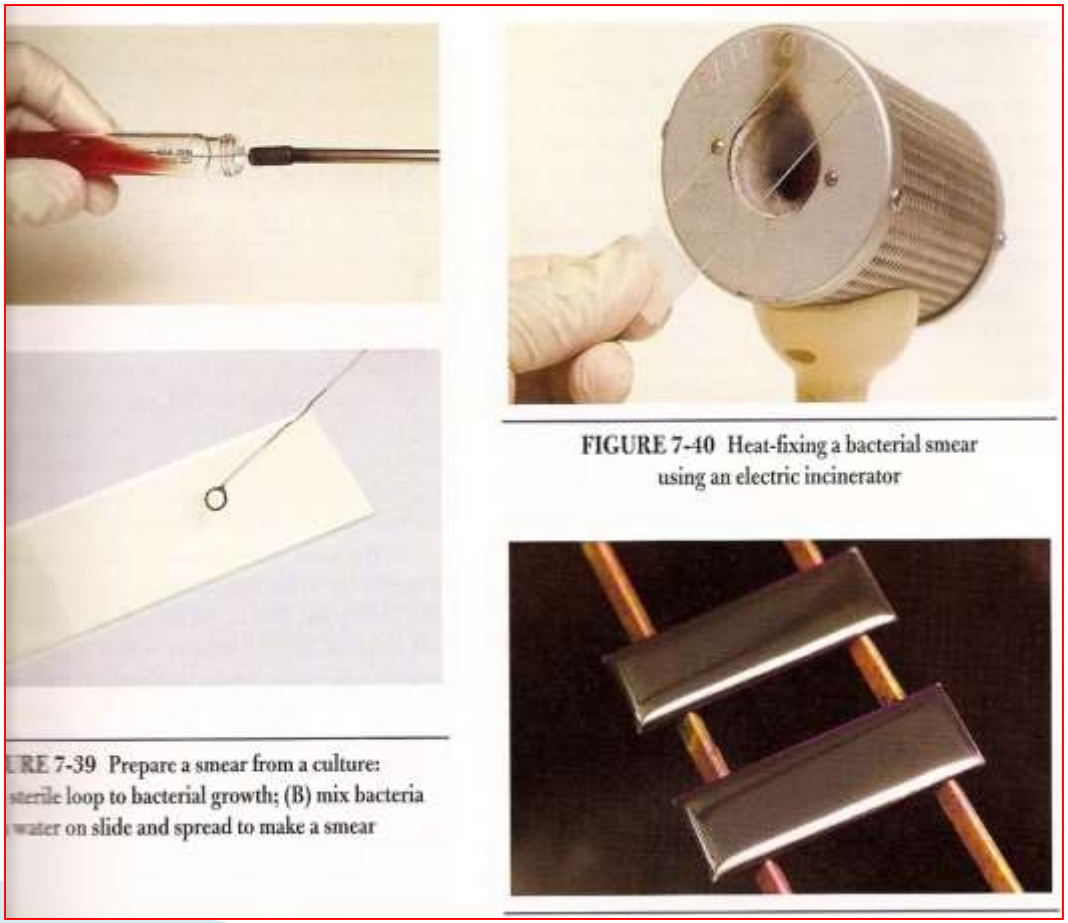
- Differentiates bacteria into two groups: Gram positive and Gram negative
- Stain mechanism is generally related to the thickness of the cell wall, pore size and permeability properties of intact cell envelope

Staining:

1. Bacterial smear
2. Stain
3. Observe the smear under the microscope

1 / Preparing the Bacterial Smear

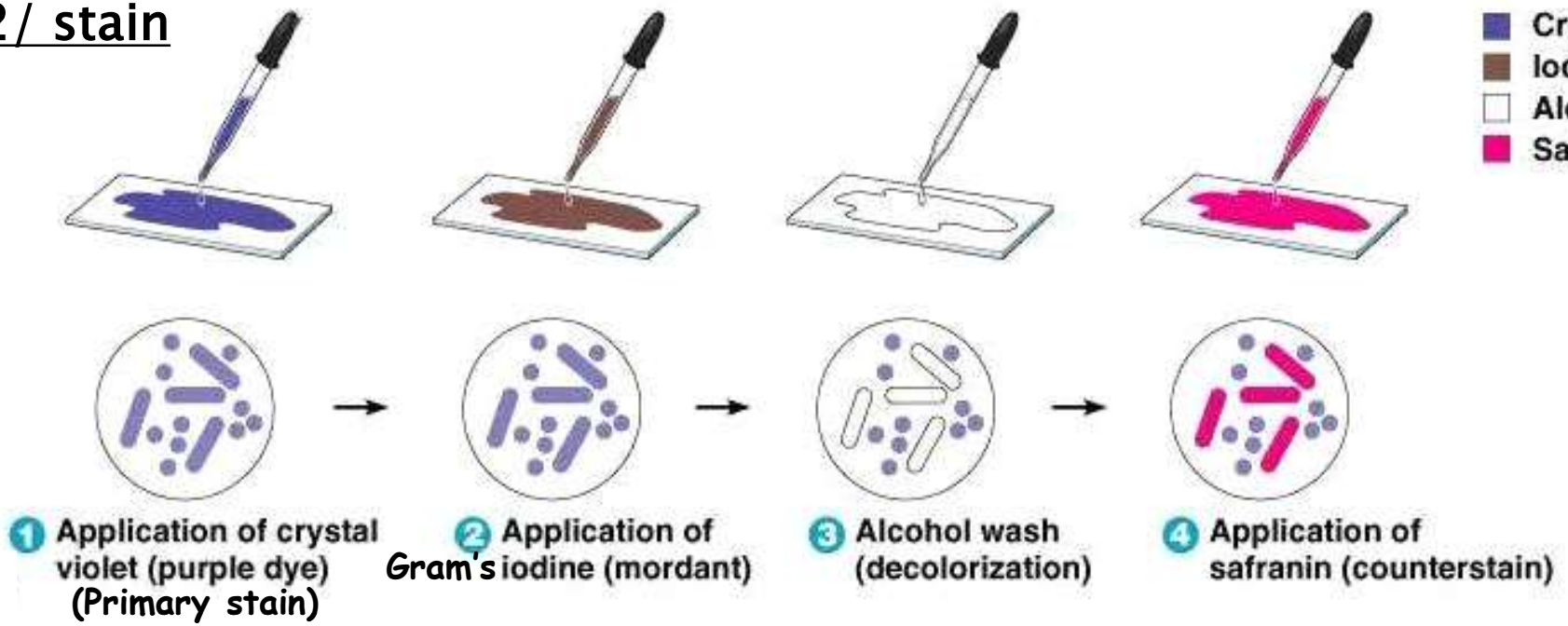
- Put on drop of normal saline in a slide
- Using a sterilized and cooled inoculation loop, obtain a very small sample of a bacterial colony.
- Gently mix the bacteria into the normal saline drop.
- Remove it to dry by air
- Heat-fixing the smear



URE 7-39 Prepare a smear from a culture:
sterile loop to bacterial growth; (B) mix bacteria
water on slide and spread to make a smear

FIGURE 7-40 Heat-fixing a bacterial smear using an electric incinerator

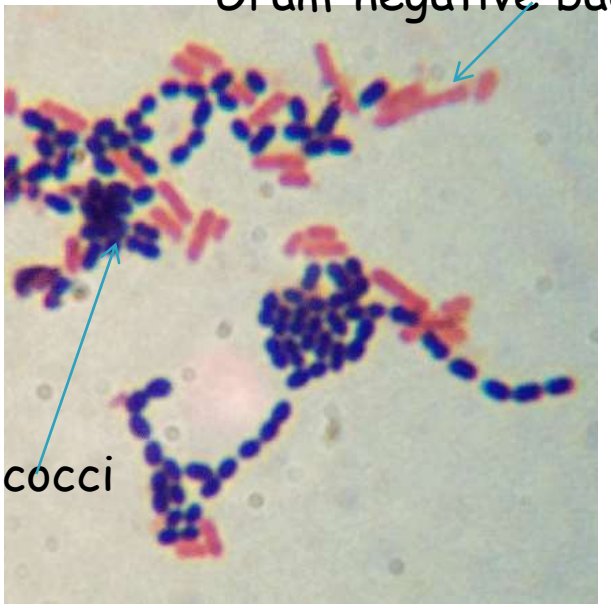
2/ stain



Crystal violet Gram's iodine Alcohol Safranin

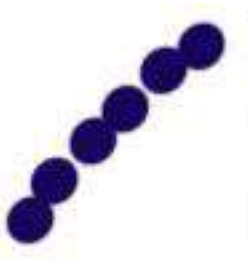
3/ Observe the Stained Smear

- Oil immersion
- Gram reactions
  - Gram (+) → purple
  - Gram (–) → pink

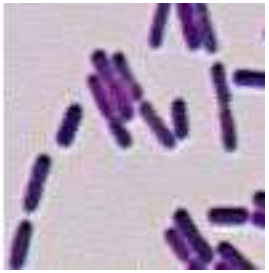


Examples of Gram Positive Bacteria

Staphylococcus aureus



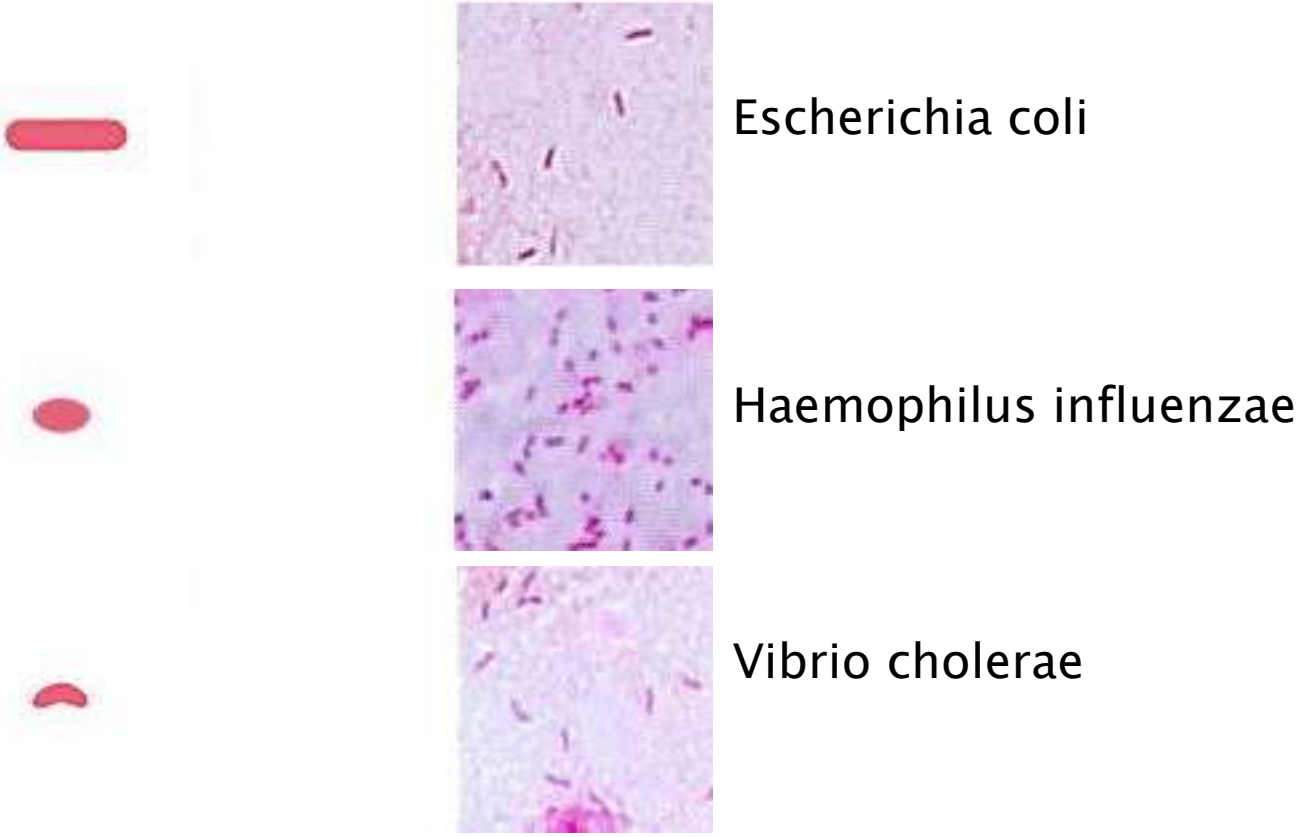
Streptococcus pyogenes



Clostridium perfringens

Listeria monocytogenes

Examples of Gram Negative Bacteria



Escherichia coli

Haemophilus influenzae

Vibrio cholerae

Antibiotics Susceptibility Testing

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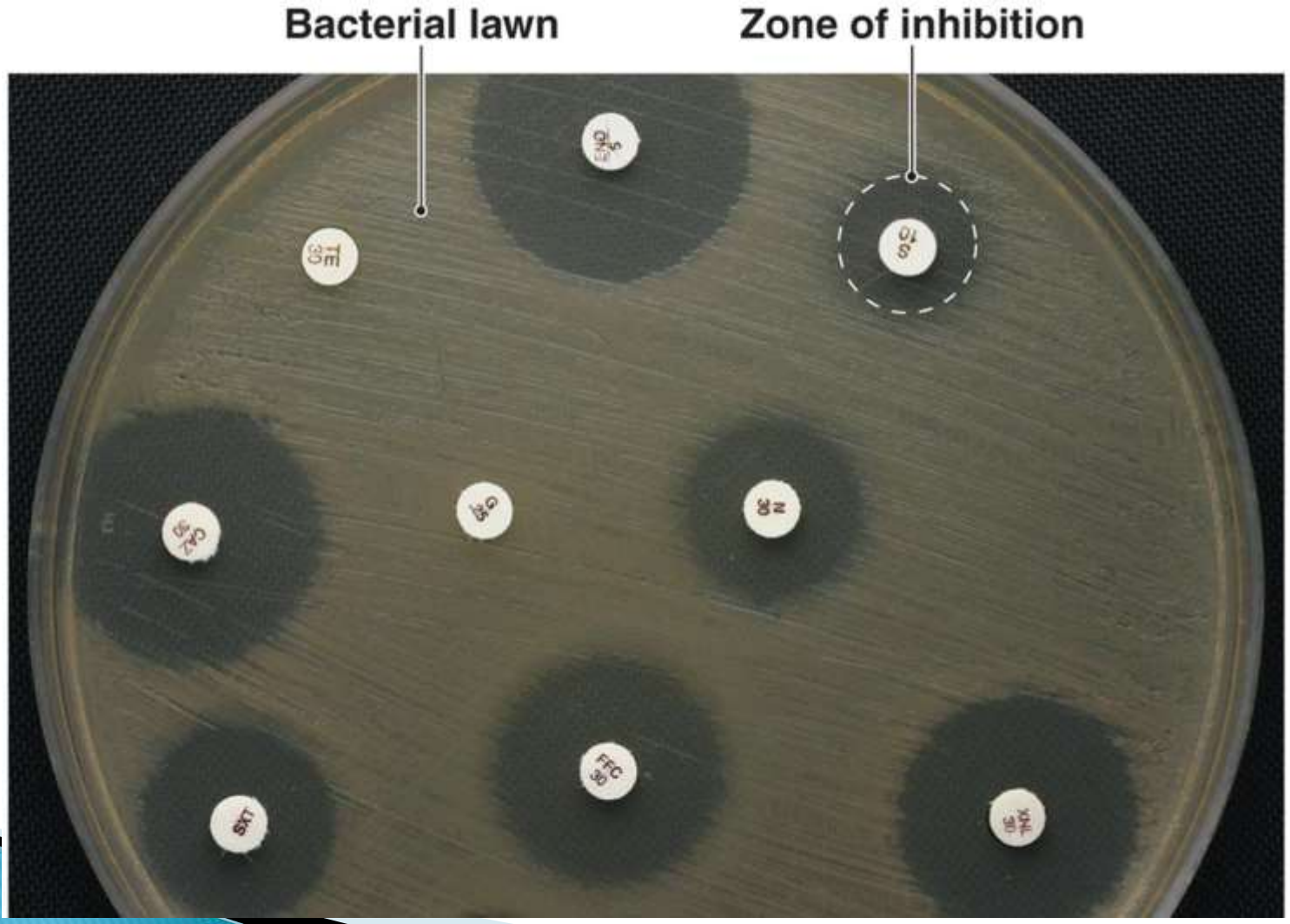
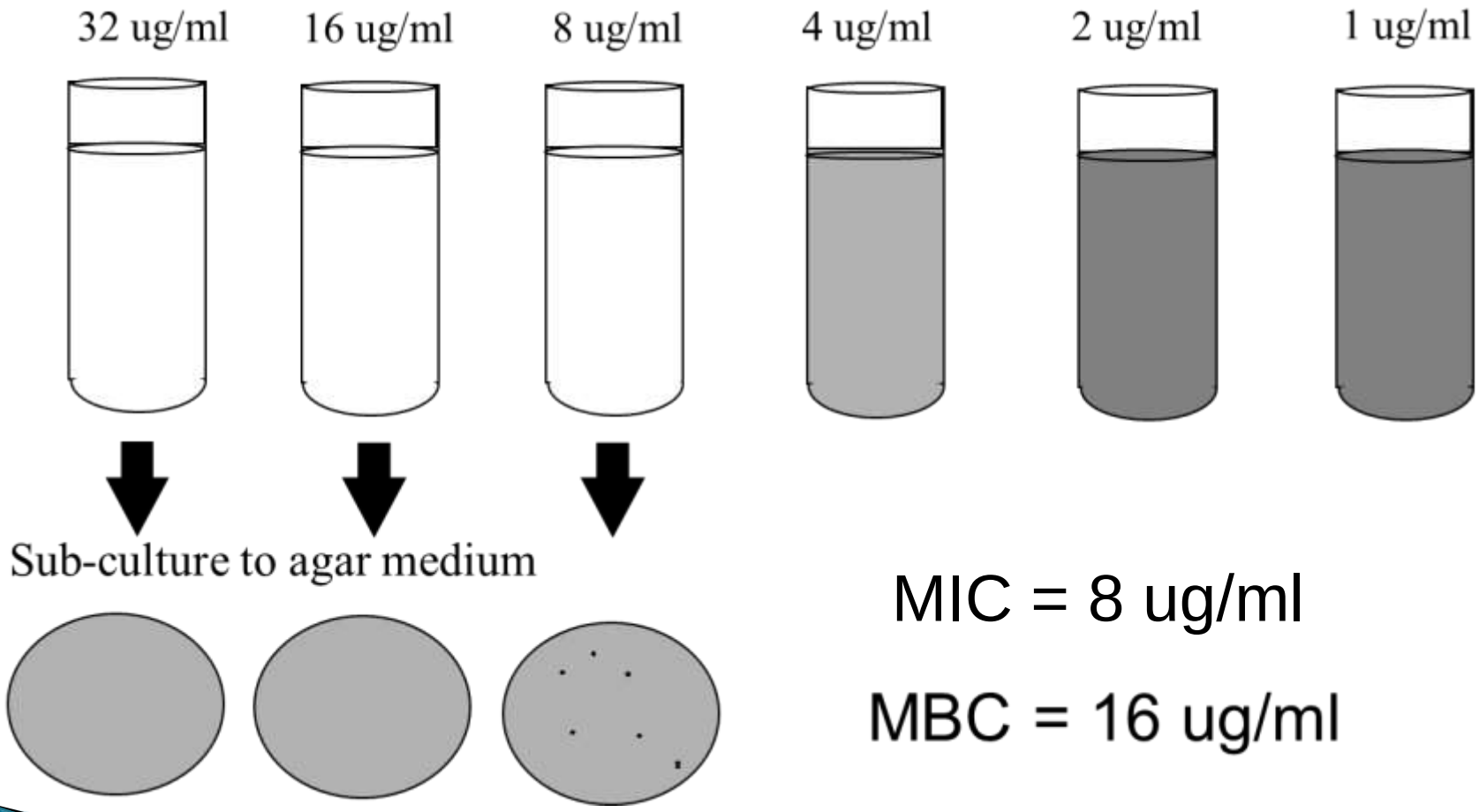
Drug efficacy can be measured by susceptibility testing Including

1. Kirby-Bauer Method (diffusion test)
2. Broth dilution test
3. The E test
4. Automatic (Vitek, Vitek 2)
5. Molecular testing of relevant genes

1. Kirby-Bauer Method (disc method)



2. Dilution Test



MIC = 8 ug/ml

MBC = 16 ug/ml

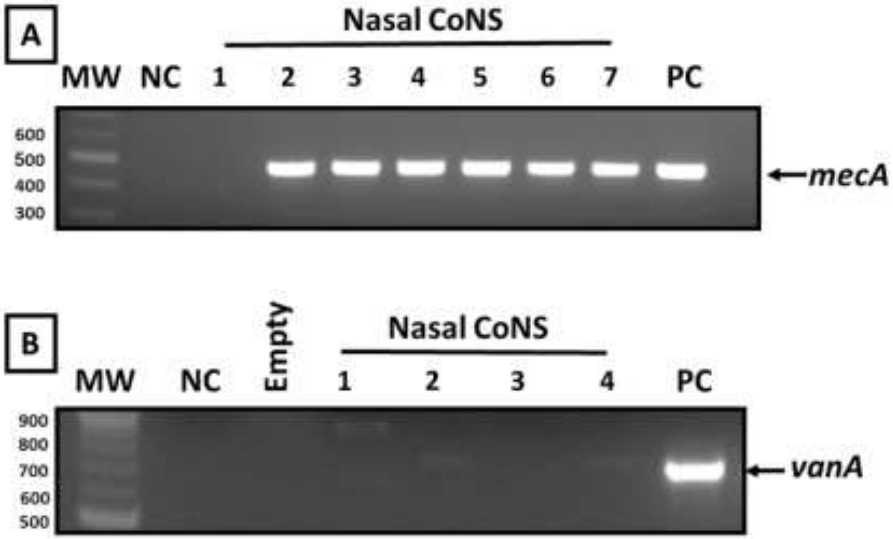
3. E test combines aspects of Kirby-Bauer and MIC tests

4. Automatic (Vitek, Vitek 2)

5- Molecular testing of relevant genes

- DNA extraction
- Study relevant genes
  - mecA – MRSA
  - vanA – VRSA
- PCR
- Gel electrophoresis

Figure 1. Analysis of PCR products by gel electrophoresis stained with ethidium bromide for *mecA*, *vanA*, and *vanB* genes in nasal CoNS samples.



Antibiotics susceptibility report reading

1

Name	: Rajesh Hinduja	Age/Gende	: 51 y / M	Collected:	08/09/2020 01:31
Patient ID	: 0010235933	Visit Type	:IP	Received :	08/09/2020 09:27
[redacted]		DOB	:20/06/1969	Reported :	08/09/2020 12:01
Accession	: 2001023647	Location	:1S0613`1SR6181`1SB6181`1PULMMED		

2

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ORDER#: D90306040
SOURCE: Sputum, Suctioned ett
ANTIBIOTICS AT COLL.:
Stain, Gram (Respiratory)                    FINAL
        Few WBC's
        Rare Mixed Respiratory Flora
Culture and Gram Stain, Aerobic, RespiratorFINAL
        Very light growth of mixed upper respiratory flora
        Heavy growth of Pseudomonas aeruginosa


        Heavy growth of Pseudomonas aeruginosa
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3

Antibiotic	Sputum culture	
	MIC	Interpretation
Ampicillin	16	I
Amoxicillin/clavulanic acid	8	S
Piperacillin/tazobactam	≤ 4	S
Cefazolin	≥ 64	R
Cefoxitin	≥ 64	R
Cefotaxime	≥ 64	R
Ceftazidime	4	S
Cefepime	≥ 64	R
Aztreonam	≥ 64	R
Imipenem	1	S
Amikacin	≥ 64	R
Gentamicin	≥ 16	R
Ciprofloxacin	≥ 4	R
Tigecycline	≥ 8	R
Trimethoprim/sulfamethoaxazole	≤ 20	S

MIC, minimum inhibitory concentration; R, resistant; S, sensitive; I, intermediate.