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AFRICAN SOCIETY FOR LABORATORY MEDICINE

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ANTIMICROBIAL RESISTANCE (AMR) COMMUNITY OF PRACTICE (CoP)



The Rise of Resistance: How Bacteria “Outsmart” Antibiotics

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Outline

- Background
- Antimicrobials overview
- Antibiotics mechanisms of action
- Acquisition of resistance
- Antibiotics mechanisms of resistance
- Beta-lactamases
 - Classification
 - Detection methods

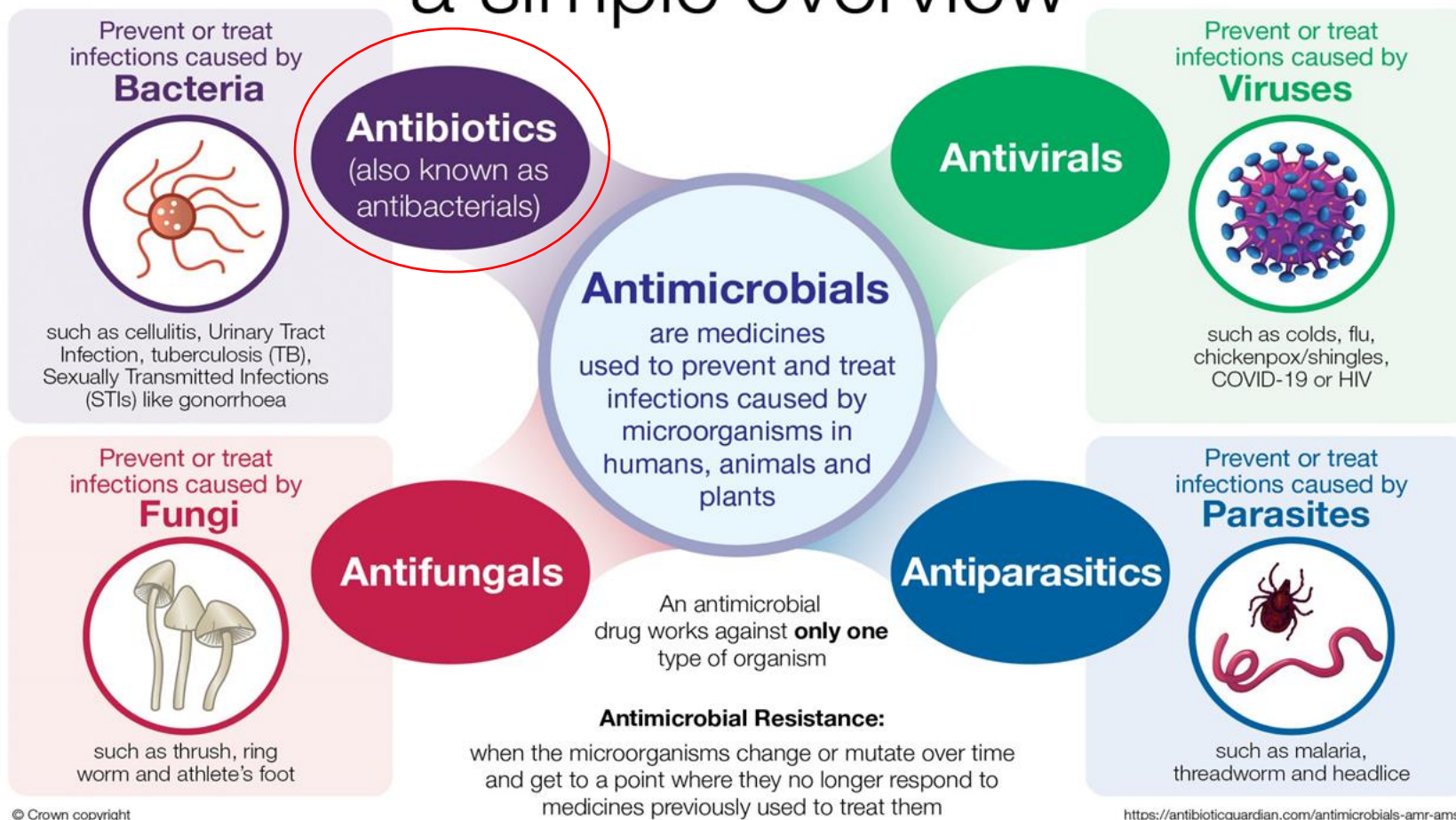
- The discovery of antibiotics, made us think the battle against infectious diseases has been won
- However, as many bacteria have developed resistance to multiple antimicrobial agents, the war now seems to be shifting in favour of the bacteria
- Antimicrobial Resistance (AMR) is a major threat to global health
- The World Health Organization (WHO) has declared it as one of the [top 10 global public health threats](#) confronting humanity

- Globally, an estimated **4.95 million deaths** were associated with drug-resistant infections, both directly and indirectly, with **1.27 million** of these deaths directly attributable to drug resistance
- This indicates that over one million people died because their infections could not be treated with any available medicines due to resistance
- **Seventy percent** of these deaths resulted from severe infections that no longer responded to first-line antibiotics used for empirical treatment



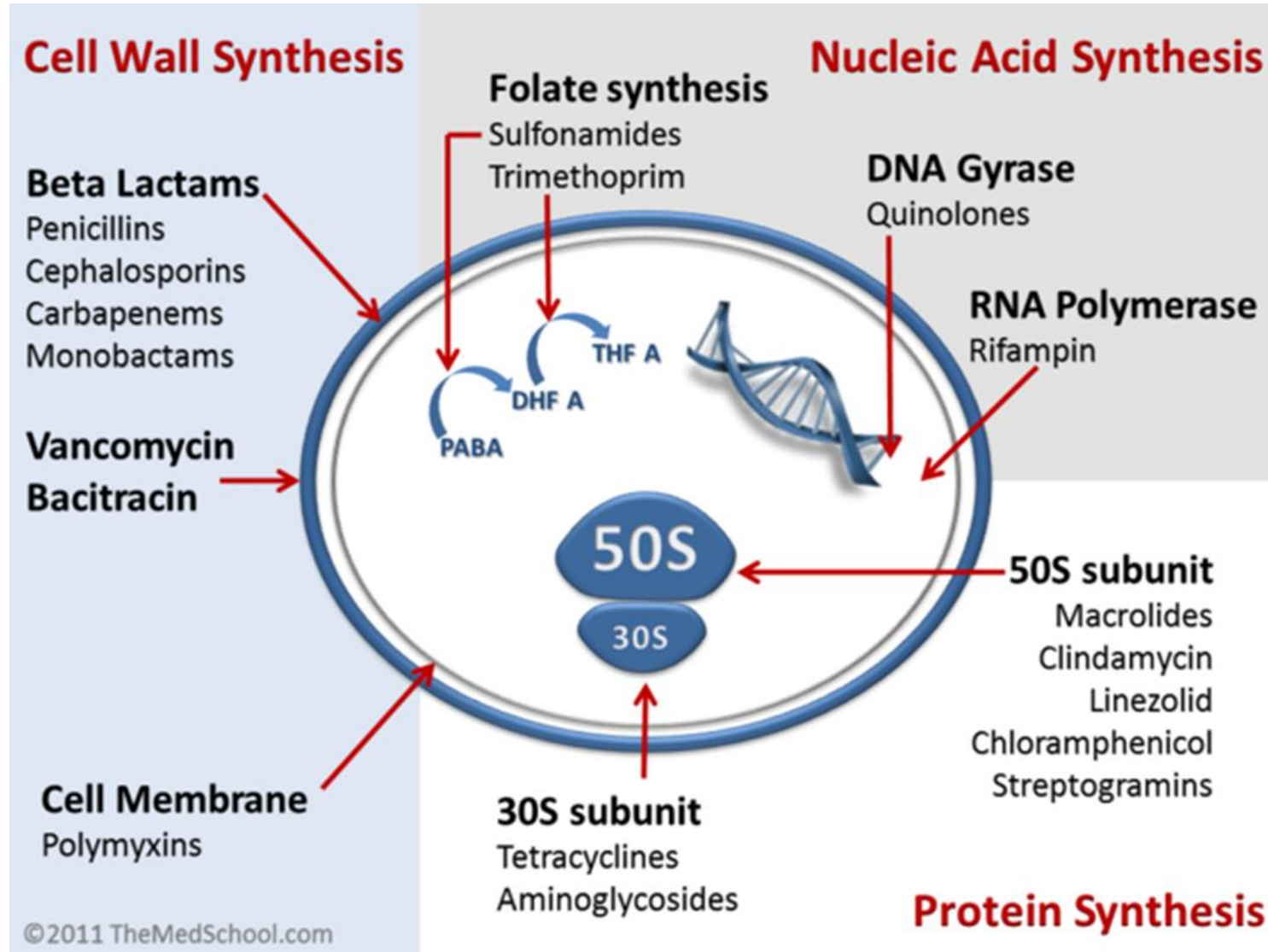
Public Health
England

Antimicrobials: a simple overview



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Mechanisms of action



Causes of resistance



Over-prescribing
of antibiotics



Patients not finishing
their treatment



Over-use of antibiotics in
livestock and fish farming



Poor infection control
in hospitals and clinics



Lack of hygiene and poor
sanitation



Lack of new antibiotics
being developed

www.who.int/drugresistance

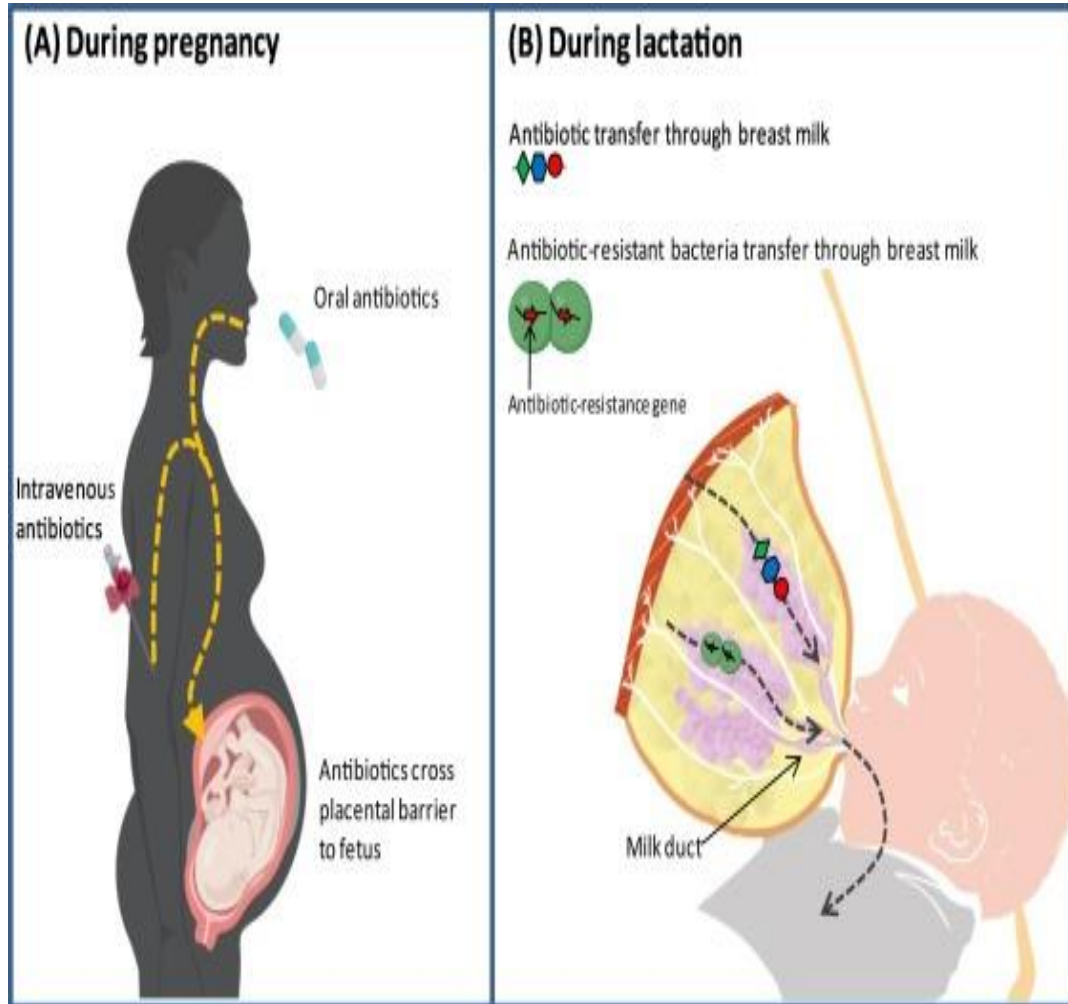
#AntibioticResistance



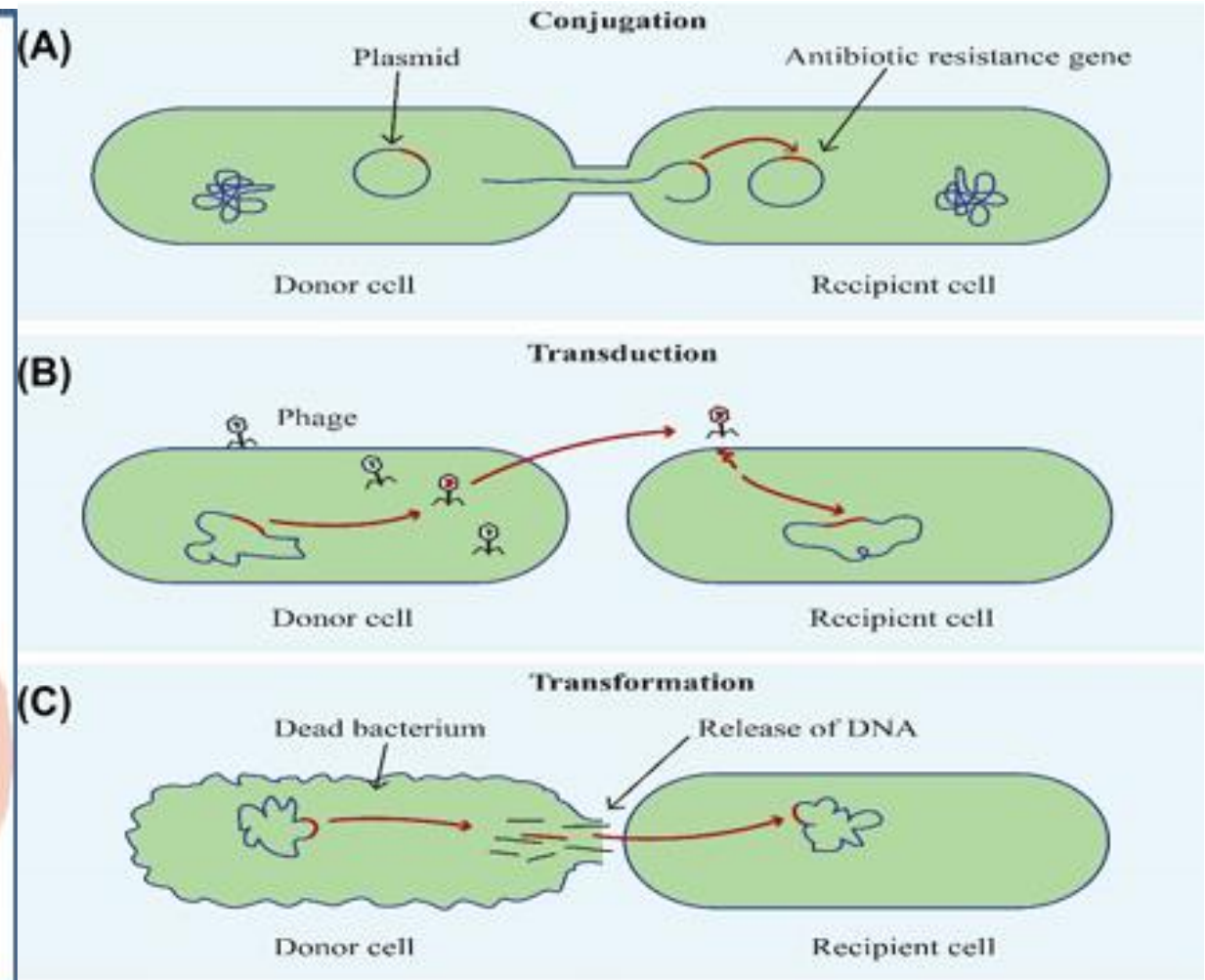
World Health
Organization

Acquisition of resistance genes

VERTICAL GENE TRANSFER



HORIZONTAL GENE TRANSFER

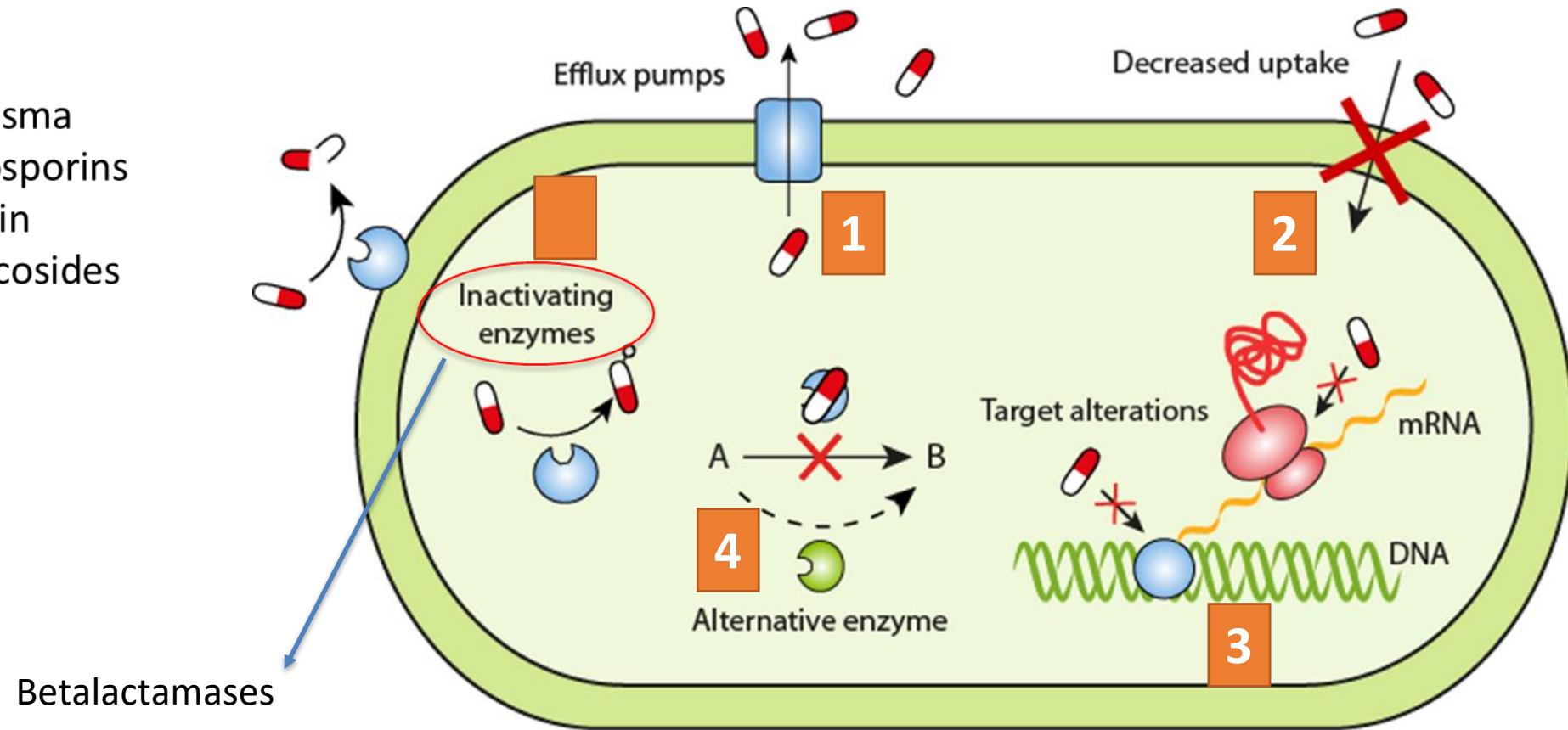


Mechanisms of resistance

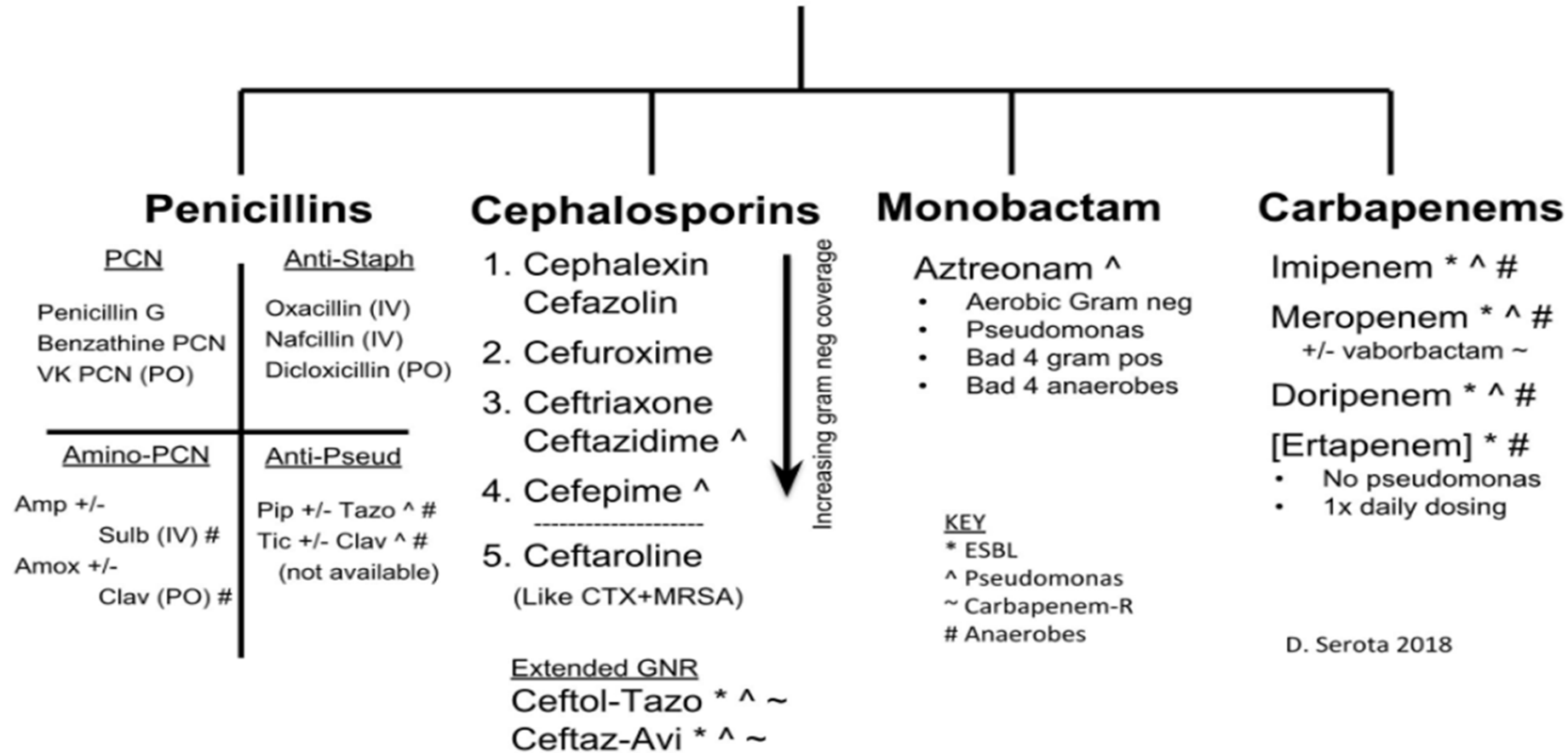
INTRINSIC RESISTANCE

- Natural
- Wild-type
- Eg: Beta-lactams-Mycoplasma
- Enterococcus-cephalosporins
- Gram neg- Vancomycin
- Anaerobes-Aminoglycosides
-

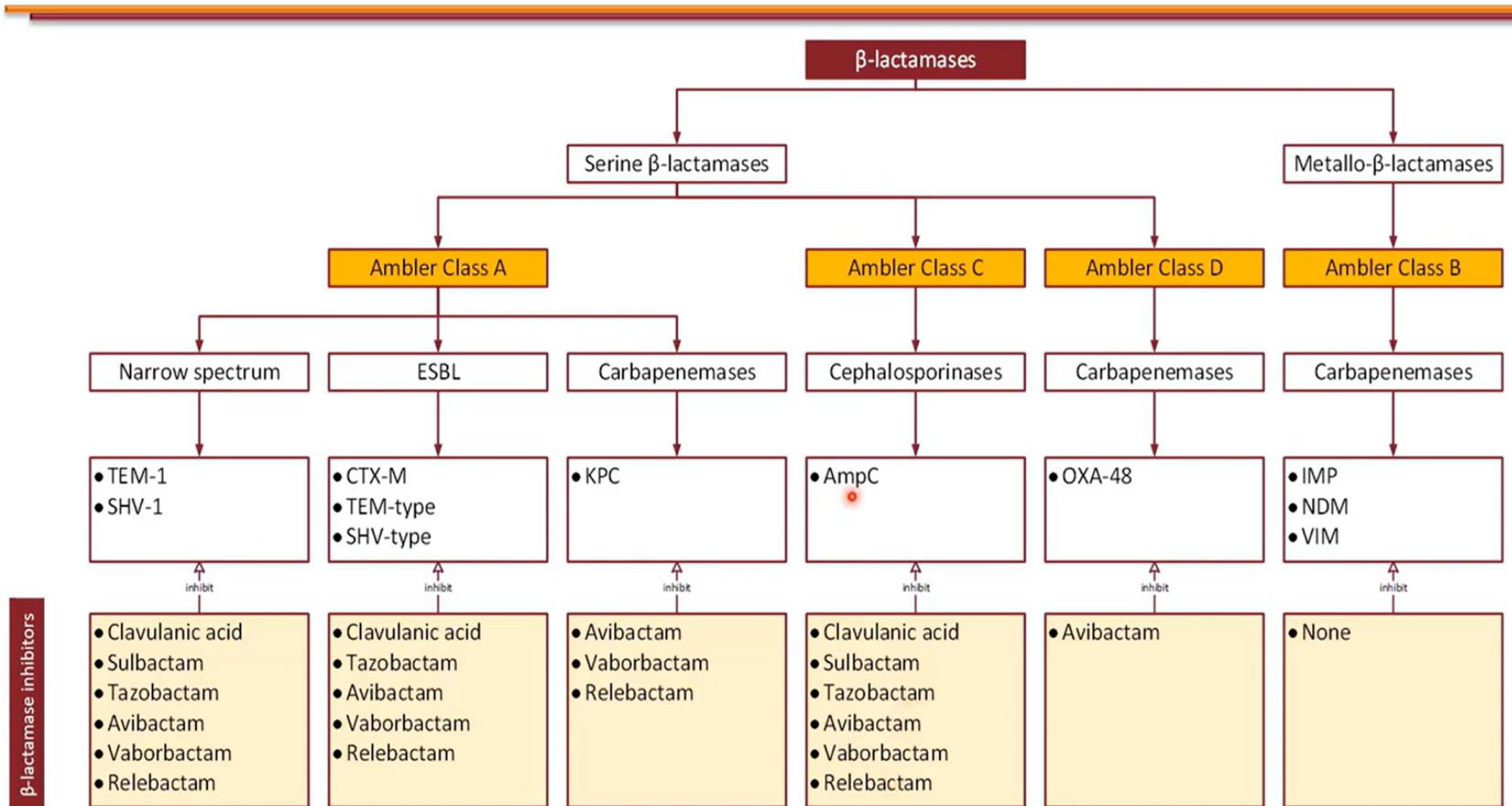
ACQUIRED RESISTANCE



Beta Lactams



Classification of β -Lactamases



- **Penicillinases (Subgroup 2a)**

- β -lactamases with a relatively limited spectrum of hydrolytic activity
- Predominant β -lactamases in Gram-positive cocci, including the staphylococci (blaZ)
- Hydrolyze benzyl-penicillin and many penicillin derivatives, with poor hydrolysis of cephalosporins, carbapenems, or monobactams
- Inhibited by beta-lactamase inhibitors

- **Subgroup 2b β -lactamases**

- Hydrolyze penicillins and early cephalosporins, such as cephaloridine and cephalothin
- Strongly inhibited by clavulanic acid and tazobactam.
- They include the TEM-1, TEM-2, and SHV-1 enzymes, the most common plasmid-mediated β -lactamases identified in the 1970s and early 1980s
- Mainly found in gram negative bacteria

• Phenotypic Methods

1. Nitrocefin Test:

- **Procedure:** Use a chromogenic cephalosporin (nitrocefin) which changes colour when hydrolyzed by beta-lactamase
- **Interpretation:** A colour change (yellow to red) indicates beta-lactamase activity

QC: *S.aureus* 29213-Positive

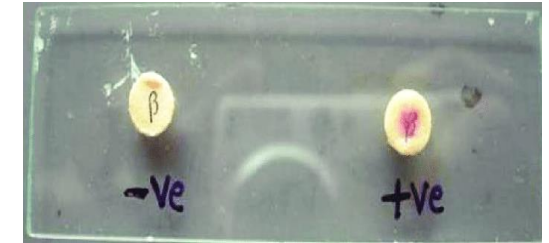
S.aureus 25923 Negative

2. Combination Disk Test:

- **Procedure:** Use disks containing a beta-lactam antibiotic alone and in combination with a beta-lactamase inhibitor.
- **Interpretation:** An increase in the zone of inhibition with the inhibitor indicates the presence of beta-lactamase

- **NOTE:** A positive test means penicillin, ampicillin, amoxicillin and piperacillin

Tests indicated for *S. aureus* isolates that test susceptible to Penicillin before reporting

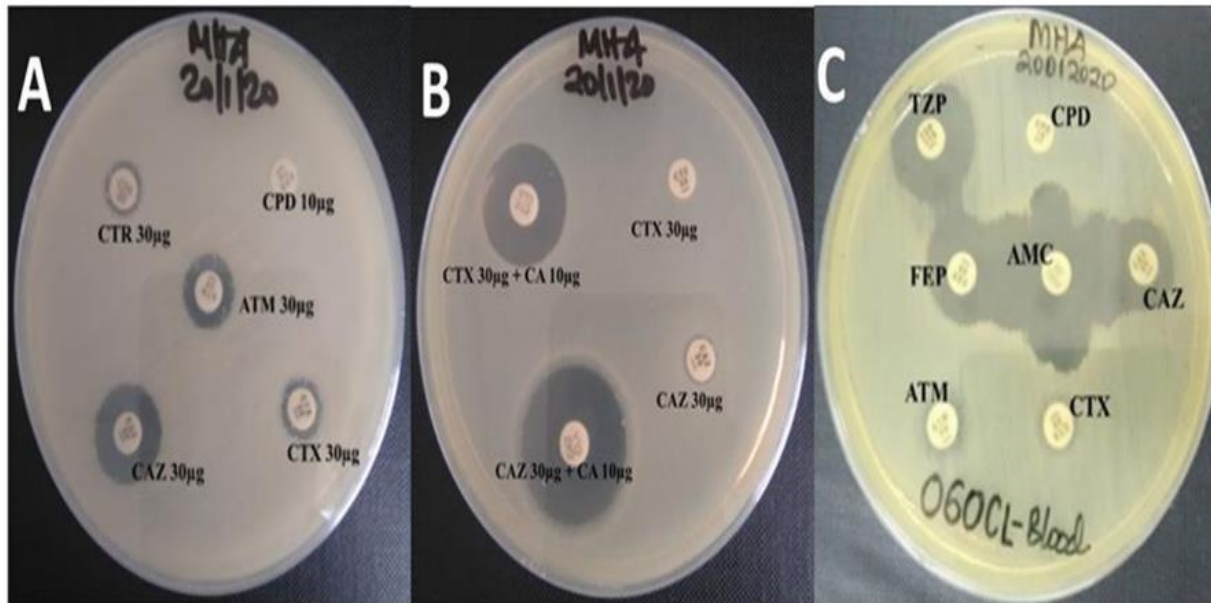


- Group 2be

- Produce extended spectrum of activity
- Also known as ESBL
- Inactivates penicillins, 1st to 3rd gen cephalosporins, monobactams but not carbapenems
- ESBL types are diverse, encoded by TEM, SHV, CTX-M variants
- Inhibited by beta-lactamase inhibitors



- **Double-Disk Synergy Test (DDST)/Clavunate inhibition Test:**
 - **Procedure:** Place a beta-lactam antibiotic disk and a beta-lactamase inhibitor disk close to each other on an agar plate inoculated with the test organism
 - **Interpretation:** Enhanced inhibition near the inhibitor disk indicates beta-lactamase production



Quality control for ESBL

- *Escherichia coli* ATCC 25922 - ≤ 2 mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.
- *Klebsiella pneumoniae* ATCC 700603 - ≥ 5 mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone.
- ≥ 3 mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.

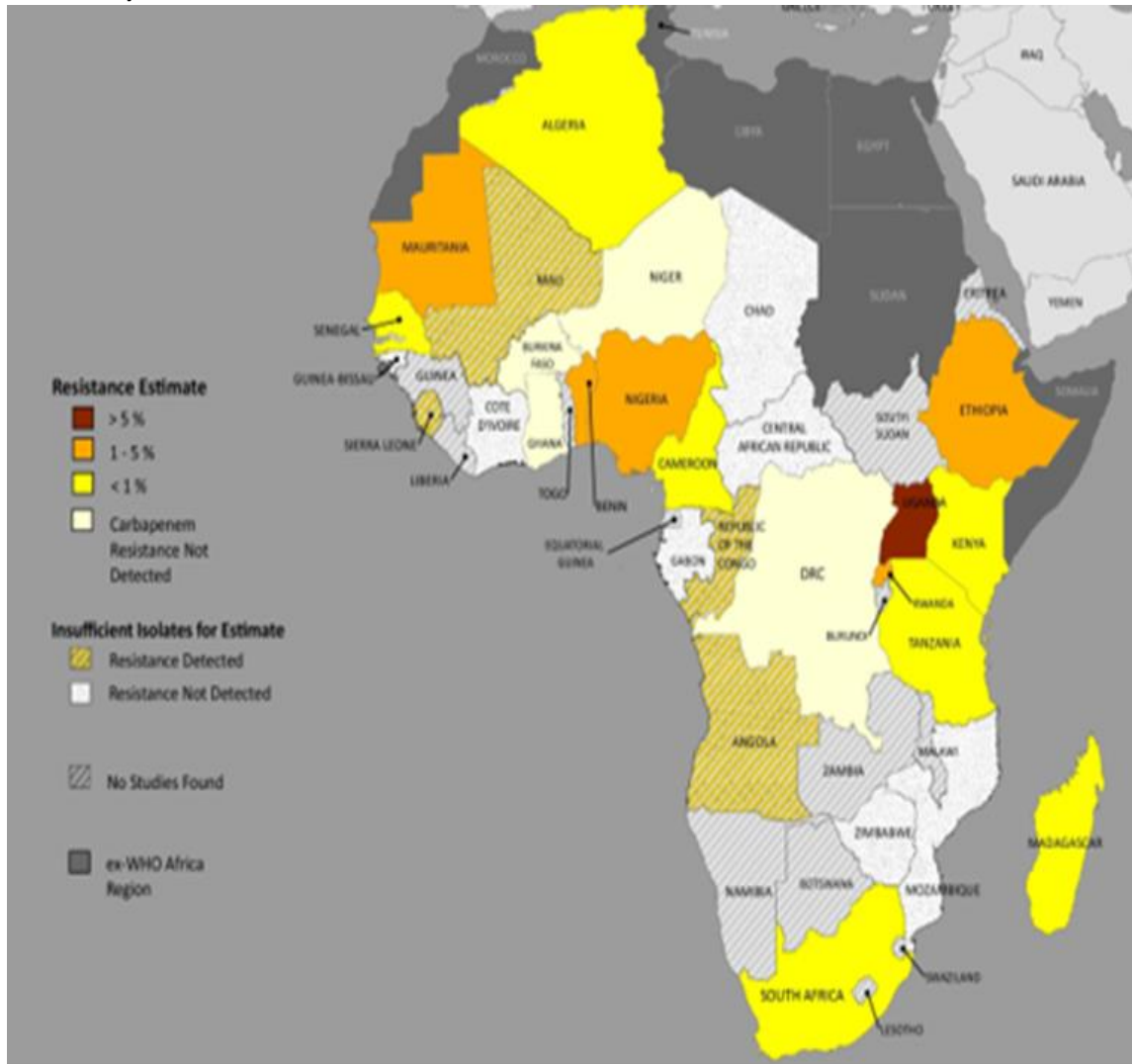


- AmpC beta-lactamases are classified as Group 1 according to the Bush-Jacoby-Medeiros classification
- Provide resistance to most penicillins, cephalosporins (except for fourth-generation cephalosporins like cefepime), cephamycins (e.g., cefoxitin), and monobactams (e.g., aztreonam)
- They do not hydrolyze carbapenems, but AmpC-producing organisms may have additional resistance mechanisms that confer resistance to these drugs
- The genes encoding AmpC beta-lactamases are either chromosomal or plasmid-mediated
 - Chromosomal AmpC genes are typically inducible and are found in species such as *Serratia marcescens*, *Providencia spp*, *Indole(+)**Proteus*, *Citrobacter freundii*, *Enterobacter spp*, and *Morganella morganii*.
 - Plasmid-mediated AmpC genes, such as blaCMY, blaACT, and blaDHA, can spread between different bacterial species.

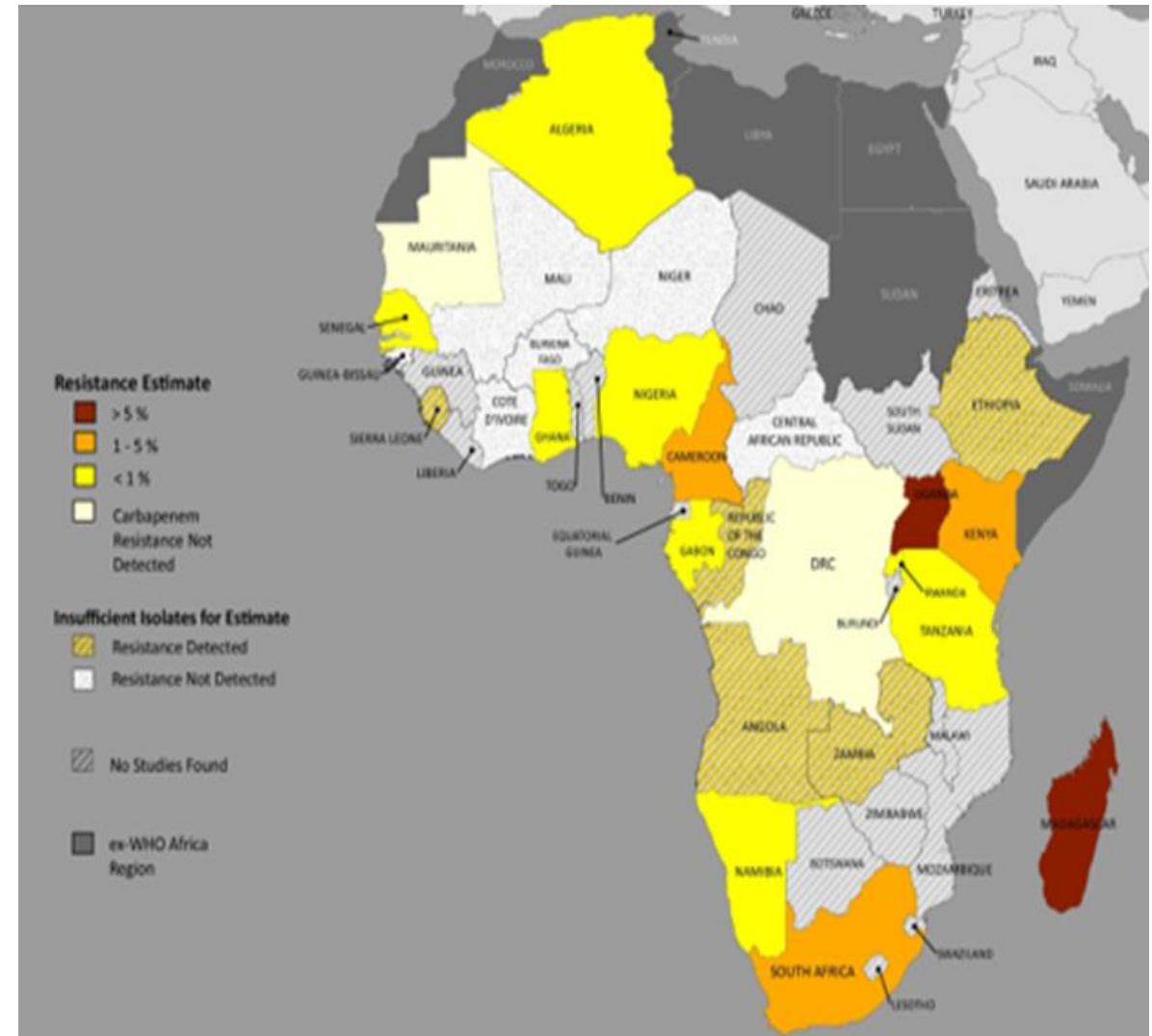
- There are no CLSI guidelines for detecting AmpC-mediated resistance in Gram-negative clinical isolates, which often leads to misleading results, particularly with phenotypic tests
- Detection of AmpC production in organisms with inducible chromosomal AmpC β -lactamase is unnecessary
- **Phenotypic Methods:**
 - Testing for AmpC production can include the use of ceftiofur disks in disk diffusion tests, which can indicate resistance due to AmpC production
- **Molecular Methods:**
 - PCR and sequencing can identify specific genes encoding AmpC beta-lactamases

Mapping of Carbapenem resistant Enterobacteriales

a)



b)



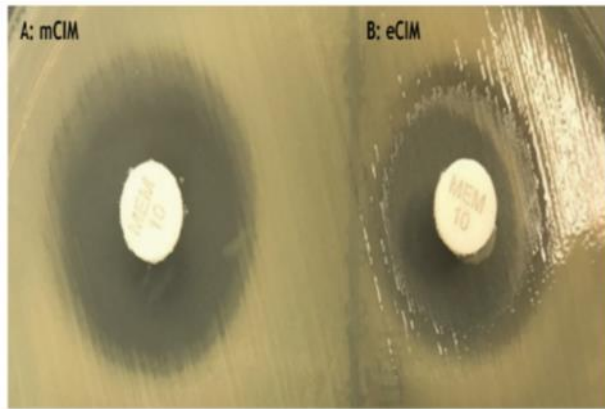
- Carbapenemase producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems
- Ertapenem non susceptibility is the most sensitive indicator of carbapenemases production
- Imipenem or meropenem MICs 2-4 ug/ml or Ertapenem MIC 2 ug/ml
- Quality control strains
 - E.coli ATCC 25922
 - *K. pneumoniae* ATCC BAA 1705 KPC positive
 - *K. pneumoniae* ATCC BAA 1706: Carbapenemase negative
 - *K. pneumoniae* ATCC BAA 2146:NDM positive (For use with eCIM)

Carbapenemases detection

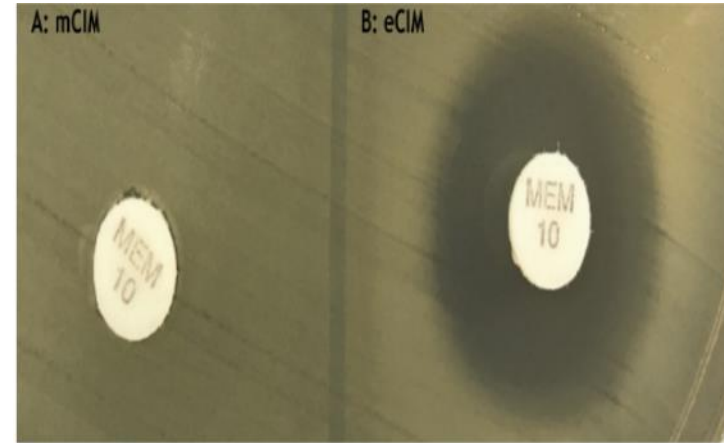
	Tests Used for Epidemiological or Infection Prevention-Related Testing			
	CarbaNP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and <i>P. aeruginosa</i> that are not susceptible to one or more carbapenems	Enterobacterales and <i>P. aeruginosa</i> that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and <i>P. aeruginosa</i> that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP or mCIM.
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	<p>Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life).</p> <p>Invalid results occur with some isolates.</p> <p>Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected.</p>	Requires overnight incubation	Requires overnight incubation	<p>Special reagents and equipment are needed.</p> <p>Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.</p>

Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method, MIC, minimal inhibitory concentration.

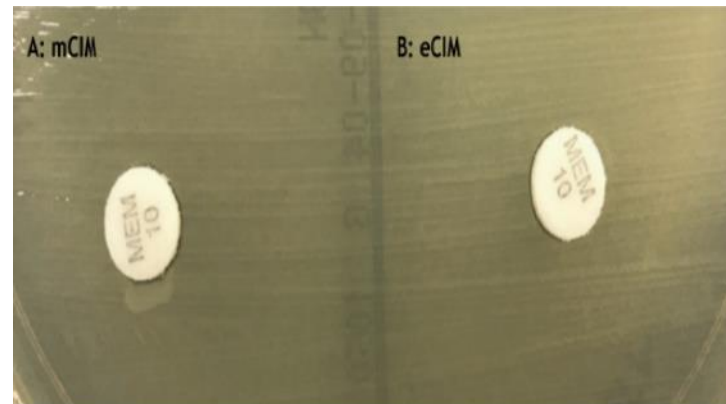
Carbapenemase detection methods



Result: Negative for carbapenemases
Report: Carbapenemase not detected



Result: Positive mCIM and eCIM
Report: Metallo-b-lactamase



Result: Positive mCIM; Negative eCIM
Report: Carbapenemase detected(Serine)

Carbapenemase detection methods

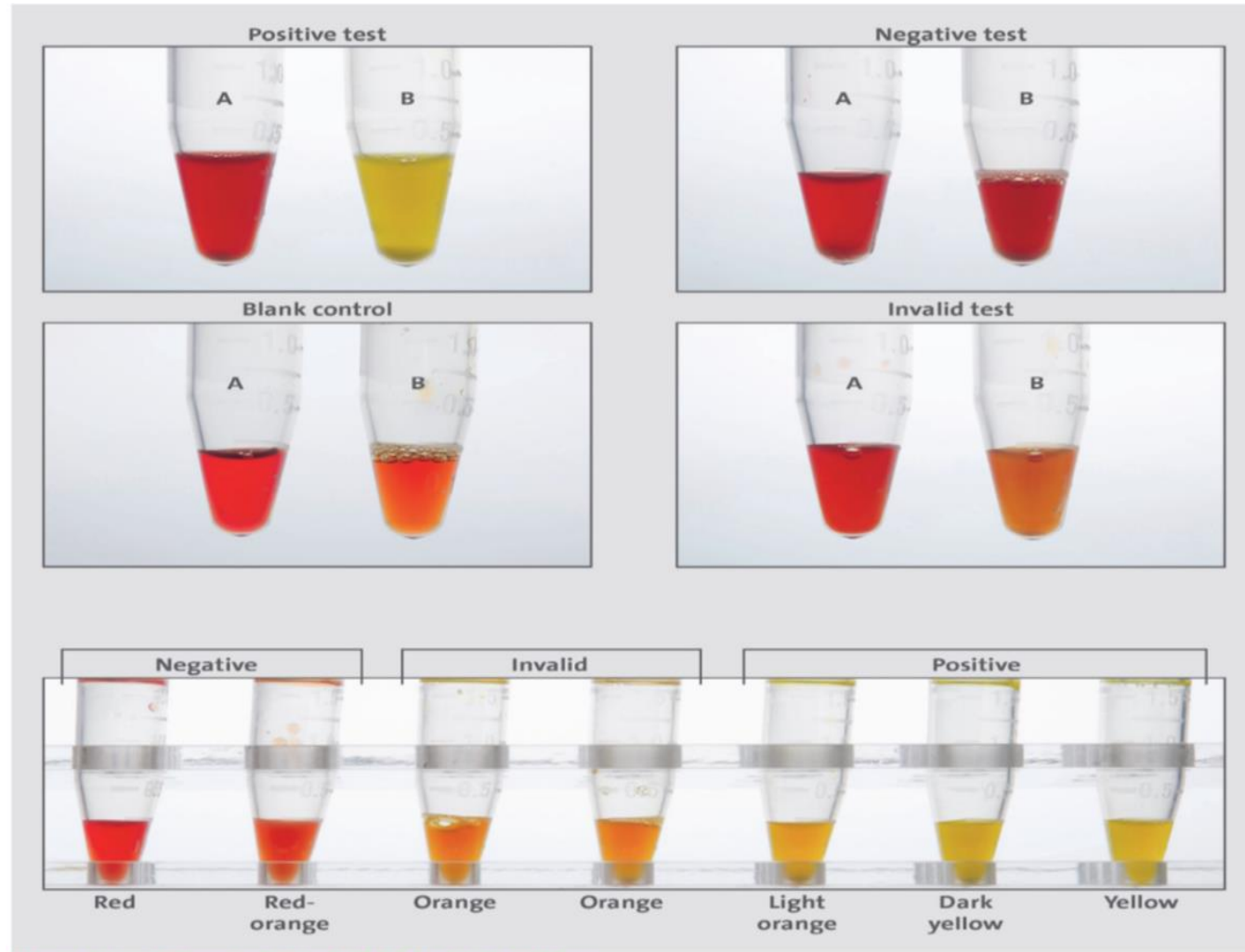


Figure 1. Interpretation of Color Reactions

Exercise 1



Organism Quantity: Selected Organism: *Klebsiella pneumoniae*

Comments:	

Identification Information	
Organism Origin	MYLA®
Selected Organism	<i>Klebsiella pneumoniae</i>
	Entered: Jul 9, 2024 12:23 CAT By: Download
Analysis Messages:	

Susceptibility Information	Card: AST-N255	Lot Number: 6552721403	Expires: May 9, 2025 12:00 CAT
	Status: Final	Analysis Time: 9.18 hours	Completed: Jul 9, 2024 21:52 CAT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>= 32	R	Imipenem	>= 16	R
Amoxicillin/Clavulanic Acid	>= 32	R	Meropenem	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Amikacin	>= 64	R
Cefuroxime	>= 64	R	Gentamicin	>= 16	R
Cefuroxime Axetil	>= 64	R	Ciprofloxacin	>= 4	R
Cefoxitin	>= 64	R	Tigecycline	4	I
Cefotaxime	>= 64	R	Nitrofurantoin	>= 512	R
Ceftazidime	>= 64	R	Colistin	<= 0.5	S
Cefepime	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ertapenem	>= 8	R			

AES Findings:	Last Modified: Oct 24, 2023 12:59 CAT Parameter Set: NHLS TSHWANE	
Confidence Level:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	CARBAPENEMASE (+ OR - ESBL), IMPERMEABILITY CARBA (+ESBL OR +HL AmpC)

Exercise 2

Organism Quantity:

Selected Organism: Klebsiella pneumoniae

Comments:	

Identification Information	
Organism Origin	MYLA®
Selected Organism	Klebsiella pneumoniae
	Entered: Jun 26, 2024 11:45 CAT By: Download
Analysis Messages:	

Susceptibility Information	Card: AST-N255	Lot Number: 6552721403	Expires: May 9, 2025 12:00 CAT
	Status: Final	Analysis Time: 8.73 hours	Completed: Jun 26, 2024 20:48 CAT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>= 32	R	Imipenem	<= 0.25	S
Amoxicillin/Clavulanic Acid	16	*R	Meropenem	<= 0.25	S
Piperacillin/Tazobactam	8	S	Amikacin	<= 2	S
Cefuroxime	>= 64	R	Gentamicin	<= 1	S
Cefuroxime Axetil	>= 64	R	Ciprofloxacin	1	R
Cefoxitin	<= 4	S	Tigecycline	2	S
Cefotaxime	>= 64	R	Nitrofurantoin	64	I
Ceftazidime	16	R	Colistin	<= 0.5	S
Cefepime	2	*R	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ertapenem	<= 0.5	S			

AES Findings:	Last Modified: Oct 24, 2023 12:59 CAT Parameter Set: NHLS TSHWANE	
Confidence Level:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	EXTENDED SPECTRUM BETA-LACTAMASE

Exercise 3



Organism Quantity:	Selected Organism: Staphylococcus aureus
Comments:	

Identification Information	
Organism Origin	MYLA®
Selected Organism	Staphylococcus aureus
	Entered: Jul 9, 2024 12:01 CAT By: Download
Analysis Messages:	
The following antibiotic(s) are not claimed: Ampicillin, Gentamicin High Level (synergy), Streptomycin High Level (synergy).	

Susceptibility Information	Card:	AST-P603	Lot Number:	4832749403	Expires:	Jun 6, 2025 12:00 CAT
	Status:	Final	Analysis Time:	12.40 hours	Completed:	Jul 10, 2024 00:45 CAT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	NEG	-	Clindamycin	<= 0.25	S
Benzylpenicillin	0.12	S	Linezolid	2	S
Ampicillin			Teicoplanin	<= 0.5	S
Oxacillin	0.5	S	Vancomycin	1	S
Gentamicin High Level (synergy)			Tetracycline	>= 16	R
Streptomycin High Level (synergy)			Tigecycline	<= 0.12	S
Gentamicin	<= 0.5	S	Fusidic Acid	<= 0.5	S
Ciprofloxacin	<= 0.5	S	Mupirocin	<= 2	
Moxifloxacin	<= 0.25	S	Rifampicin	<= 0.5	S
Inducible Clindamycin Resistance	NEG	-	Trimethoprim/ Sulfamethoxazole	>= 320	R
Erythromycin	<= 0.25	S			

Exercise 4

Organism Quantity:	Selected Organism: Staphylococcus aureus					
Comments:						
Identification Information						
Organism Origin	MYLA®					
Selected Organism	Staphylococcus aureus					
	Entered: Jul 9, 2024 12:01 CAT		By: Download			
Analysis Messages:						
The following antibiotic(s) are not claimed: Ampicillin, Gentamicin High Level (synergy), Streptomycin High Level (synergy),						
Susceptibility Information	Card:	AST-P603	Lot Number:	4832749403	Expires:	Jun 6, 2025 12:00 CAT
	Status:	Final	Analysis Time:	12.65 hours	Completed:	Jul 10, 2024 01:00 CAT
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation	
Cefoxitin Screen	NEG	-	Clindamycin	<= 0.25	S	
Benzylpenicillin	>= 0.5	R	Linezolid	2	S	
Ampicillin			Teicoplanin	<= 0.5	S	
Oxacillin	0.5	S	Vancomycin	<= 0.5	S	
Gentamicin High Level (synergy)			Tetracycline	<= 1	S	
Streptomycin High Level (synergy)			Tigecycline	<= 0.12	S	
Gentamicin	4	*R	Fusidic Acid	<= 0.5	S	
Ciprofloxacin	<= 0.5	S	Mupirocin	<= 2		
Moxifloxacin	<= 0.25	S	Rifampicin	<= 0.5	S	
Inducible Clindamycin Resistance	NEG	-	Trimethoprim/Sulfamethoxazole	160	R	
Erythromycin	<= 0.25	S				

Exercise 5



Organism Quantity: Selected Organism: Staphylococcus aureus

Comments:	[REDACTED]

Identification Information	
Organism Origin	MYLA®
Selected Organism	Staphylococcus aureus
Entered:	Jul 10, 2024 14:44 CAT By: Download

Analysis Messages:
The following antibiotic(s) are not claimed:
Ampicillin, Gentamicin High Level (synergy), Streptomycin High Level (synergy).

Susceptibility Information	Card:	AST-P603	Lot Number:	4832749403	Expires:	Jun 6, 2025 12:00 CAT
	Status:	Final	Analysis Time:	12.87 hours	Completed:	Jul 11, 2024 04:00 CAT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Clindamycin	<= 0.25	*R
Benzylpenicillin	>= 0.5	R	Linezolid	2	S
Ampicillin			Teicoplanin	<= 0.5	S
Oxacillin	>= 4	R	Vancomycin	<= 0.5	S
Gentamicin High Level (synergy)			Tetracycline	>= 16	R
Streptomycin High Level (synergy)			Tigecycline	0.25	S
Gentamicin	>= 16	R	Fusidic Acid	<= 0.5	S
Ciprofloxacin	>= 8	R	Mupirocin	>= 8	
Moxifloxacin	1	I	Rifampicin	<= 0.5	S
[REDACTED]	[REDACTED]	[REDACTED]	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ervitromycin	>= 8	R			

Exercise 5



Organism Quantity: _____ Selected Organism: Staphylococcus aureus

Comments:

MRSA

Identification Information	
Organism Origin	MYLA®
Selected Organism	Staphylococcus aureus
	Entered: Jul 10, 2024 14:44 CAT By: Download
Analysis Messages:	
The following antibiotic(s) are not claimed: Ampicillin, Gentamicin High Level (synergy), Streptomycin High Level (synergy).	
This isolate is presumed to be resistant based on detection of inducible clindamycin resistance.	

Susceptibility Information	Card: AST-P603	Lot Number: 4832749403	Expires: Jun 6, 2025 12:00 CAT
	Status: Final	Analysis Time: 12.87 hours	Completed: Jul 11, 2024 04:00 CAT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Clindamycin	<= 0.25	*R
Benzylpenicillin	>= 0.5	R	Linezolid	2	S
Ampicillin			Teicoplanin	<= 0.5	S
Oxacillin	>= 4	R	Vancomycin	<= 0.5	S
Gentamicin High Level (synergy)			Tetracycline	>= 16	R
Streptomycin High Level (synergy)			Tigecycline	0.25	S
Gentamicin	>= 16	R	Fusidic Acid	<= 0.5	S
Ciprofloxacin	>= 8	R	Mupirocin	>= 8	
Moxifloxacin	1	I	Rifampicin	<= 0.5	S
Inducible Clindamycin Resistance	POS	+	Trimethoprim/ Sulfamethoxazole	>= 320	R
Erythromycin	>= 8	R			

*= AES modified **= User modified

AES Findings:	Last Modified: Oct 24, 2023 12:59 CAT Parameter Set: NHLS TSHWANE	
Confidence Level:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	MUPIROCIN	LOW LEVEL RESISTANCE,HIGH LEVEL RESISTANCE

Exercise 6



Organism Quantity:	Selected Organism: <i>Serratia marcescens</i>					
Comments:						
Identification Information						
Organism Origin	MYLA®					
Selected Organism	<i>Serratia marcescens</i>					
	Entered: Jul 5, 2024 16:26 CAT		By: Download			
Analysis Messages:						
The following antibiotic(s) are suppressed from analysis: Ampicillin, Piperacillin/Tazobactam, Imipenem.						
Susceptibility Information	Card:	AST-N255	Lot Number:	6552721403	Expires:	May 9, 2025 12:00 CAT
	Status:	Final	Analysis Time:	15.85 hours	Completed:	Jul 6, 2024 08:40 CAT
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation	
Ampicillin			Imipenem			
Amoxicillin/Clavulanic Acid	16	*R	Meropenem	<= 0.25	S	
Piperacillin/Tazobactam			Amikacin	<= 2	*R	
Cefuroxime	16	*R	Gentamicin	<= 1	S	
Cefuroxime Axetil	16	*R	Ciprofloxacin	<= 0.25	S	
Cefoxitin	8	*R	Tigecycline	1	*R	
Cefotaxime	<= 1	S	Nitrofurantoin	256	R	
Ceftazidime	<= 1	S	Colistin	>= 16	R	
Cefepime	<= 1	S	Trimethoprim/ Sulfamethoxazole	<= 20	S	
Ertapenem	<= 0.5	S				

- Antimicrobial Resistance (AMR) is a public health threat
- It is associated with high morbidity and mortality
- Widespread use of beta-lactams has led to development of resistance
- The choice of testing method to be used is based on the easy of use, and timeliness
- Appropriate use of antibiotic at the appropriate dosage and for the appropriate duration—is one important means of reducing the selective pressure that helps resistant organisms emerge
- Early appropriate laboratory detection of resistance will assist in prompt appropriate management of patients

ASLM

AFRICAN SOCIETY FOR LABORATORY MEDICINE

ADVANCING THE LABORATORY PROFESSION AND NETWORKS IN AFRICA

ANTIMICROBIAL RESISTANCE (AMR) COMMUNITY OF PRACTICE (CoP)



THANK YOU



NATIONAL INSTITUTE FOR
COMMUNICABLE DISEASES

Division of the National Health Laboratory Service