# Application of CLSI in AST Reporting

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**Antimicrobial susceptibility test (AST)** is the most important task of Microbiology laboratory.

Accurate interpretation of AST results help to initiate appropriate antibiotic therapy and also help preparing the antibiogram, infection control purpose etc.

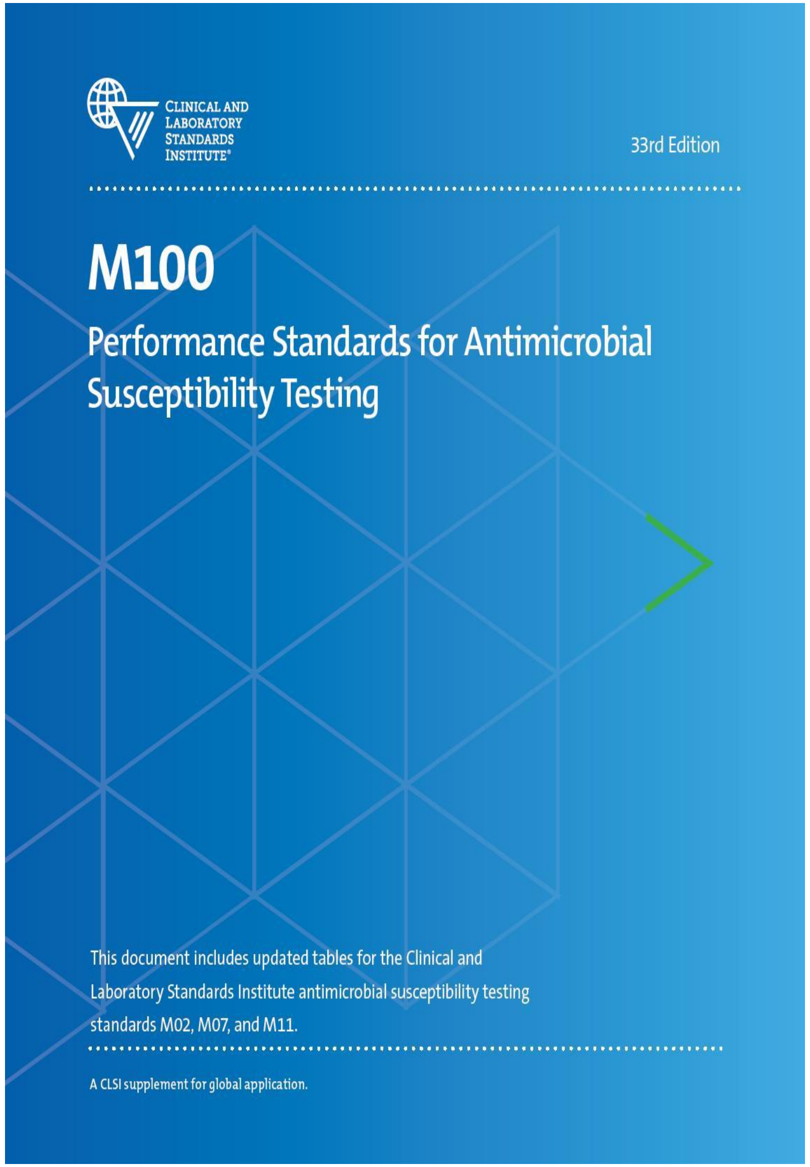
Erroneous interpretation of AST results lead to therapeutic failure, error in estimating true  AMR burden.

# Antimicrobial panel selection guidelines

Two standard guidelines available that provides recommendation for accurate interpretation of AST results

-Clinical Laboratory Standard Institute(CLSI)

-European Committee on Antimicrobial Susceptibility testing (EUCAST)



# Selection of antimicrobial panel for AST

- Based on CLSI/EUCAST
- Consensus between Microbiology and clinical department
- Yearly updated

# AST panel categorized into several Test/Report Groups up to CLSI M100,2022

| CLSI Test/Report Group | Definition |
|---|---|
| Group A | Primary Test And Reported |
| Group B | Optional Primary Test, Selectively Reported |
| Group C | Supplemental, Selectively Reported |
| Group U | Primary tested, For Urine Only |
| Group Inv. ("investigational") | Not yet approved by US FDA |
| Group O ("other") | Have clinical indication but are generally not routinely tested /reported in USA. |

# CLSI M100,2023 updated **AST panel category into TEST/REPORT Tiers**

## Tier Based Approach

- Clinical efficacy
- Prevalence of resistance
- Minimizing emergence of resistance
- FDA indications
- Current recommendations on first line and alternative drugs
- Cost

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### IDSA Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections: Version 1.0

Published by IDSA, 3/7/2022

A Focus on Extended-Spectrum β-lactamase Producing Enterobacterales, Carbapenem-Resistant Enterobacterales, and *Pseudomonas aeruginosa* with Difficult-to-Treat Resistance

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### IDSA Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections: Version 2.0

Published by IDSA, 3/31/2022

A focus on AmpC β-lactamase-Producing Enterobacterales, Carbapenem-Resistant *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* Infections

# TEST/REPORT Tiers and additional designation

**Tier 1 :** Antimicrobial agent that are appropriate for routine, primary testing And reporting.

**Tier 2 :** Antimicrobial agent that are appropriate routine, primary testing but may follow cascade reporting rule.

**Tier 3 :** Antimicrobial agent that are appropriate routine, primary testing in institutions that serve patients at high risk for MDROs but should follow cascade reporting rule.

**Tier 4 :** Antimicrobial agent that  warrant testing and reporting by clinician request if other tier agents are not optimal.

**Urine Only(U):** Primary tested, For Urine Only

**Group Inv.** (“investigational”): Not yet approved by US FDA

**Group O (“other”):** Clinically indicated but are generally not tested in US.

- **Recommendations for reporting Tier 2 Agents**

  -When the organism is resistant to agents in Tier 1

- **Recommendations for reporting Tier 3 Agents**

  -When the organism is resistant to agents in Tier 1 and 2

- **Recommendations for reporting Tier 4 Agents-**

  -Unavailability of preferred drug for clinical use
  -Patients underlying condition, including allergies
  -Resistance to Tier 1,2 and 3
  -Polymicrobial infection
  -infection control purposes as an epidemiological aid

## Group U ("urine") includes certain antimicrobial agents that

- Used only or primarily for treating UTIs: (e.g., nitrofurantoin,, Trimethoprim,sulfamethoxazole,fosfomycin)
- Should not be reported against pathogens from other sites.

## Exception:

- **Cefazolin** which has UTI and Systemic BP (for ENB, Tier 1 and U cat.)
- **Norfloxacin:** Although O category, to be tested for urine-only isolates
- **Broader anbx, pathogen specific group U-**
  - Ciprofloxacin and levofloxacin –*Enterococcus*
  - Tetracycline – *Acinetobacter, Enterococcus,* Other non- ENB
  - Pseudomonas aeruginosa-Amikacin

# Tier Based Approach

| CLSI Tier | Testing | Reporting |
|---|---|---|
| 1 | Routine | All |
| 2 (General) | Routine | Cascade |
| 3 (High risk for MDROs) | Routine or by request | Cascade |
| 4 | By request | By request |
| U only | Routine | As appropriate |
| Other<br>(Mostly not in the list of first choice or alternative drugs) | By request | By request |
| Inv<br>(No FDA approval) | By request | By request |

- **Selective Reporting**

  AST results for a particular bug-drug combination are obtained but results are not reported in the electronic health record.

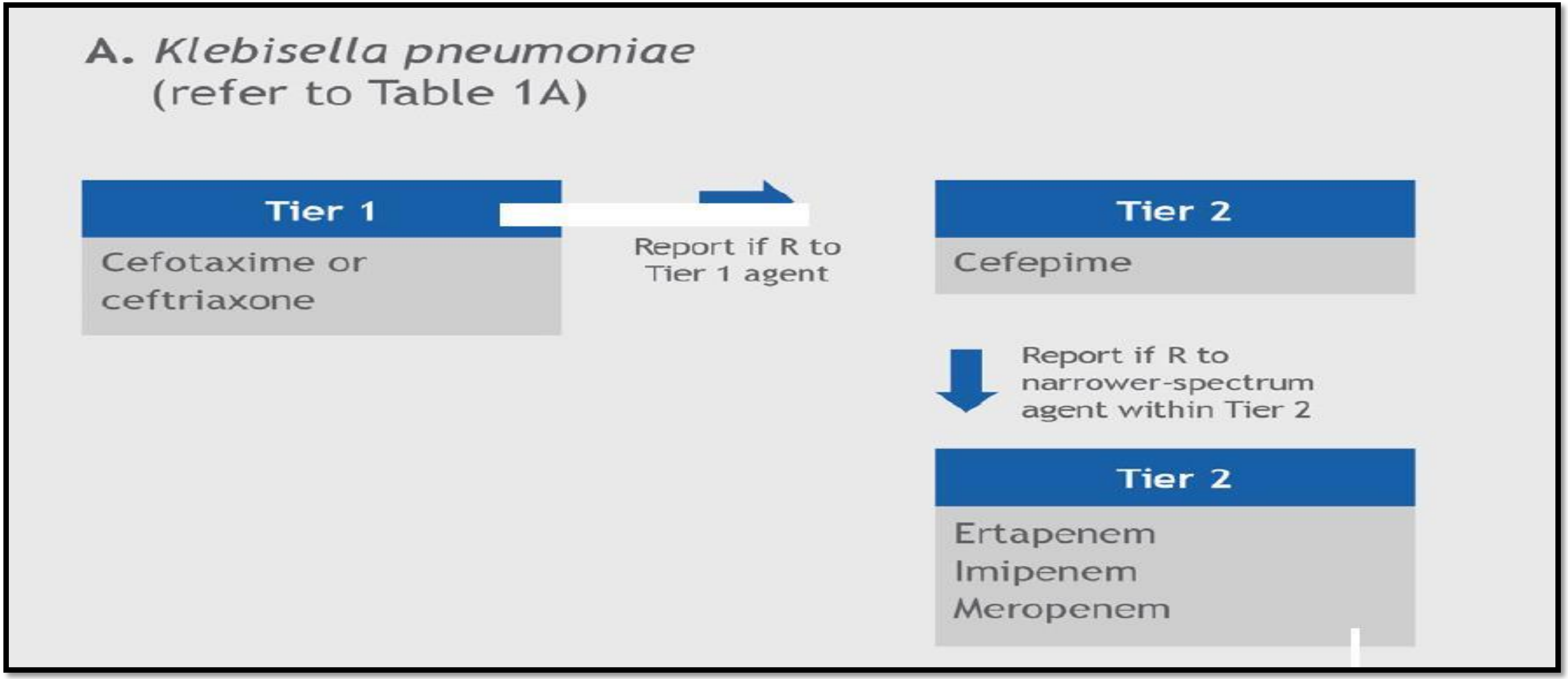
- **Cascade Reporting**

  AST results for a particular bug-drug combination are obtained but results are not reported for broader-spectrum agents unless the bug is resistant to narrow-spectrum agents. Cascade reporting is a subset of selective reporting.

- **Importance**

  Selective and Cascade Reporting are done to encourage appropriate antimicrobial agent use.

# Example of cascade reporting

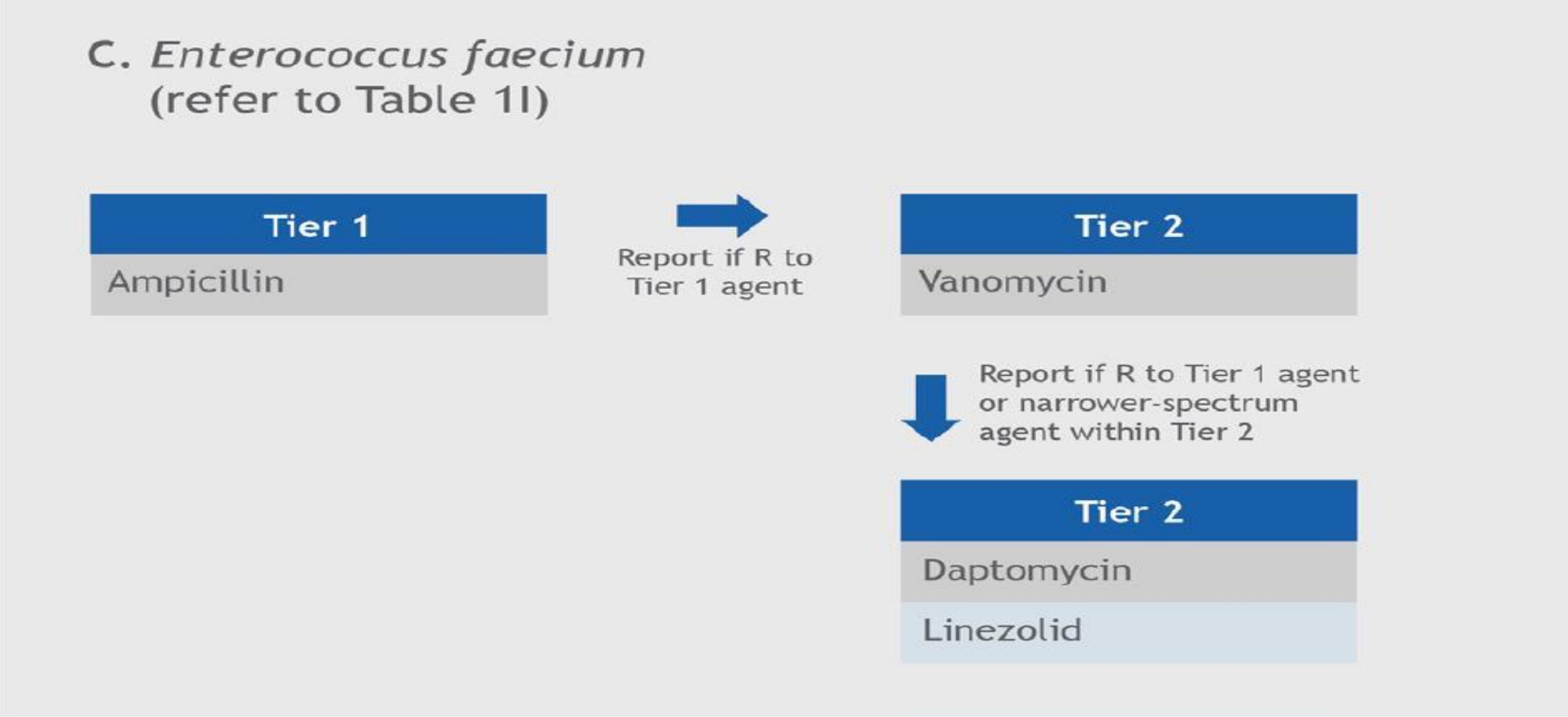


# Cascade reporting – Between Tiers

**B.** *Klebisella pneumoniae*
(refer to Table 1A)

| Tier 1 | | Tier 2 | | Tier 3 |
|---|---|---|---|---|
| Cefotaxime or ceftriaxone | Report if R to Tier 1 agent | Cefepime | Report if R to Tier 1 and Tier 2 agents | Cefiderocol |
| | | Ertapenem<br>Imipenem<br>Meropenem | | Ceftazidime-avibactam |
| | | | | Imipenem-relebactam |
| | | | | Meropenem-vaborbactam |

# Cascade reporting – Within Tiers



# Antibiotic panel for different Gram negative bacilli (CLSI)

| Tier | Enterobacterales | Pseudomonas aeruginosa | Acinetobacter | Other non- ENB |
|---|---|---|---|---|
| Tier 1 | Ampicillin, Cefazolin, Cefotaxime or ceftriaxone, Amox-clav, Amp-sul, Pip-taz, Genta,<br>Ciproflox, Levoflox<br>Trimethoprim-sulfamethoxazole | Ceftazidim, Cefepime<br>Pip-taz ,<br>Tobra, Cefepime Cipro, Levo | Amp-sul, Cefta, Cefepime<br>Cipro, Levo, Genta, Tobra | Ceftazidime Genta, tobra, Pip-taz<br>Trimethoprim-sulfamethoxazole |
| Tier 2 | Cefuroxime, Cefepime<br>Carbapenems<br>Amikacin,Tobra<br>Cefoxitin,cefotetan,<br>Tetracycline | Carbapenem(Imi, mero) | Carbapenem<br>(Imi, mero)<br>Amikacin Piptaz<br>Trimethoprim-sulfamethoxazole<br>Minocycline | Cefepime<br>Carbapenem(Imi, mero)<br>Amikacin<br>Ciproflox, Levoflox<br>Minocycline |
| Tier 3 | Cefta-avib,Cefiderocol<br>Imi-rele,Mero-Vabo<br>Plazomycin | Cefiderocol,Cefta-avib<br>Ceftolozane-taz<br>Imi-rele | Cefiderocol | |
| Tier 4 | Aztreonam,Ceftarolin,<br>Ceftazidime | Aztreonam | Cefotaxime<br>Ceftriaxone<br>Doxycycline<br>Colistin or<br>polymyxinB | Cefotaxime<br>Ceftriaxone |

# Antibiotic panel for different Gram positive cocci (CLSI)

| Tier | Staphylococcus | Enterococcus | βH Streptococci | S. pneumoniae |
|---|---|---|---|---|
| **Tier 1** | Azithro/clarithro/erythro Clindamycin, Oxacillin, Cefoxitin(surrogate for oxacillin),<br>Doxy, Minocycline ,Tetracycline<br>Cotrimoxazole, Vancomycin | Ampicillin ,Penicillin | Erythromycin Clindamycin Ampicillin or Penicillin | Erythromycin Penicillin (Ox disk) Cotrimoxazole Cefotaxime or ceftriaxon |
| **Tier2** | Penicillin,<br>Daptomycin , Linezolid, | Vancomycin<br>High Level Gentamicin,<br>Daptomycin, Linezolid | Tetracycline | Clindamycin, Doxy, tetracycline ,Levo, moxifloxacin Meropenem Vancomycin |
| **Tier3** | Ceftaroline, tedizolid, Rifampin Lefamulin | Streptomycin(high level resistance testing only),Tedizolid | Cefotaxime or ceftriaxon Vancomycin | |
| **Tier 4** | Cipro/levo, moxiflox Gentamicin, Dalbavancin, Orita, Telavancin | Dalba, Orita, Telavancin | Cefepime,Ceftaroline Daptomycin Levoflox Linezolid, tedizolid Dalba, Orita, Telavancin | Amoxycillin,AmoxyclaCefuroxime Ceftaroline,<br>Erta, imipenem<br>Linezolid,<br>rifamp,Cefuroxime |
| **Urine only** | Nitrofurantoin | T1-Nitrofurantoi-<br>T2-Cipro/levo<br>T3-Fosfo ( only for E.feacalis urinary isolates),Tetra | | |

# GENERAL TERMINOLOGIES

# (AST INTERPRETATIVE CATEGORY)

# (CLSI M100)

# Clinical Breakpoint

- MIC or zone diameter value
- Used to categorize an organism as one of the Interpretive Categories with respect to an antimicrobial agent
- Also called as Clinical Breakpoint

1. Microbiology characteristics
2. PK-PD parameters
3. Clinical outcome data

MIC distribution of a large no. of isolates

if the drug at standard dose attains the desired conc.

If the drug at is clinically effective

| CLSI M100 | Zone diameter (mm) | | | MIC (µg/mL) | | |
|---|---|---|---|---|---|---|
| Enterobacterales | S | I | R | S | I | R |
| Ampicillin | ≥17 | 14-16 | ≤13 | ≤8 | 16 | ≥32 |

**Different types of non clinical breakpoint have been proposed**

Epidemiological cut-off value:

PK-PD breakpoint:

| Parameters | Clinical BP | ECOFF/ ECV BP | PK-PD BP |
|---|---|---|---|
| MIC distribution data | ✓ | ✓ | X |
| PK-PD data | ✓ | X | ✓ |
| Clinical outcome data | ✓ | X | X |

## Breakpoint developing Guidelines:

- CLSI
- EUCAST
- Others: FDA, research articles
- Not to be used interchangeably

# AST interpretative categories (CLSI)

| Therapeutic success on | S | I | I^ | SDD | R |
|---|---|---|---|---|---|
| Standard dosage | ✓ | X | X | X | X |
| Increased dosage | - | ± | ± | ✓ | X |
| Standard dosage in urine | ✓ | ± | ✓ | ± | X |
| Technical errors | - | ± | ± | ± | - |

| AST interpretative categories and their definitions | |
|---|---|
| S | Isolate is inhibited *in-vivo* when drug is given at standard dosage |
| I | • Uncertain therapeutic effect (In-vivo response rates may be lower than for susceptible isolates when given in standard dose)<br>• May be active at sites where the drug is physiologically concentrated<br>• May be active when a high dose/frequency of drug is used<br>• Represents a buffer zone to prevent small, uncontrolled technical errors |
| SDD | Isolate is inhibited when drug is given at increased dosage<br>• ↑dose/frequency or both<br>• Literature (clinical trial) supported, Safe to use |
| I^ | Isolate is inhibited when drug is given at standard dose at urinary site<br>The drug is physiologically concentrated in the urine |
| R | Isolate is NOT in-vivo inhibited when drug is given at standard/increased dosage |
| NS | Isolate with ZD/MIC falls outside susceptible BP and<br>For which there is no resistant BP designated |

## SDD available for the following organism/drug combinations

- For Enterobacterales- Cefepime, Piperacillin and piperacillin-tazobactam
- For Staphylococcus aureus- Ceftaroline
- For Enterococcus faecium- Daptomycin

# Non-susceptible (NS) - CLSI M100

- Only a susceptible breakpoint is designated
- Because of the absence or rare occurrence of resistant strains.
- Non-susceptible does not necessarily mean that the isolate has a resistance mechanism
- Not to be confused with **not-susceptible** (I plus R categories)

# EUCAST Interpretative categories

| Testing results shows | Includes |
| --- | --- |
| The isolate is susceptible | S and I |
| The isolate is susceptible at standard dosing | S |
| The isolate is susceptible only at increased exposure | I |
| The isolate is resistant | R |
| The isolate falls in the range of area of technical uncertainty (not an interpretative category) | ATU |

# Equivalent agent vs Surrogate marker

## Equivalent agent

Testing for one agent (agent A) predict the susceptibility result (S/I/R) of another closely related (agent B) of the same class and vice –versa

## Surrogate agent

Test for the one agent (called surrogate marker) predicts the AST result (S/I/R) of another closely related agent /group (called the agent of interest), but not vice –versa.

# Equivalent agent vs Surrogate marker

As per CLSI

| Characteristics | Equivalent agent test | Surrogate marker test |
|---|---|---|
| Prediction of S and R results | Vice-versa (A↔B) | One directional (A⟶B) |
| Test for one agent can predict for | Another agent of the same class | One or many agents of the same class |
| Used for treatment | Both agents are used | Surrogate agent is not used |
| Testing method for the agent of interest (agent B) | Available | Either not available or has performance issues |

# List of Equivalent agents

As per CLSI

| Equivalent agents | Organism |
|---|---|
| Cefotaxime or ceftriaxone | Enterobacterales |
| Colistin or polymyxin B | Enterobacterales, *P. aeruginosa*, *A. baumannii* |
| Penicillin or penicillinase-labile Pn(ampi, amox)* | *Staphylococcus* |
| Azithromycin, clarithromycin, erythromycin | |
| Ciprofloxacin or levofloxacin | |
| Ampicillin or amoxicillin* | Anaerobes |
| Ampicillin or amoxicillin* | *Haemophilus* |
| Cefotaxime or ceftazidime or ceftriaxone | |
| Cefdinir or cefixime or cefpodoxime | |
| Ertapenem or imipenem | |
| Ciprofloxacin or levofloxacin or moxifloxacin | |
| Penicillin or ampicillin | β hemolytic streptococci |
| Cefepime or cefotaxime or ceftriaxone | |
| Cefotaxime or ceftriaxone | *N. meningitidis* |

As per CLSI

# List of Surrogate agents

| Surrogate agents | Organism | Agents used for treatment |
|---|---|---|
| Cefazolin<br>(S result only) | *E. coli, Klebsiella, Proteus mirabilis* | Oral cephalosporins<br>(uncomplicated UTI) |
| Cefoxitin | MRSA (mec A) | All beta-lactams |
| Oxacillin<br>1µg disk | Pneumococcus | Penicillin (if oxacillin is found susceptible) |
| Pefloxacin | *Salmonella* | Ciprofloxacin |

# Predict susceptibility

- There are number of other antimicrobial agent  mention in CLSI, which can be used to predict the susceptibility to other agents but do not fit with the definitions of surrogate markers and equivalent agents.

- A comment  should always be added in patient report about clinical application of surrogate agent, equivalent agent and Predict susceptibility.

# Examples of predicting agents

As per CLSI

| Agent tested | Predict susceptibility to |
| --- | --- |
| Enterococci susceptible to penicillin (only S results) | Ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam |
| Enterococci susceptible to ampicillin (S and R results) | Amoxicillin, ampi-sulb, amox-clav, and pip-taz, imipenem (E. faecalis only) |
| Tetracycline (only S results) | Doxycycline and minocycline |
| S. pneumoniae to levofloxacin(only S results) | Gemifloxacin and moxifloxacin |
| S. pneumoniae to erythromycin (S and R) | Azithromycin, clarithromycin |
| Ampicillin for Enterobacterales (S and R) | Amoxicillin |
| Beta-lactam (only S results) | Corresponding beta lactam-beta lactamase inhibitors |

# Site-specific exclusion of antimicrobials from reporting

| Sample | Do not report |
| --- | --- |
| CSF | Agents administered by oral route only<br>1st- and 2nd-generation cephalosporins and cephamycins<br>Clindamycin<br>Macrolides<br>Tetracyclines<br>Fluoroquinolones<br>Carbapenems (Doripenem, imipenem, ertapenem)<br>Lefamulin |
| Urine | Clindamycin, Macrolide<br>Chloramphenicol |
| Respiratory specimen | Daptomycin, colistin (systemic) |

# Intrinsic resistance (IR)

- Intrinsic resistance  is the innate ability of bacteria  to resist the action of an antimicrobial agent.

- IR agent should be excluded from AST panel

- IR is always species-specific.so correct identification is important

- IR must be mention in patient report as an additional comment

Following members of Enterobacterales are also resistant to following antibiotics:

| | Ampicillin | Amoxicillin- clavulanate | Ampicillin- sulbactam | Ticarcillin | Cephalosporins I: Cefazolin, Cephalothin | Cephamycins: Cefoxitin, Cefotetan | Cephalosporin II: Cefuroxime | Tetracyclines | Tigecycline | Nitrofurantoin | Polymyxin B Colistin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Citrobacter freundii* | R | R | R | | R | R | R | | | | |
| *Citrobacter koseri, Citrobacter amalonaticus* group | R | | | R | | | | | | | |
| *Escherichia hermannii* | R | | | R | | | | | | | |
| *Klebsiella* (*formerly Enterobacter*) *aerogenes* | R | R | R | | R | R | | | | | |
| *Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella variicola* | R | | | R | | | | | | | |
| *Morganella morganii* | R | R | | | R | | R | | R | R | R |
| *Proteus mirabilis* | There is no intrinsic resistance to penicillins and cephalosporins in this organism. | | | | | | | R | R | R | R |
| *Proteus penneri* | R | | | | R | | R | R | R | R | R |
| *Proteus vulgaris* | R | | | | R | | R | R | R | R | R |
| *Serratia marcescens* | R | R | R | | R | R | R | | | R | R |
| *Yersinia enterocolitica* | R | R | | R | R | | | | | | |

**Table 8: Intrinsic antimicrobial resistance[3,15]**

| Organisms | Intrinsic resistance to the following antimicrobial agents |
|---|---|
| *Enterobacteriaceae* | Members of family *Enterobacteriaceae* are intrinsically resistant to antimicrobials specific for Gram-positive organisms such as: clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), lipoglycopeptides (oritavancin, teicoplanin, and telavancin), linezolid, tedizolid, quinupristin-dalfopristin, rifampin, and macrolides (erythromycin, clarithromycin, and azithromycin) |
| | Exceptions: *Salmonella* and *Shigella* spp. are susceptible azithromycin |
| *Klebsiella pneumonia* | Same as for *Enterobacteriaceae* plus ampicillin and ticarcillin |
| *Citrobactér* species | Same as for *Enterobacteriaceae* plus ampicillin, first and second generation cephalosporins, cephamycins, amoxicillin-clavulanate and ampicillin-sulbactam |
| *Enterobacter* species | Same as for *Enterobacteriaceae* plus ampicillin, first generation cephalosporins and cephamycins, amoxicillin clavulanate, ampicillin sulbactam |
| Proteeae tribe | Same as for *Enterobacteriaceae* plus ampicillin, first and second generation cephalosporins, tetracyclines, tigecycline, nitrofurantoin and polymyxins (polymyxin B and colistin) |
| *Salmonella* species | Same as for *Enterobacteriaceae* plus aminoglycosides, first and second generation cephalosporins |
| *Shigella* species | Same as for *Enterobacteriaceae* plus aminoglycosides, first and second generation cephalosporins, and cephamycins |
| *Serratia marcescens* | Same as for *Enterobacteriaceae* plus ampicillin, first and second generation cephalosporins, cephamycins, amoxicillin-clavulanate, ampicillin-sulbactam, nitrofurantoin and polymyxins (Polymyxin B and colistin) |
| *Yersinia enterocolitica* | Same as for *Enterobacteriaceae* plus ampicillin, ticarcillin, first generation cephalosporins and amoxicillin-clavulanate |
| NF-GNB | NF-GNB are intrinsically resistant to penicillin (i.e, benzyl penicillin), cephalosporins I (cephalothin, cefazolin), cephalosporin II (cefuroxime), cephamycins (cefoxitin, cefotetan), clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), linezolid, macrolides, quinupristin-dalfopristin, and rifampin |
| *Pseudomonas aeruginosa* | Same as for NF-GNB, plus ampicillin, ceftriaxone, amoxicillin-clavulanate, ampicillin-sulbactam, Ertapenem, tetracyclines, tigecycline, co-trimoxazole and chloramphenicol |
| *A. baumannii* | Same as for NF-GNB, plus ampicillin, amoxicillin, amoxicillin-clavulanate, ertapenem, aztreonam, chloramphenicol and fosfomycin |
| *S. maltophilia* | Same as for NF-GNB, plus ampicillin, amoxicillin, cefotaxime, ceftriaxone, cefepime, amoxicillin-clavulanate, aztreonam, imipenem, meropenem, ertapenem, polymyxins (polymyxin B and colistin), aminoglycosides, chloramphenicol and fosfomycin |
| *B. cepacia* complex | Same as for NF-GNB, plus ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, ertapenem, polymyxins (polymyxin B and colistin) and fosfomycin. Therefore, these drugs should not be used in therapy |
| Gram-positive bacteria | Gram-positive bacteria are intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid |
| *S. aureus* | Same as for other Gram-positive bacteria |
| *Enterococcus* species | Same as for other Gram-positive bacteria plus cephalosporins, aminoglycosides, clindamycin and co-trimoxazole |

*A. baumannii: Acinetobacter baumannii; B. cepacia: Burkholderia cepacia; S. maltophilia Stenotrophomonas maltophilia;* NF-GNB: Nonfermentative Gram-negative bacteria

# Exceptional resistance phenotypes:

--Exceptional resistance phenotypes are phenotypes of resistance of some bacterial species to particular antimicrobial agents that haven't yet been reported or are very rare.

--Exceptional resistance phenotypes should be checked.

--If they're confirmed locally, the isolate should be further studied to verify the exceptional phenotype, and sent to a reference laboratory for confirmation.

| | Organisms | Exceptional phenotypes (Resistant to) |
|---|---|---|
| Gram-negative bacteria | Any Enterobacteriaceae (except Proteus and *Serratia marcescens*) | Resistant to Colistin |
| | *Salmonella Typhi* | Resistant to carbapenems |
| | *Pseudomonas aeruginosa* and *Acinetobacter spp* | Resistant to colistin |
| | *Haemophilus influenzae* | Resistant to any third-generation cephalosporin, carbapenems, fluoroquinolones |
| | *Moraxella catarrhalis* | Resistant to any third-generation cephalosporin and/or fluoroquinolone |
| | *Neisseria meningitidis* | Resistant to any third generation cephalosporins and/or fluoroquinolones |
| | *Neisseria gonorrhoeae* | Resistant to spectinomycin |
| Gram-positive bacteria | *Staphylococcus aureus* | Resistant to vancomycin, teicoplanin, daptomycin, linezolid, quinupristin-dalfopristin or tigecycline. |
| | *Coagulase-negative staphylococci* | Resistant to vancomycin, daptomycin, linezolid, quinupristin-dalfopristin or tigecycline. |
| | *Corynebacterium spp* | Resistant to vancomycin, teicoplanin, daptomycin, linezolid, quinupristin-dalfopristin or tigecycline. |
| | *Streptococcus pneumoniae* | Resistant to carbapenems, vancomycin, teicoplanin, daptomycin, linezolid, quinupristin-dalfopristin, tigecycline or rifampicin |
| | Group A, B, C and G β-haemolytic *streptococci* | Resistant to penicillin, cephalosporins, vancomycin, teicoplanin, daptomycin, linezolid, quinupristin-dalfopristin or tigecycline |
| | *Enterococcus spp.* | Resistant to daptomycin, linezolid or tigecycline. Resistant to teicoplanin but not vancomycin. |
| | *Enterococcus faecalis* | Resistant to ampicillin |

# Reporting of Gram-negative Bacilli

## Both CLSI and EUCAST provide standard recommendations for interpretation of AST against several antibiotic.

- **Doses regimen-** Both guidelines provide dosage regimens necessary to achieve plasma drug conc.

- It is strongly recommended that laboratory share this information with clinical team.

### Susceptible dose dependent for Piptaz – Enterobacterales

| Disk Diffusion Breakpoints (mm) | MIC Breakpoints (mm) | Interpretive category |
|---|---|---|
| ≥25 | ≤8/4 | S |
| 21-24 | 16/4 | SDD |
| ≤20 | ≥32/4 | R |

3.375-4.5 g administered every 6 hourly as 30 minutes infusion

4.5 g-every 6-h as 3-h infusion/ 4.5g -every 8 h as 4 h infusion

## Recording of zone diameter

- Practice of interpreting result by approximate idea without measuring zone diameter strictly prohibited.
- Lab should have a breakpoint chart using guidelines
- Zone diameter breakpoint of same antimicrobial disc may differ among different organisms

| MEROPENEM | S (in mm) | I (in mm) | R (in mm) |
|---|---|---|---|
| Enterobacterales | ≥23 | 20-22 | ≤19 |
| *Pseudomonas aeruginosa* | ≥19 | 16-18 | ≤15 |
| *Pseudomonas species* | - | - | - |
| *Acinetobacter species* | ≥18 | 15-17 | ≤14 |
| *Burkholderia cepacia* | ≥20 | 16-19 | ≤15 |

# Enterobacterales

## Table 1A. Enterobacterales (not including inducible AmpC producers and *Salmonella/Shigella*)[a]

| Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting | Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution | Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution | Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors |
|---|---|---|---|
| Ampicillin | | | |
| Cefazolin | Cefuroxime | | |
| Cefotaxime or ceftriaxone[b] | Cefepime[c] | | |
| | Ertapenem<br>Imipenem<br>Meropenem | Cefiderocol | |
| | | Ceftazidime-avibactam | |
| | | Imipenem-relebactam | |
| | | Meropenem-vaborbactam | |
| Amoxicillin-clavulanate<br>Ampicillin-sulbactam | | | |
| Piperacillin-tazobactam | | | |
| Gentamicin | Tobramycin | Plazomicin | |
| | Amikacin | | |
| Ciprofloxacin<br>Levofloxacin | | | |
| Trimethoprim-sulfamethoxazole | | | |
| | Cefotetan<br>Cefoxitin | | |
| | Tetracycline[d] | | |
| | | | Aztreonam |
| | | | Ceftaroline[b] |
| | | | Ceftazidime[b] |
| | | | Ceftolozane-tazobactam |
| **Urine Only** | | | |
| Cefazolin (surrogate for uncomplicated UTI)[e] | | | |
| Nitrofurantoin | | | |
| | | Fosfomycin[f] (*Escherichia coli*) | |

Abbreviations: MDRO, multidrug-resistant organism; UTI, urinary tract infection.

# Changes in CLSI 2023 Enterobacterales/Aminoglycosides/Pg76

| Aminoglycosides | 2022 | | | | | | 2023 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DD (mm) | | | MIC (µg/mL) | | | DD (mm) | | | MIC (µg/mL) | | |
| | S (≥) | I | R (≤) | S (≤) | I | R | S (≥) | I | R (≤) | S | I | R |
| Gentamicin (10µg) | 15 | 13-14 | 12 | 4 | 8 | 16 | **18** | **15-17** | **14** | **2** | **4** | **8** |
| Tobramycin (10µg) | 15 | 13-14 | 12 | 4 | 8 | 16 | **17** | **13-16** | **12** | **2** | **4** | **8** |
| Amikacin (30µg) | 17 | 15-16 | 14 | 16 | 32 | 64 | **20** | **17-19** | **16** | **4** | **8** | **16** |
| Plazomicin (30µg) | - | - | - | - | - | - | **18** | **15-17** | **14** | **2** | **4** | **8** |
| Kanamycin (30µg) | 18 | 14-17 | 13 | 16 | 32 | 64 | 18 | 14-17 | 13 | 16 | 32 | 64 |
| Netilmicin (30µg) | 15 | 13-14 | 12 | 8 | 16 | 32 | 15 | 13-14 | 12 | 8 | 16 | 32 |
| Streptomycin (10µg) | 15 | 12-14 | 11 | - | - | - | 15 | 12-14 | 11 | - | - | - |

# Reporting of specific beta-lactamases resistance mechanism (ESBL, AmpC, carbapenemase )

- As per CLSI and EUCAST,additional testing for detection of ESBL, AmpC and carbapenemase testing and subsequent editing of the result from susceptable to resistant is not necessary.

- Because Current breakpoints of beta lactam drugs have revise in such a way that it will detects all clinically important resistance mechanisms.

- Isolate tested S using clinical breakpoints but positive for beta lactamases should be reported as S and beta lactamases production should not influence the category of susceptibility of result.

# Indication for ESBL, AmpC, carbapenemase testing

- Should be tested only for epidemiological, public health and infection control purpose

(24) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary for the dosage indicated below. When using current breakpoints, routine ESBL testing is not necessary before reporting results. However, **in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders, laboratories may decide to perform phenotypic or genotypic testing for ESBLs, and the results may be used to guide therapeutic management or** for epidemiological or infection prevention purposes. **Limitations of phenotypic and genotypic methods must be considered (see Table 3A introductory text).[4]**

# AmpC β-lactamases (CLSI 2023 Pg 27)

- **Repeat AST for AmpC producers:** Organisms which are potential inducible AmpC producers (**eg. SPICE**) may develop resistance to beta lactams during therapy. If beta lactams that are found susceptible in vitro are used for therapy, repeat routine AST (after 3 days) is strongly recommended.

- **Spice organisms**-S.Marcescens, P.aeruginosa, Indole positive proteae(Providencia and Morganella),**Citrobacter freundii complex and Enterobacter (E.cloacae and K aerogenes).**

# Interpretation of type of β-lactamase production

| Penicillins | Oxacillins | 1st/2nd gen. cephems | 3rd gen. Cephem | 4th gen. Cephem | Aztreonam | BLBLI | Carbapenem | Ambler class | Bush Jacoby group | Interpretation (type of β-lactamase production) |
|---|---|---|---|---|---|---|---|---|---|---|
| R | S | S | S | S | S | S | S | A | 2a | Penicillinase |
| R | R | R | S | S | S | S | S | A | 2b | Early cephalosporinases |
| R | R | R | R | R | R | S | S | A | 2be | ESBL (e.g. TEM3, SHV2, CTXM15) |
| R | R | R | R | R | R | R | S | A | 2ber | ESBL variant (R to BLBLI) e.g. TEM50 |
| R | R | R | R | R | S | S | S | A | 2e | ESBL variant (aztreonam sparing) e.g. CepA |
| R | R | S | S | S | S | S | S | D | 2d | Oxacillinase |
| R | R | R/S | R/S | R/S | R | R/S | R | D | 2df | Carbapenemase (Oxa type) |
| R | R | R | R | R | R | S | R | A | 2f | Carbapenemase (serine β-lactamase) e.g. kpc |
| R | R | R | R | S | R | R | S | C | 1 | AmpC β-lactamase |
| R | R | R | R | R | S | R | R | B | 3 | Carbapenemase (metallo β-lactamase)<br>Examples: ndm, vim, imp |

# Reporting of Colistin

- Colistin breakpoint available for Enterobacterales , *P. aeruginosa, Acinetobacter baumanni in* CLSI and EUCAST.

- In addition EUCAST has Breakpoint available for other Acinetobacter and other pseudomonus species.

- **Equivalent agent**- Colistin and Polymyxin B are equivalent agent, Therefore,colistin results can be extrapolated for polymyxin B.

# Warnings/ comments to be added to polymyxin B/ colistin reports by CLSI Subcommittee)

1. Clinical and PK/PD data demonstrate this agent is of limited clinical efficacy
2. **Restricted use only: If** available, alternative [non-polymyxin] agents are strongly preferred. If not available, should be use in combination with another active antimicrobials.
3. **Doses:** Colistin and polymyxin B should be given with a loading dose and maximum renally adjusted doses or maximum recommended doses respectively.
4. **For pneumonia**-When given systemically, this drug is unlikely to be effective for pneumonia.

# Reporting of Fosfomycin

CLSI –
Disc diffusion (DD) and MIC  breakpoints(BP) are available for only UTI isolates of *E.coli and E. faecalis*, and not for other  members of Enterobacterales and *Enterococcus*

EUCAST –
Oral DD & MIC breakpoints - only UTI *E.coli* isolates
IV DD breakpoints – *E.coli* (any clinical specimen)
For other Enterobacterales (any clinical specimen)- MIC

## Reporting of Tigecycline

--No clinical break point available in CLSI

---Breakpoint value differ of FDA and EUCAST differ from each other.

| Organism | Disk Diffusion Breakpoints | | | MIC Breakpoints | | |
|---|---|---|---|---|---|---|
| | S (mm) | I (mm) | R | S (µg/ml) | I (µg/ml) | R |
| Enterobacterales | | | | | | |
| EUCAST (*E.coli, C.koseri*) | ≥18 | - | <18 | ≤0.5 | - | >0.5 |
| Zone diameter breakpoints validated for *E. coli* only. For *C. koseri*, use an MIC method | | | | | | |
| FDA** | ≥19 | 15-18 | ≤14 | ≤2 | 4 | ≥8 |
| *Pseudomonas* spp | - | - | - | - | - | - |
| *Acinetobacter* spp | - | - | - | - | - | - |

# Antibiotic panel for Salmonella and shigella

## Table 1B. *Salmonella* and *Shigella* spp.[a,b]

| Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting | Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution | Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution | Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors |
|---|---|---|---|
| Ampicillin | | | |
| Ciprofloxacin<br>Levofloxacin | | | |
| Trimethoprim-sulfamethoxazole | | | |
| Cefotaxime or ceftriaxone | | | Ertapenem[c]<br>Imipenem[c]<br>Meropenem[c] |
| | Azithromycin[d] | | |
| | | | Tetracycline[e] |

Abbreviation: MDRO, multidrug-resistant organism.

# CLSI 2022, p/34

| Organism | Isolate source | AST |
|---|---|---|
| Typhoidal *Salmonella* *Shigella* | Intestinal isolates | Ampicillin<br>Fluoroquinolone<br>Cotrimoxazole |
| Non- Typhoidal *Salmonella* | Intestinal isolates | AST not required |
| Typhoidal *Salmonella* & NTS | Extraintestinal isolates | 3rd-generation cephalosporin<br>Chloramphenicol<br>Azithromycin (*S.* Typhi) |

## Clinically ineffective agents-

- For salmonella and shigella 1st and 2nd generation cephalosporin, cephamycin and aminoglycoside may appear susceptible in vitro but not effective clinically and should not tested or reported .

- Azithromycin-Enterobacterales except for salmonella and shigella are intrinsic resistance to azithromycin and do not have breakpoints.

- Azithromycin- salmonella breakpoint available only for s.typhi not for others.

# *Pseudomonas aeruginosa*

## Table 1C. *Pseudomonas aeruginosa*

| Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting | Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution | Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution. | Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors |
|---|---|---|---|
| Ceftazidime | Imipenem | Cefiderocol | |
| Cefepime | Meropenem | Ceftazidime-avibactam | |
| Piperacillin-tazobactam | | Ceftolozane-tazobactam | |
| | | Imipenem-relebactam | |
| Tobramycin | | | |
| Ciprofloxacin<br>Levofloxacin | | | |
| | | | Aztreonam |
| **Urine Only** | | | |
| | Amikacin | | |

Abbreviation: MDRO, multidrug-resistant organism.

# Changes in CLSI 2023 Pseudomonas aeruginosa/Piptaz/Pg68

| | 2022 | | | | | | 2023 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DD (mm) | | | MIC (µg/mL) | | | DD (mm) | | | MIC (µg/mL) | | |
| | S (≥) | I | R (≤) | S (≤) | I | R | S (≥) | I | R (≤) | S | I | R |
| Piptaz (100/10 µg) | 21 | 15-20 | 14 | 16/4 | 32/4 - 64/4 | 128/4 | 22 | 18-21 | 17 | 16/4 | 32/4 | 64/4 |

S breakpoint - 4.5 g administered every 6 hourly as 30 minutes infusion

# Changes in CLSI 2023 :Pseudomonas aeruginosa/Aminoglycocide/Pg78

| Aminoglycosides | 2022 | | | | | | 2023 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DD (mm) | | | MIC (µg/mL) | | | DD (mm) | | | MIC (µg/mL) | | |
| | S (≥) | I | R (≤) | S (≤) | I | R | S (≥) | I | R (≤) | S | I | R |
| Tobramycin (10 µg) | 15 | 13-14 | 12 | 4 | 8 | 16 | **19** | **13-18** | **12** | **1** | **2** | **4** |
| Amikacin (30 µg) | 17 | 15-16 | 14 | 16 | 32 | 64 | 17 | 15-16 | 14 | 16 | 32 | 64 |
| Gentamicin (10 µg) | 15 | 13-14 | 12 | 4 | 8 | 16 | - | - | | | | - |

**URINE BP ONLY**

Non-Urinary site – Amikacin as monotherapy has worst clinical outcomes; hence always advice for combo therapy

## Table 1G. Other Non-Enterobacterales[a,b]

| Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting | Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution | Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution | Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors |
|---|---|---|---|
| Ceftazidime | Cefepime | | |
| | Imipenem<br>Meropenem | | |
| Gentamicin<br>Tobramycin | Amikacin | | |
| Piperacillin-tazobactam | | | |
| Trimethoprim-sulfamethoxazole | | | |
| | Aztreonam | | |
| | Ciprofloxacin<br>Levofloxacin | | |
| | Minocycline | | |
| | | | Cefotaxime<br>Ceftriaxone |
| **Urine Only** | | | |
| Tetracycline[c] | | | |

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

# Reporting of **Gram-positive Cocci**

# *Staphylococcus*

**Reporting of penicillin**

- **Isolates tested resistant**- Can be resistant to penicillinase – labile penicillin.
- **Isolates tested susceptible -** must be tested for beta lactamase production before reporting AST results.
- **β-lactamase testing can be performed by penicillin zone edge test**
  - ❖ **If test is positive**, then all labile penicillinase labile penicillins are reported as resistant.
  - ❖ **If test is negative**, then such isolates are considered susceptible to penicillin as well as beta lactam agents having anti staphylococcal activity.

# Detection of methicillin (oxacillin) resistance in staphylococci

| Organism | Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp. | | | | |
|---|---|---|---|---|---|
| | Cefoxitin MIC | Cefoxitin disk diffusion | Oxacillin MIC | Oxacillin disk diffusion | Oxacillin salt agar |
| *S. aureus* | Yes (16-20 h) | Yes (16-18 h) | Yes (24 h) | No | Yes (24 h) |
| *S. lugdunensis* | Yes (16-20 h) | Yes (16-18 h) | Yes (24 h) | No | No |
| *S. epidermidis* | No | Yes (24 h) | Yes (24 h) | Yes (16-18 h) | No |
| *S. pseudintermedius* | No | No | Yes (24 h) | Yes (16-18 h) | No |
| *S. schleiferi* | No | No | Yes (24 h) | Yes (16-18 h) | No |
| *Staphylococcus* spp. (not listed above **or not** identified to the species level) | No | Yes[a] (24 h) | Yes[a] (24 h) | No | No |

## Interpretation of Oxacillin (or cefoxitin) S results

- Penicillinase-stable penicillins: E.g. cloxacillin, nafcillin etc.
- BL-BLI agents: E.g. AMC, AMS, Piptaz
- Oral & Parenteral cephems
  - Except for cefixime, ceftazidime, ceftazidime-avibactam, ceftibuten and ceftolozane-tazobactam (no staphylococcal activity)
- Carbapenems (doripenem, ertapenem, imipenem, meropenem)
- MRSA can be susceptible to ceftaroline and ceftobiprole and the therefore need to be tested

# Interpretation of Oxacillin (or cefoxitin) R results

- Isolates that test resistant to Oxacillin (or cefoxitin) should be reported as Methicillin (oxacillin) resistant.

- These isolates are resistant to all beta lactam drug except fifth generation cephalosporins.

## Vancomycin susceptibility testing in *S.aureus* –

### Vancomycin disc diffusion test is not recommended, because:

- Does not differentiate VSSA and VISA
- Does not differentiate among VS-CoNS, VI-CoNS, and VR-CoNS

### Recommended testing method

- MIC (Preferably BMD)-Ideal
- Vancomycin screen agar

### Repeat testing -

VSSA can turn to be VISA during course of prolonged therapy

- **Vancomycin resistance is extremely uncommon. Such isolate must be sent to reference lab.**

## Reporting of Inducible clindamycin resistance

- If Erythromycin disk is adjacent and D phenomenon is observe
- Erythro/R, Clinda/S or I ⟶

**Report clindamycin as resistant; as resistance may develop during therapy**

  - Clindamycin may still be used for short-term therapy of less serious SSTIs as resistance is unlikely to develop during such therapy

# *Enterococcus*

## Reporting penicillin and ampicillin in case of Enterococci

- **Enterococci susceptible to penicillin** are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin- tazobactam for non–beta-lactamase-producing enterococci.
- However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required.
- **Ampicillin susceptibility tests should be used** to predict the activity of amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, and PIPTAZ among non–beta-lactamase producing enterococci
- **Lab should mention as a comment**

- For Enterococci aminoglycosides may appear active in vitro but ineffective in vivo (intrinsic resistance).

- When aminoglycosides given along with cell active agents, can demonstrate synergy can such combination therapy may be effective in vivo.

- Indication - Endocarditis, non endovascular bacteremia and meningitis

- Combination therapy not for localised infection(UTI)

- HLAR(High level aminoglycoside resistance)-Synergy can be test by 120 microgram disc of high level gentamicin and 300 microgram streptomycin.

# *Streptococcus pneumoniae*

## Meningitis vs non-meningitis breakpoint

- For CSF isolates → interpretation using meningitis breakpoint
- For isolates from other specimens:
- ?? whether the features of meningitis are present or not –i.e. Clinical presentation, biochemical or cytological findings of CSF.

A. Features suggestive of meningitis →use meningitis breakpoint
B. Findings suggestive of meningitis not clear or not available → Interpret using both meningitis and non-meningitis breakpoints and report separately
C. Findings are clear cut not suggestive of meningitis →interpret using both meningitis and nonmeningitis breakpoints and report separately.

| | S | I | R | Comments |
|---|---|---|---|---|
| Penicillin DD (1µg Ox) (non-meningitis) | ≥20 | - | - | ≤ 19 mm- Test for Penicillin and cefotaxime, Ci, or mero MIC |
| Penicillin MIC (meningitis) | ≤ 0.06 | - | ≥ 0.12 | 3MU @ 4 h. |
| Penicillin MIC (non-meningitis) | ≤2 | 4 | ≥8 | If S - 2MU @ 4 h; MIC-I , then 3MU @ 4 h |
| Ceftriaxone (meningitis) | ≤ 0.5 | 1 | ≥2 | Use of cefotaxime / ceftriaxone in meningitis, requires therapy with max doses. |
| Ceftriaxone (non-meningitis) | ≤1 | 2 | ≥4 | |
| Vancomycin DD (any) | ≥17 | - | - | |
| Vancomycin MIC(any) | ≤1 | - | - | |

# Take home message

- Antibiotic panel  selection according to guideline

- Selective Reporting and Cascade Reporting

- Comment regarding AST interpretative categories, doses regimen, equivalent agent, surrogate agent, Predict susceptibility, Intrinsic resistance, Repeat AST testing.


"Knowing is not enough;
we must apply.
Willing is not enough;
we must do."
- Johan Wolfgang von Goethe
THANK
YOU