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Measuring MIC and Clinical Breakpoints for Veterinary Antibiotics – Essential to Antibiotic Stewardship –

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Affiliations, Conflicts of Interests, and Disclosures: Mark G. Papich

Disclosures related to my presentation:

- Employee: North Carolina State University, College of Veterinary Medicine
- Active grants/Research contracts: Merck Animal Health.
- Consulting contracts: Elanco, Zoetis, Merck, Dechra, Verté Therapeutics
- Unpaid volunteer: CLSI-VAST Subcommittee, AVMA Committee on Antibiotics, USP Expert Committee on Antibiotics (BIO4) Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB)
- Books / Royalties: Author of books relating to drug therapy published by Elsevier and Wiley-Blackwell
- I will discuss drugs/dosages that are currently NOT approved for use in animals

How to interpret a susceptibility test report

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

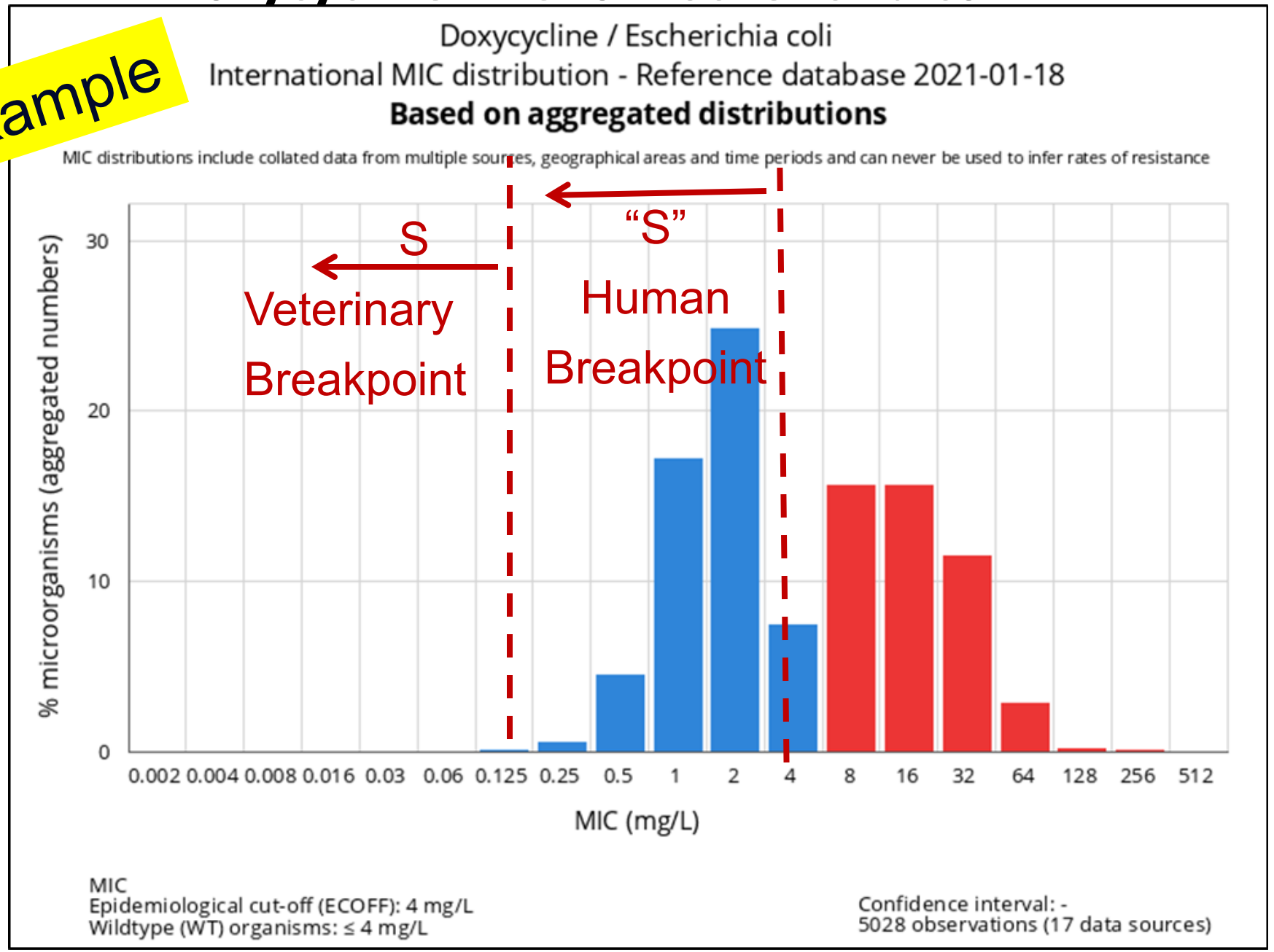
Use laboratories that rely on approved CLSI standards for testing

Veterinary Antimicrobial Susceptibility Testing subcommittee (VAST)

- Since 1993…
- Establish Interpretive Categories and breakpoints for susceptibility testing
- Publish standard methods, breakpoints, and quality control
- Update existing standards
- Educate health care professionals

Doxycycline MIC for *Escherichia coli*

An example

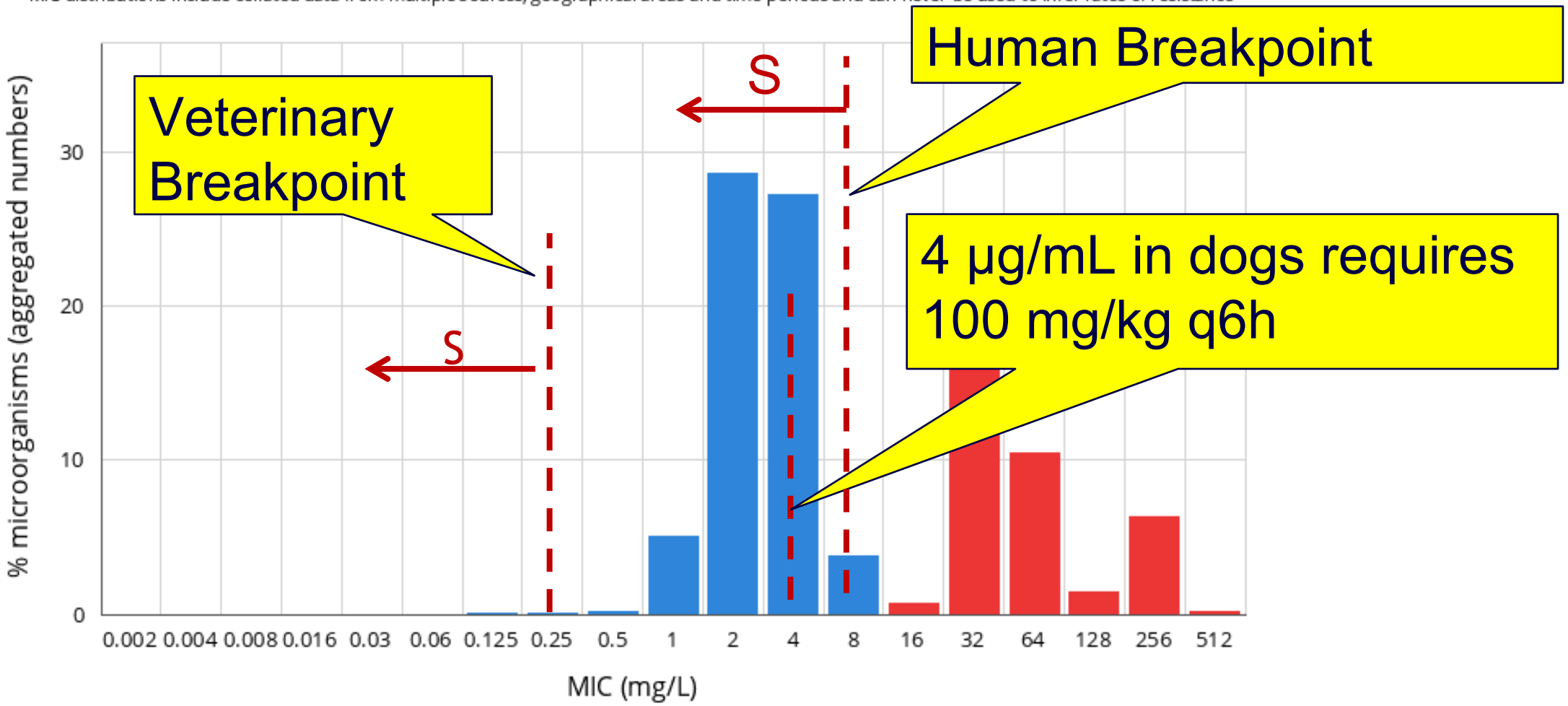


Ampicillin: *Escherichia coli*

International MIC distribution - Reference database 2025-01-07

Based on aggregated distributions

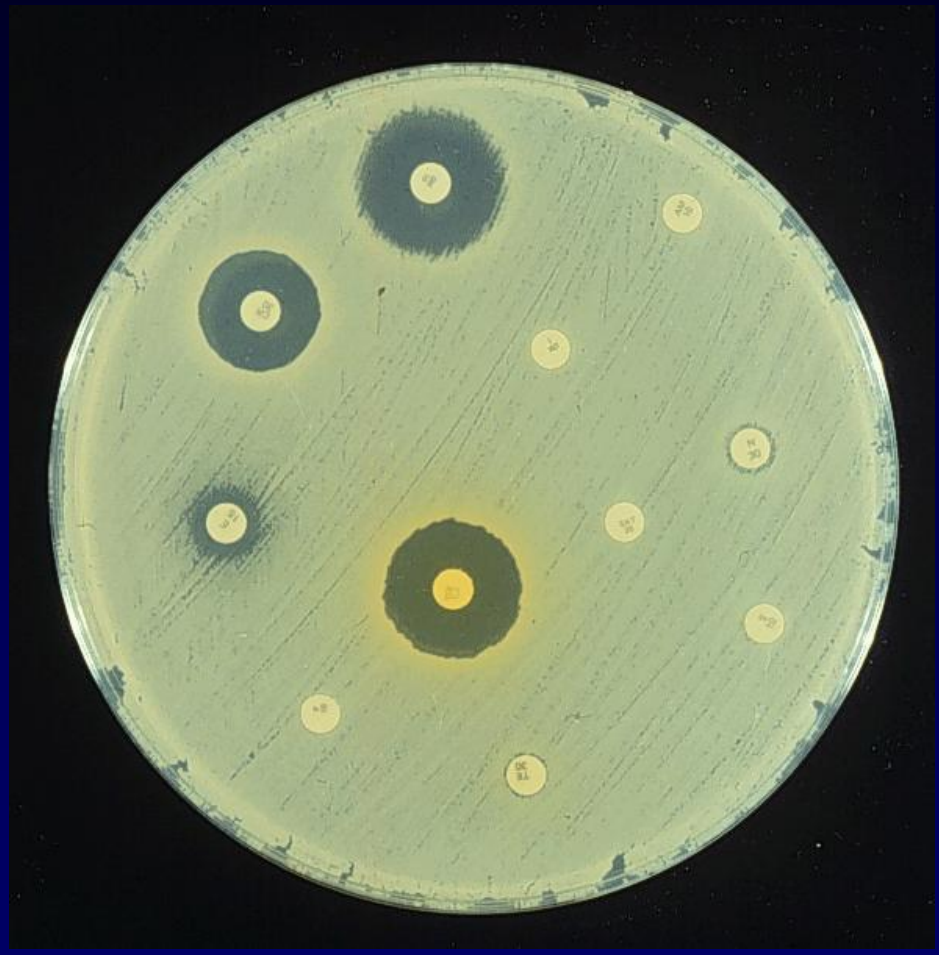
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): 8 mg/L
Wildtype (WT) organisms: ≤ 8 mg/L

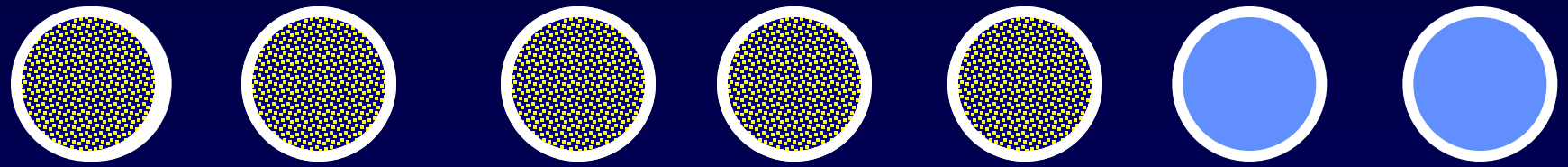
Confidence interval: 4 - 16
105483 observations (53 data sources)

Susceptibility Testing



MIC Dilution Test

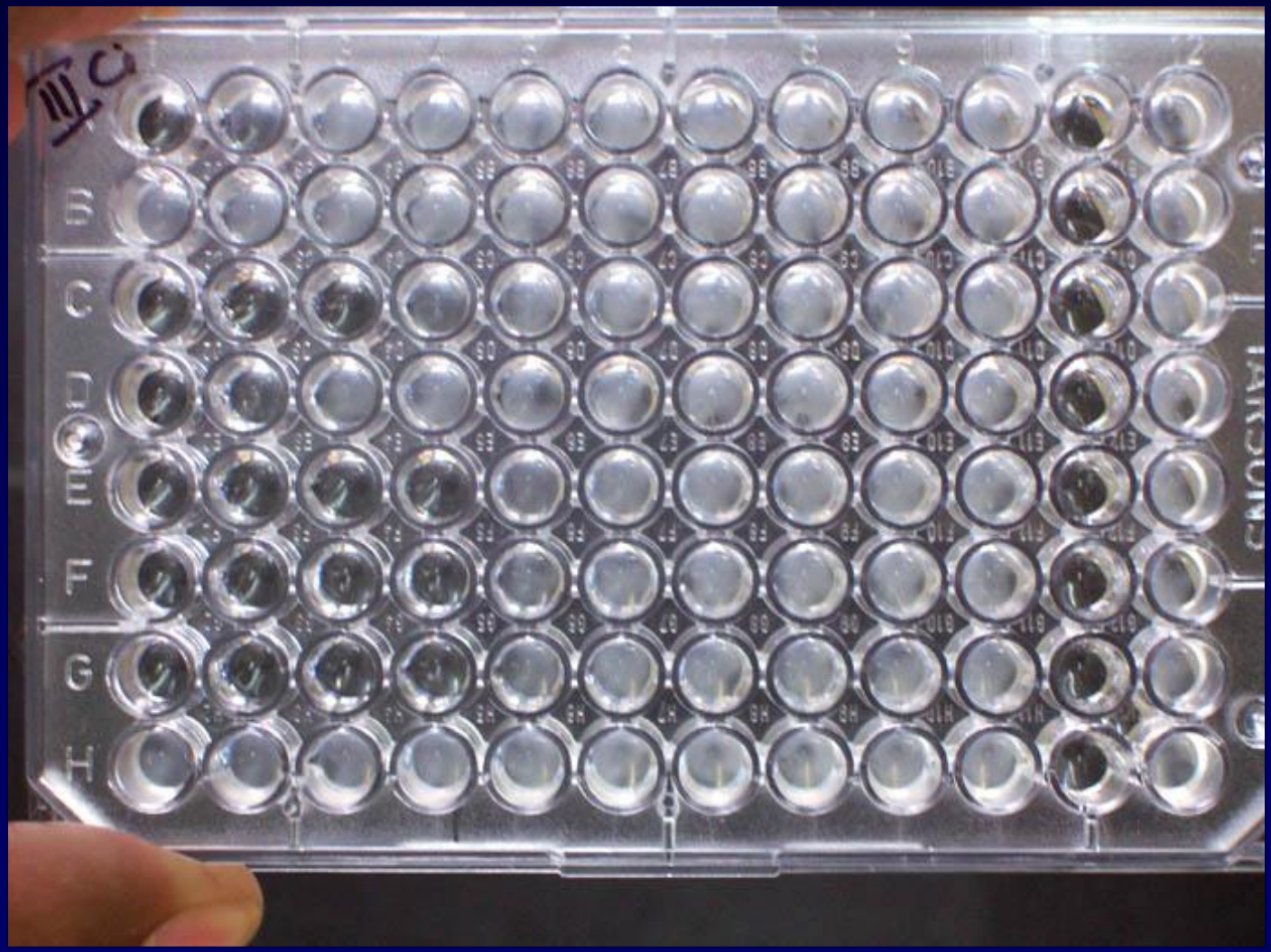
Susceptible or Resistant ?



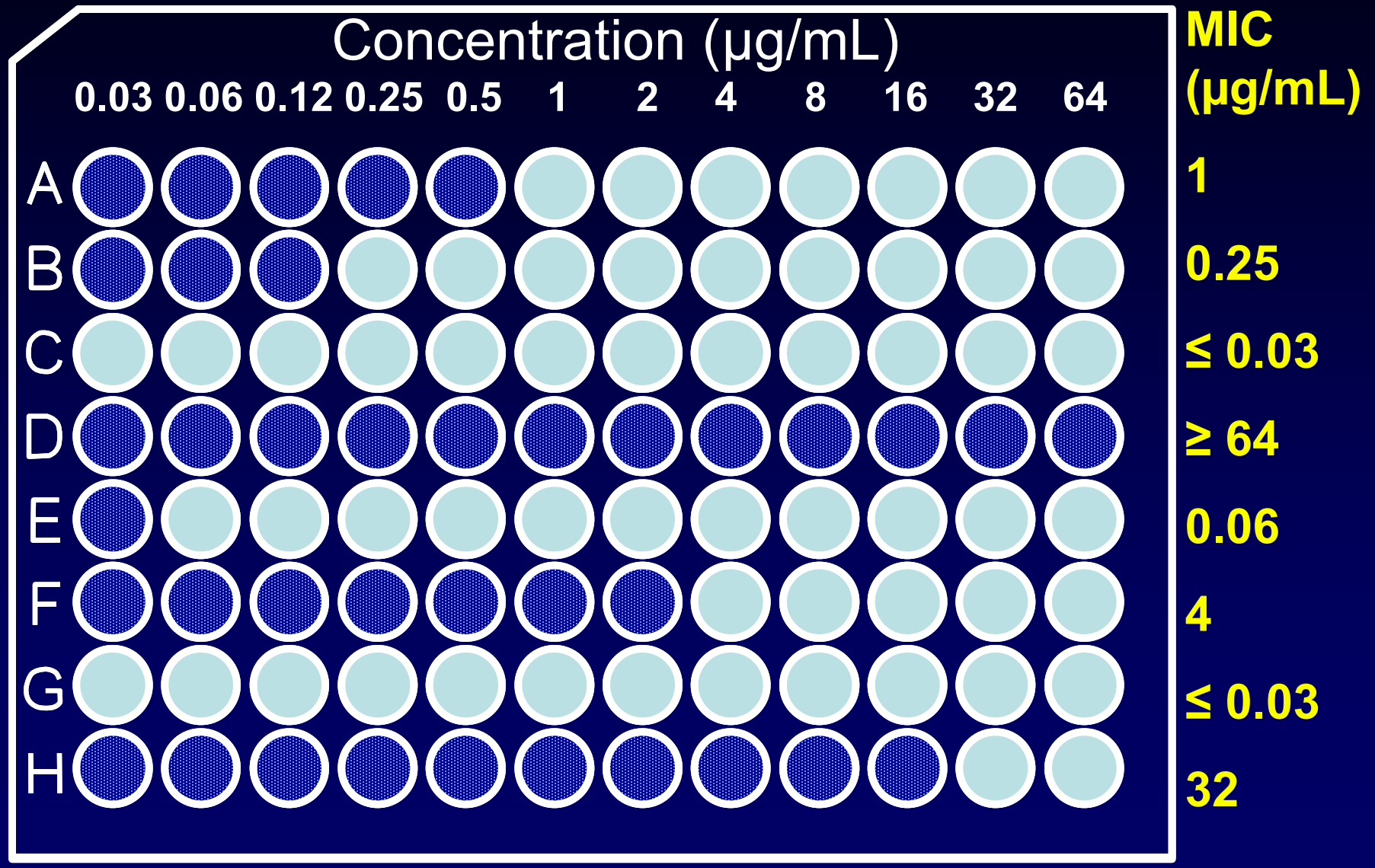
0.25 ug/mL | 0.5 ug/mL | 1.0 ug/mL | 2.0 ug/mL | 4.0 ug/mL | 8.0 ug/mL | 16.0 ug/mL

Breakpoint

Microbroth Dilution Test



MIC Determination: Dilution Test



CLSI Vet01, 7th ed, 2024

Antimicrobial	Susceptible (µg/ml)	Resistant (µg/mL)
Ampicillin	≤ 0.25	≥0.5
Amox/Clavulanate	≤0.25/0.12	≥1/0.5
Cefazolin	≤2	≥8
Cephalexin	≤2	≥8
Chloramphenicol	≤2	≥8
Clindamycin	≤0.5	≥4
Enrofloxacin	≤0.06 (+SDD)	≥0.5
Gentamicin	≤2	≥8
Imipenem (human)	≤1	≥4
Marbofloxacin	≤0.12 (+SDD)	≥0.5
Rifampin	≤1	≥4
Doxycycline	≤0.12	≥0.5
Piperacillin/Tazobactam	≤ 8/4	≥ 32/4

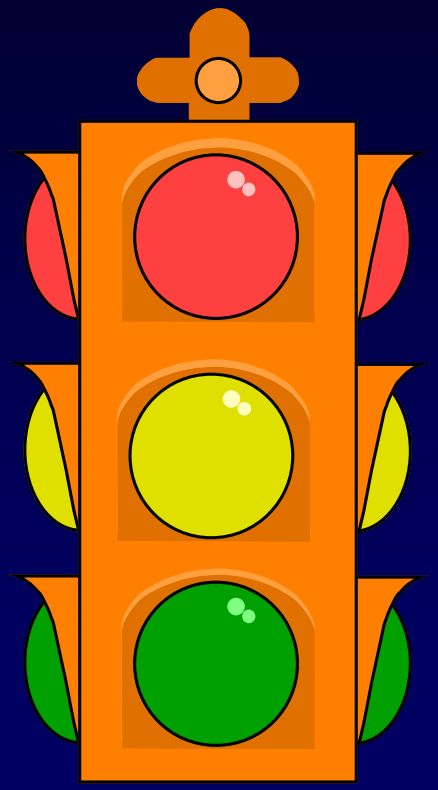
Recently updated

Recently updated

Recently updated

Interpretive Categories for Breakpoints

- Resistant
- Intermediate
- Susceptible



Susceptibility Testing
Interpretive Categories

- Susceptible (S) – a category defined by a breakpoint that implies that isolates with an MIC at or below the susceptible breakpoint are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy.

Susceptibility Testing Interpretive Categories

- Resistant (R) – a category defined by a breakpoint that implies that isolates with an MIC at or above the resistant breakpoint are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs that fall in the range in which specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

Susceptibility Testing
Interpretive Categories

- Intermediate (I) –A category defined by a breakpoint that implies that isolates with an MIC at or above the intermediate breakpoint but below the resistant breakpoint, may exhibit lower response rates than susceptible isolates with normal dosage schedules.

Susceptibility Testing
Interpretive Categories

- Intermediate (I) –While the MIC may approach usually attainable blood and tissue concentration of the agent, this may not be enough for clinical efficacy. The intermediate category may imply clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used.

Susceptibility Testing
Interpretive Categories

Susceptible Dose-Dependent (SDD) Category

- *As the name implies – depends on the dose.*
- SDD - a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosage regimen that is used.

New category.
Some laboratories may not report.

Susceptibility Testing
Interpretive Categories

Susceptible Dose-Dependent (SDD) Category

- It is necessary to use a dosage regimen (ie, higher doses, more frequent doses, or both, or infusion) that results in higher drug exposure than that achieved with the dose that was used to establish the susceptible breakpoint.

Wild-Type vs Non Wild-Type

(Will not be included on a test report.)

Wild-Type (WT) vs Non Wild-type (NWT)

- Wild-type (WT) – a category based on an epidemiological cutoff value (ECV) that describes isolates with no mechanisms of acquired resistance or reduced susceptibility for the antimicrobial agent being evaluated.

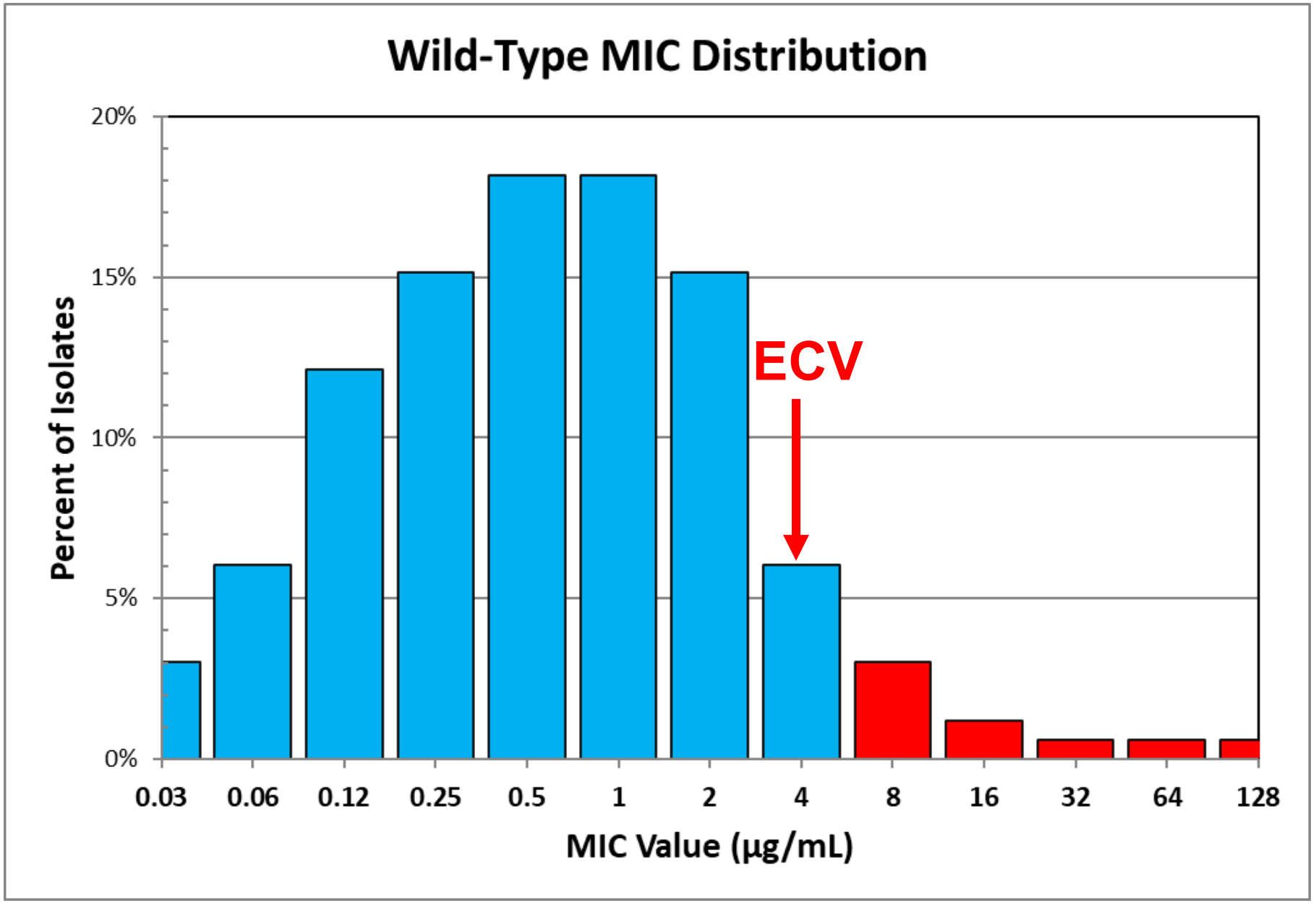
- Non wildtype (NWT) – a category based on an epidemiological cutoff value (ECV) that describes isolates with presumed or known mechanisms of acquired resistance and reduced susceptibility for the antimicrobial agent being evaluated.

Wild-Type (WT) vs Non Wild-type (NWT)

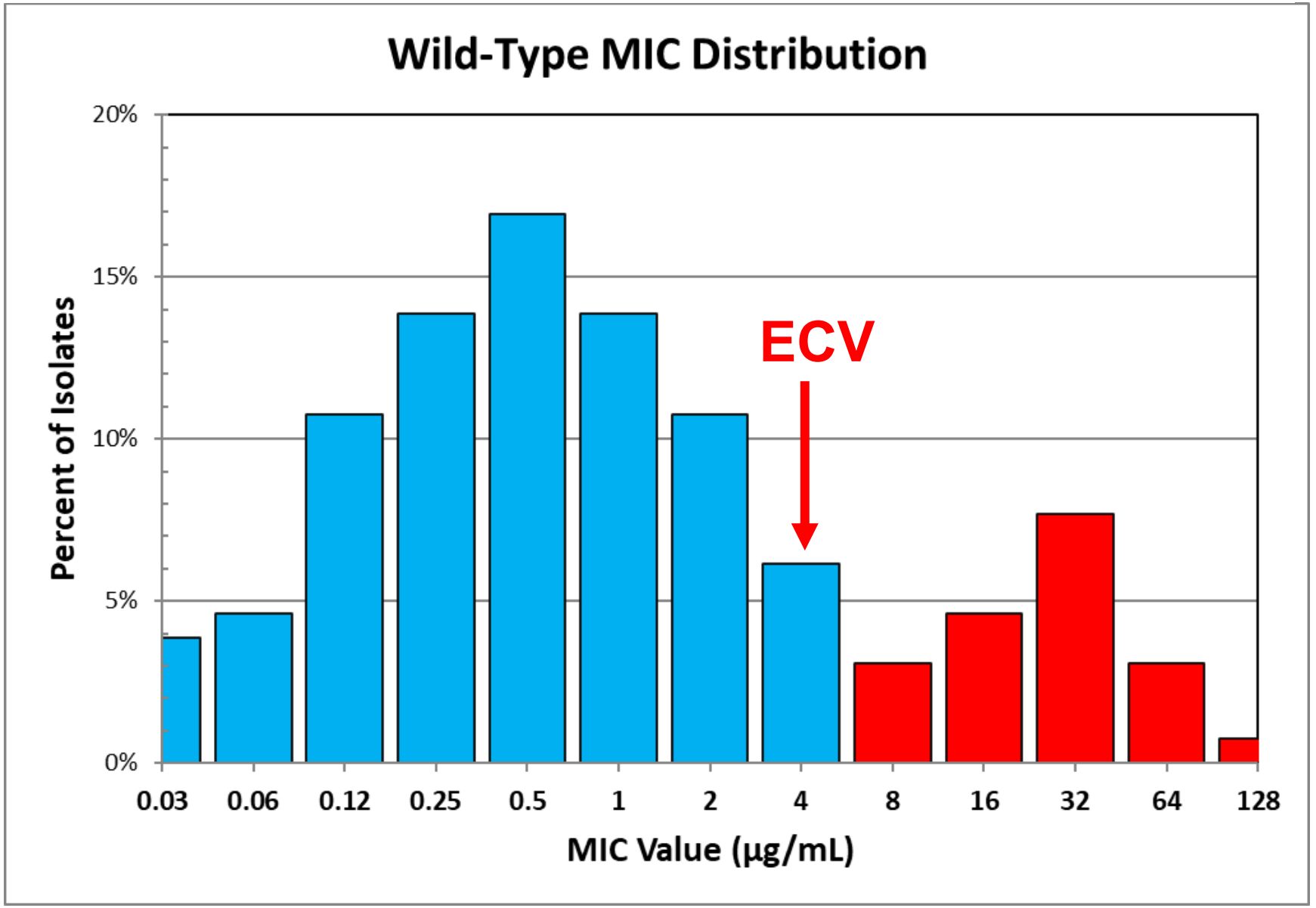
- Epidemiological Cutoff Values
  - ECV: The Clinical and Laboratory Standards Institute (CLSI, www.CLSI.org)
  - ECOFF: European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.EUCAST.org)

- The ECV is the MIC value that separates microbial populations into those with and without acquired and/or mutational resistance based on phenotypes – not the presence (or not) of a resistance gene.

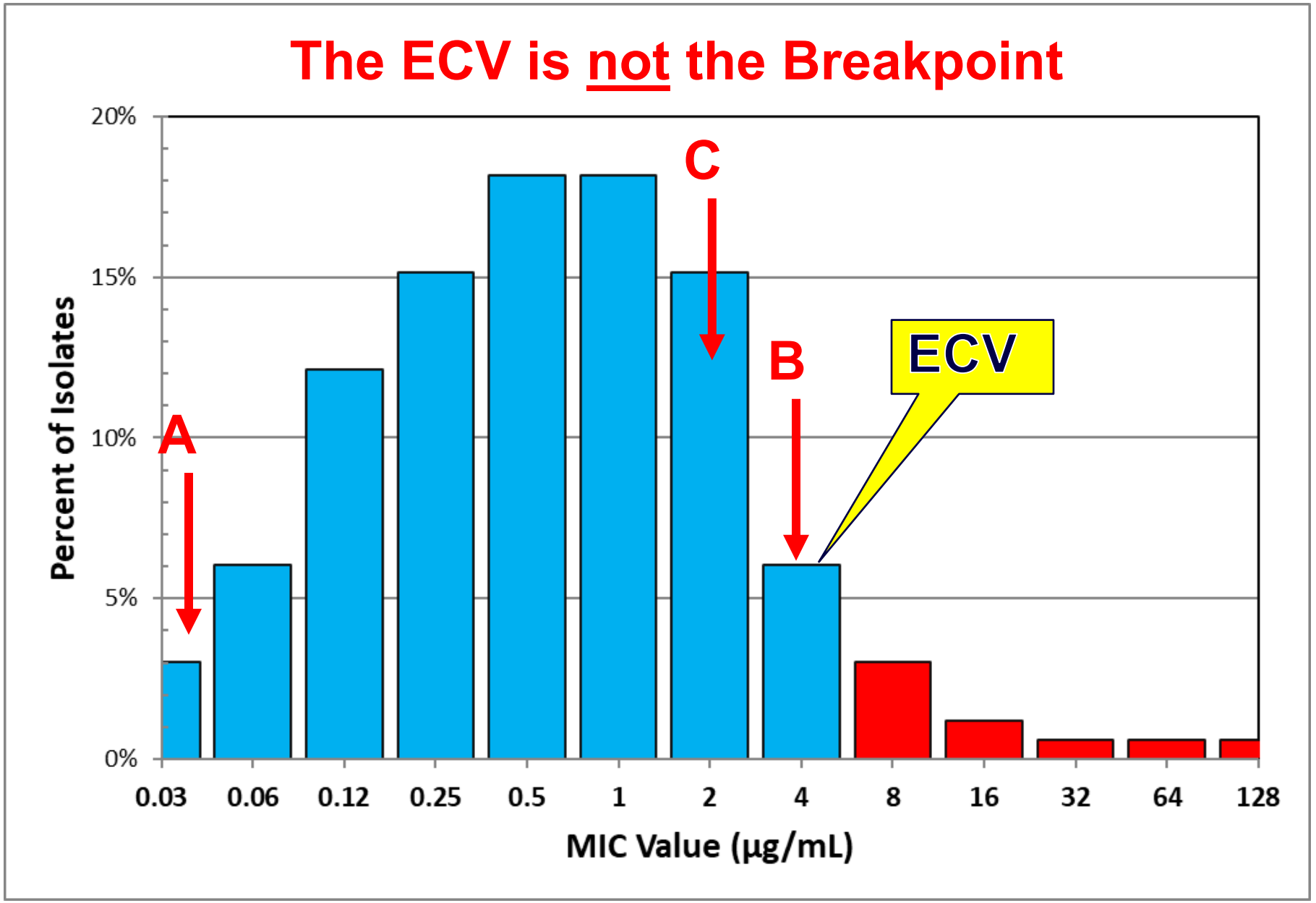
Uni-Modal WT Distribution



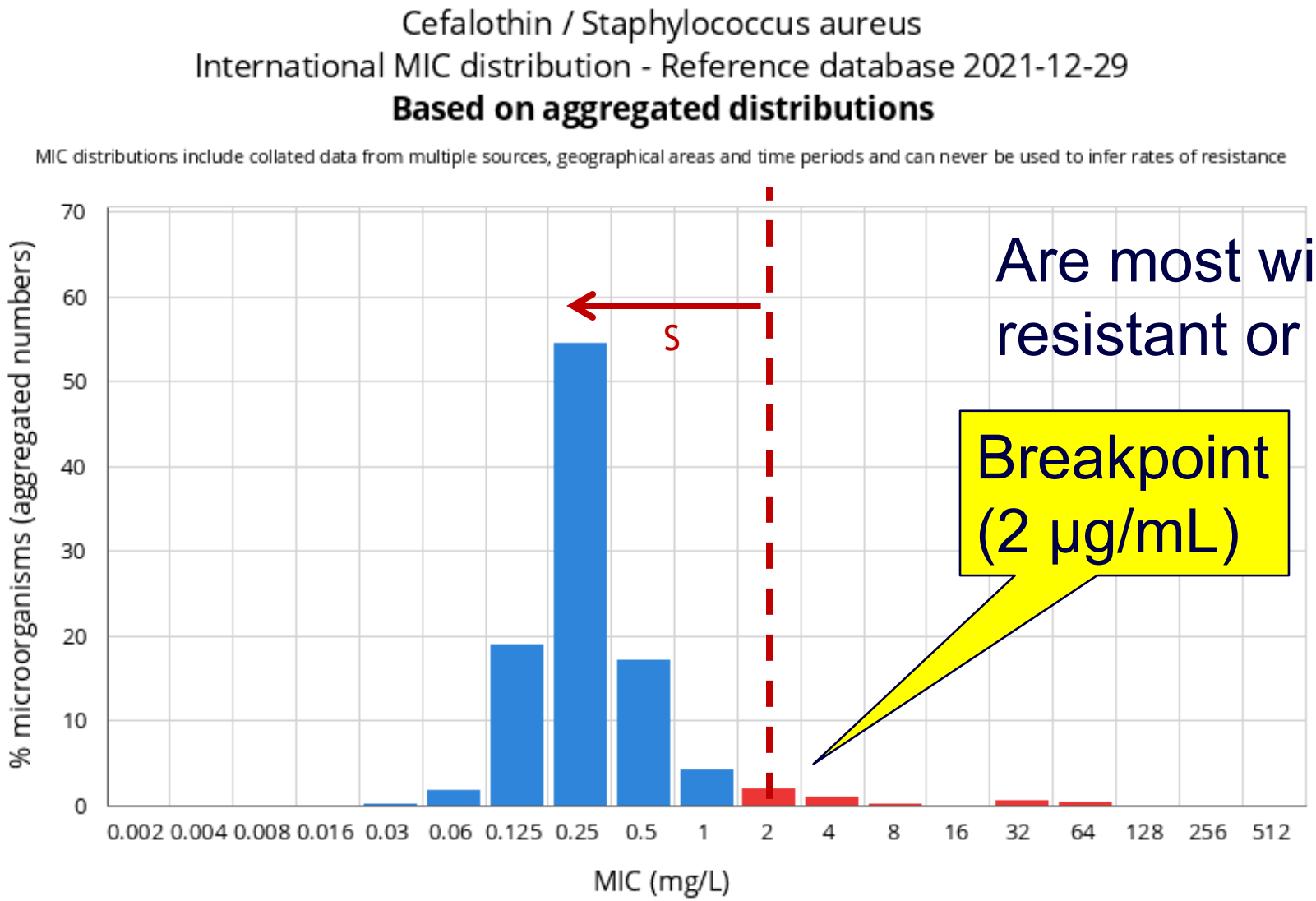
Bi-Modal WT Distribution



Uni-Modal WT Distribution



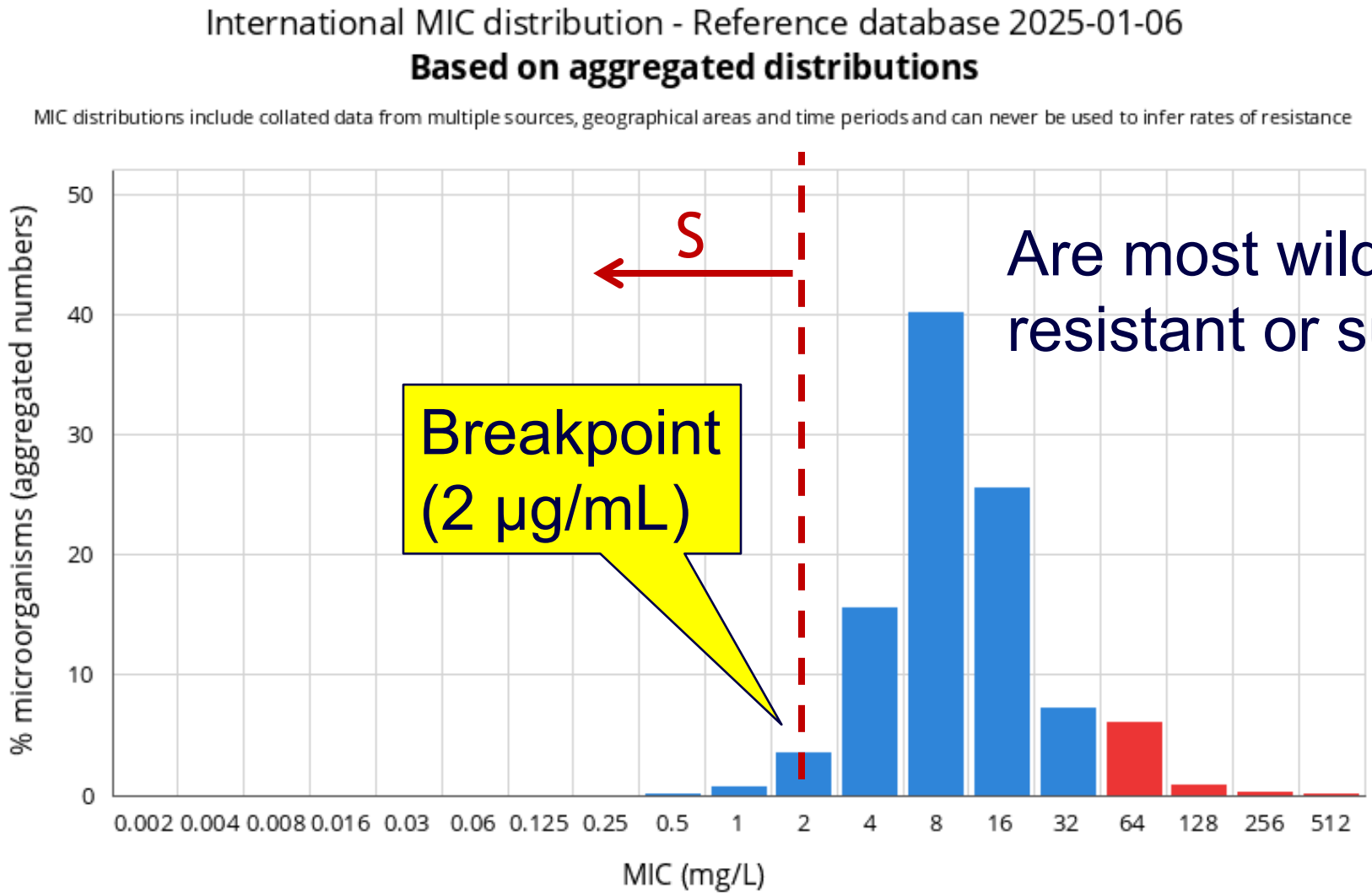
1st-Generation Cephalosporin/*Staph. aureus*



Are most wildtype strains resistant or susceptible?

Breakpoint (2 µg/mL)

1st-Generation Cephalosporin/ *E. coli*



Are most wildtype strains resistant or susceptible?

MIC
Epidemiological cut-off (ECOFF): 32 mg/L
Wildtype (WT) organisms: ≤ 32 mg/L

Confidence interval: 16 - 64
6433 observations (15 data sources)

Examples of Reports

Culture Result: *Staphylococcus pseudintermedius* (skin from Dog)

Drug	MIC	Breakpoint	Interpretation
Penicillin G	≥ 0.5	≤ 0.12	R
Oxacillin	≥ 4	≤ 0.5	R
Amoxicillin-Clav	≥ 16	≤ 0.25	R
Cefovecin	≥ 2	≤ 0.5	R
Cephalexin	≥ 32	≤ 2	R
Amikacin	1	≤ 4	S
Enrofloxacin	≥ 0.5	≤ 0.06	R
Erythromycin	≥ 4	≤ 0.5	R
Clindamycin	≥ 8	≤ 0.5	R
Doxycycline	2	≤ 0.12	R
Chloramphenicol	8	≤ 2	R
Trimethoprim-Sulfa	≥ 320	≤ 40	R
Rifampin	≤ 0.5	≤ 1	S

Methicillin-resistant

Recently updated.

Check minocycline?

Recently updated.

Methicillin-Resistant Staphylococcus (MRS)

- MRS – methicillin and oxacillin resistant
- Mediated by the *mecA* gene
- Resistant penicillin binding protein (PBP 2a
- Mediates resistance to all other β-lactam antibiotics
  - Resistant to all penicillins, ampicillin, amoxicillin, cephalosporins, and carbapenems (imipenem).

Culture Result: *Staphylococcus pseudintermedius* (skin from Dog)

Drug	MIC	Breakpoint	Interpretation
Penicillin G	≥ 0.5	≤ 0.12	R
Oxacillin	≥ 4	≤ 0.5	R
Amoxicillin-Clav	≥ 16	≤ 0.25	R
Cefotaxim	≥ 2	≤ 0.5	R
Cephalexin	≥ 32	≤ 2	R
Amikacin	1	≤ 4	S
Chloramphenicol	8	≤ 2	R
Trimethoprim-Sulfa	≥ 320	≤ 40	R
Rifampin	≤ 0.5	≤ 1	S

Methicillin-resistant

Recently updated.

Follow-up testing showed susceptibility to vancomycin and linezolid

Check minocycline?

Recently updated.

Culture Result: Escherichia coli (pneumonia)

Drug	MIC	Breakpoint	Interpretation
Cefpodoxime	≥ 16	≤ 2	R
Cefovecin	2	≤ 0.5	R
Ceftazidime	1	≤ 4	S
Doxycycline	2	≤ 0.12	R
Enrofloxacin	≥ 4	≤ 0.5	R
Gentamicin	≥ 16	≤ 2	R
Imipenem	0.5	≤ 1	S
Amikacin	2	≤ 4	S
Piperacillin/Tazo	2	≤ 8	S

Likely an ESBL isolate (need to confirm with lab)
Only options are injections.

substitute meropenem,

Culture Result: *Escherichia coli* (UTI in Dog)

Drug	MIC	Breakpoint	Interpretation
Ampicillin	4	≤ 8	S
Amoxicillin/Clav	4	≤ 8	S
Cefazolin	2	≤ 16	S
Cefpodoxime	1	≤ 2	S
Cefovecin	1	≤ 2	S
Ceftazidime	1	≤ 4	S
Marbofloxacin	0.12	≤ 0.12	S
Enrofloxacin	≤ 0.06	≤ 0.06	S
Gentamicin	≤ 0.5	≤ 2	S
Imipenem	≤ 0.12	≤ 1	S
Amikacin	2	≤ 4	S
Piperacillin/Tazo	2	≤ 8	S

Cephalexin OK

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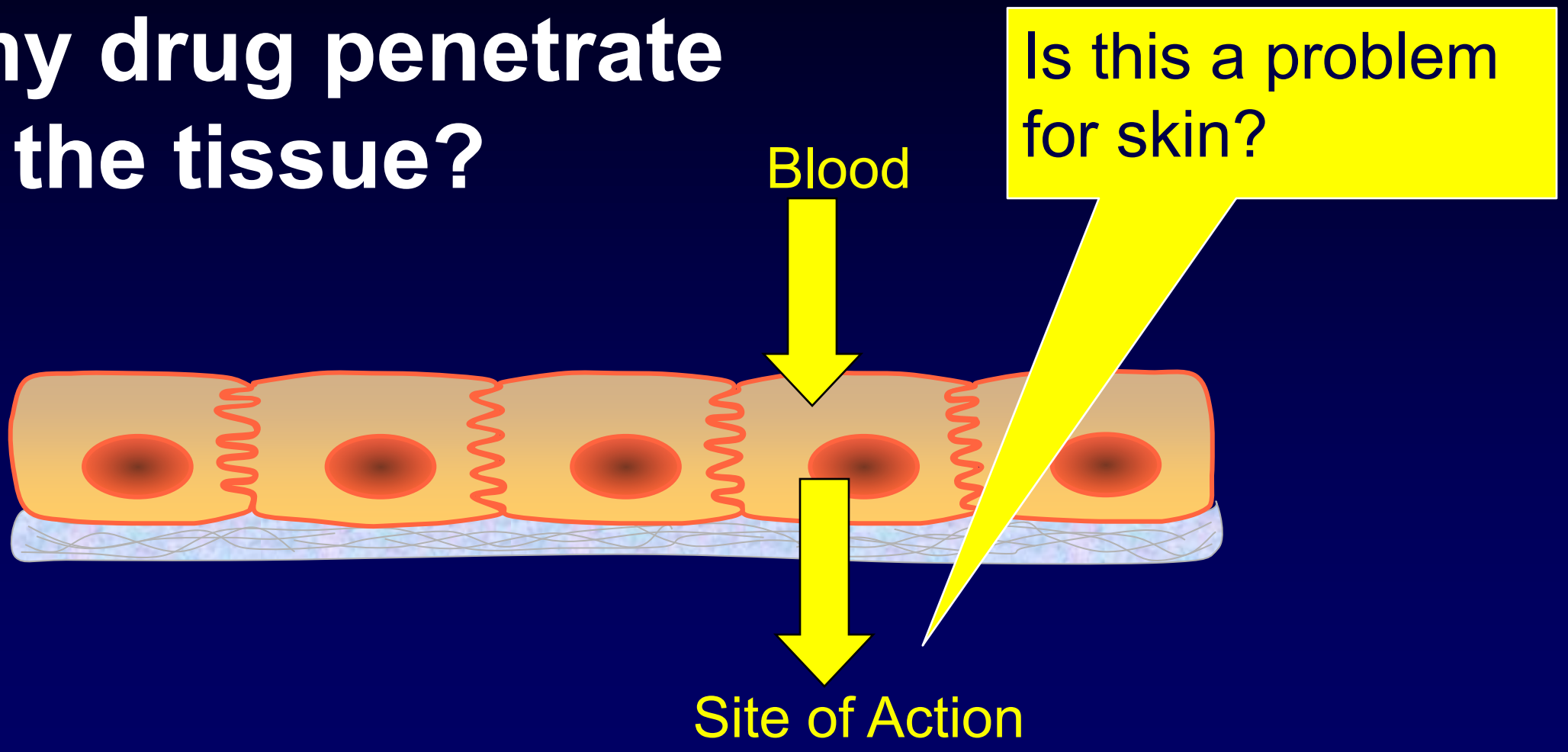
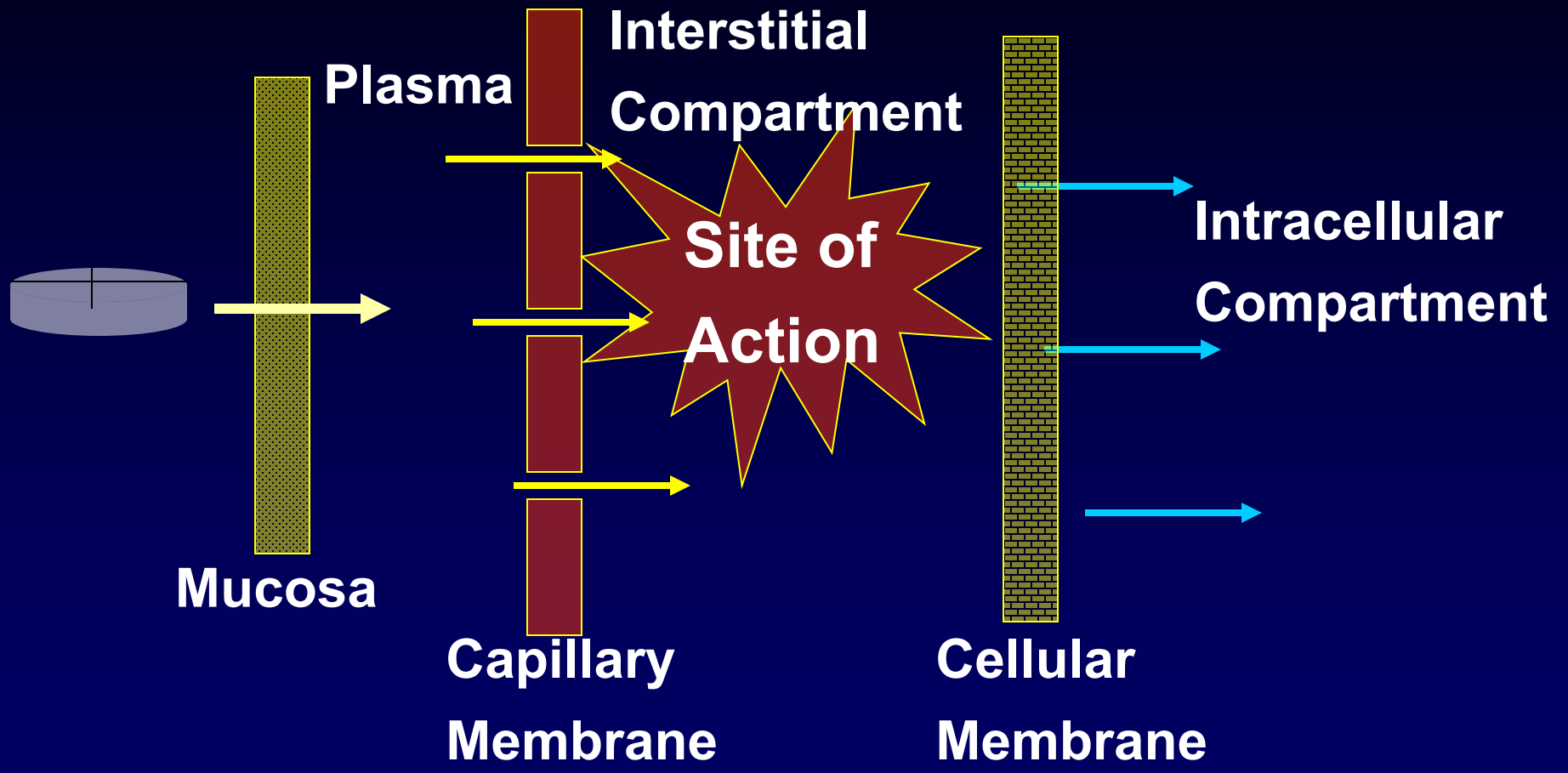
What are CLSI standards?

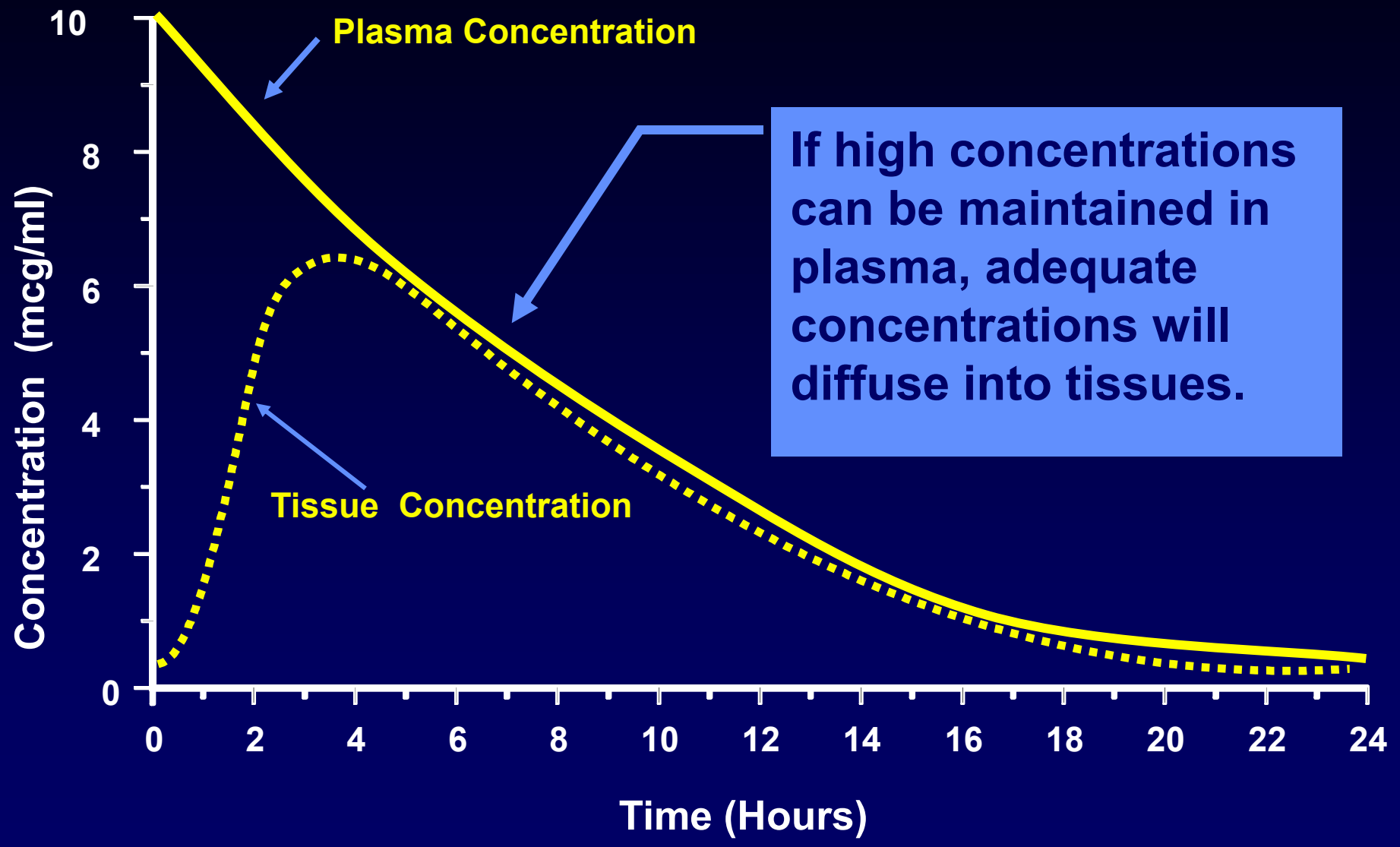
Do the breakpoints apply to specific tissues?

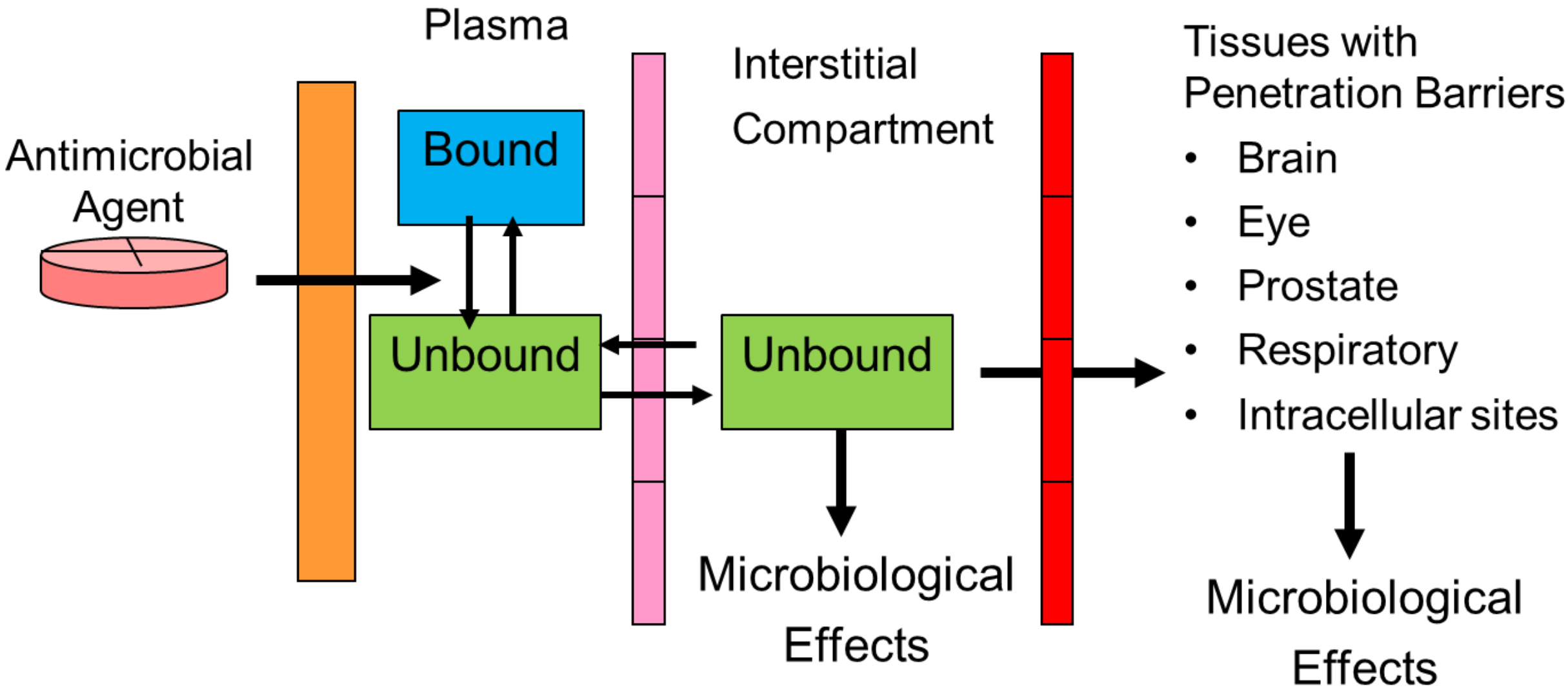
Did my drug penetrate the tissue?



Antimicrobial Target Sites




Plasma Concentration
Tissue Concentration
If high concentrations can be maintained in plasma, adequate concentrations will diffuse into tissues.
Concentration (mcg/ml)
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (Hours)


Plasma
Interstitial Compartment
Tissues with Penetration Barriers
Antimicrobial Agent
Bound
Unbound
Unbound
Brain
Eye
Prostate
Respiratory
Intracellular sites
Microbiological Effects
Microbiological Effects

Barriers to Drug Diffusion

Where are the barriers to drug diffusion?

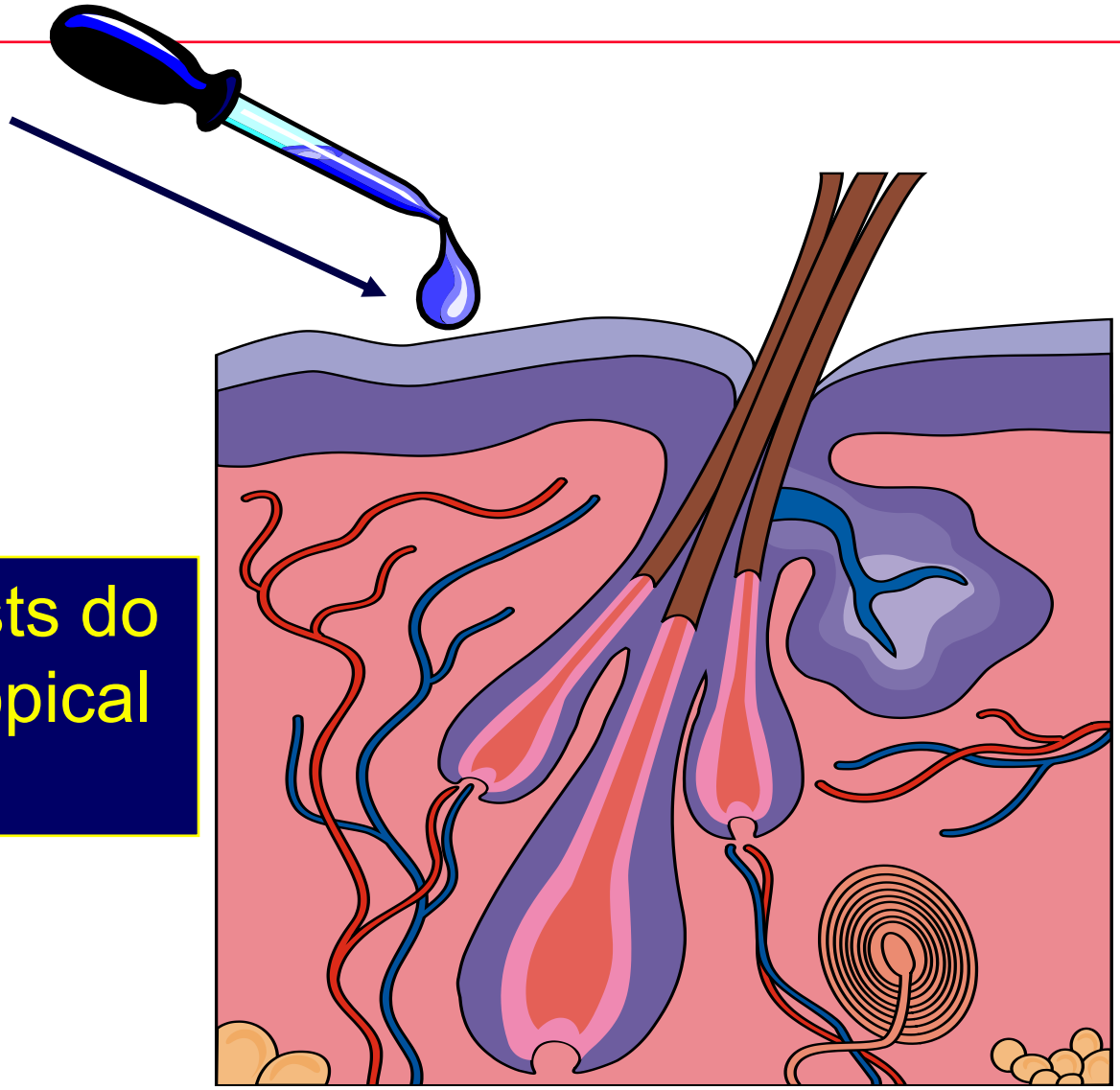
- Central nervous system (blood-brain barrier)
- Eye (blood-ocular barrier)
- Prostate (blood-prostate barrier)
- Intracellular sites
- Respiratory tract (epithelium)

Exceptions for Interpretive Categories

Topical Antibiotics Drug Delivery

Concentration at infection site exceeds plasma concentration.

★ In vitro susceptibility tests do not predict efficacy from topical administration.

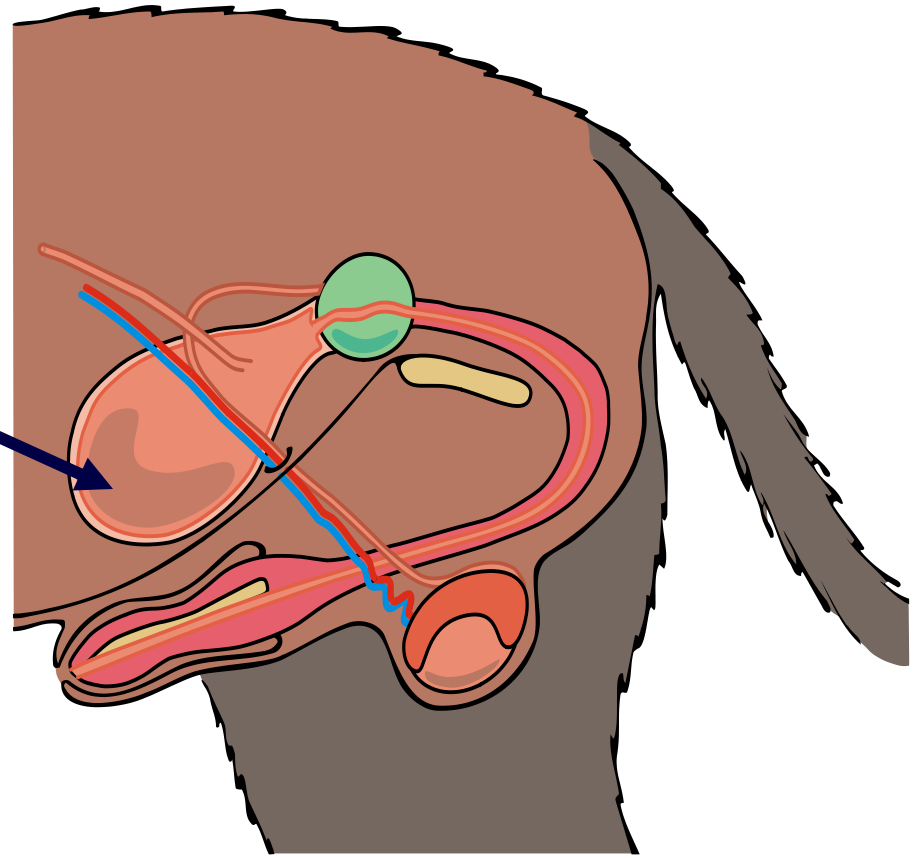


Breakpoints for Urinary Tract Infections

Urinary Tract Infections:

High urine concentrations improve activity of many antibiotics

High urine drug concentrations.



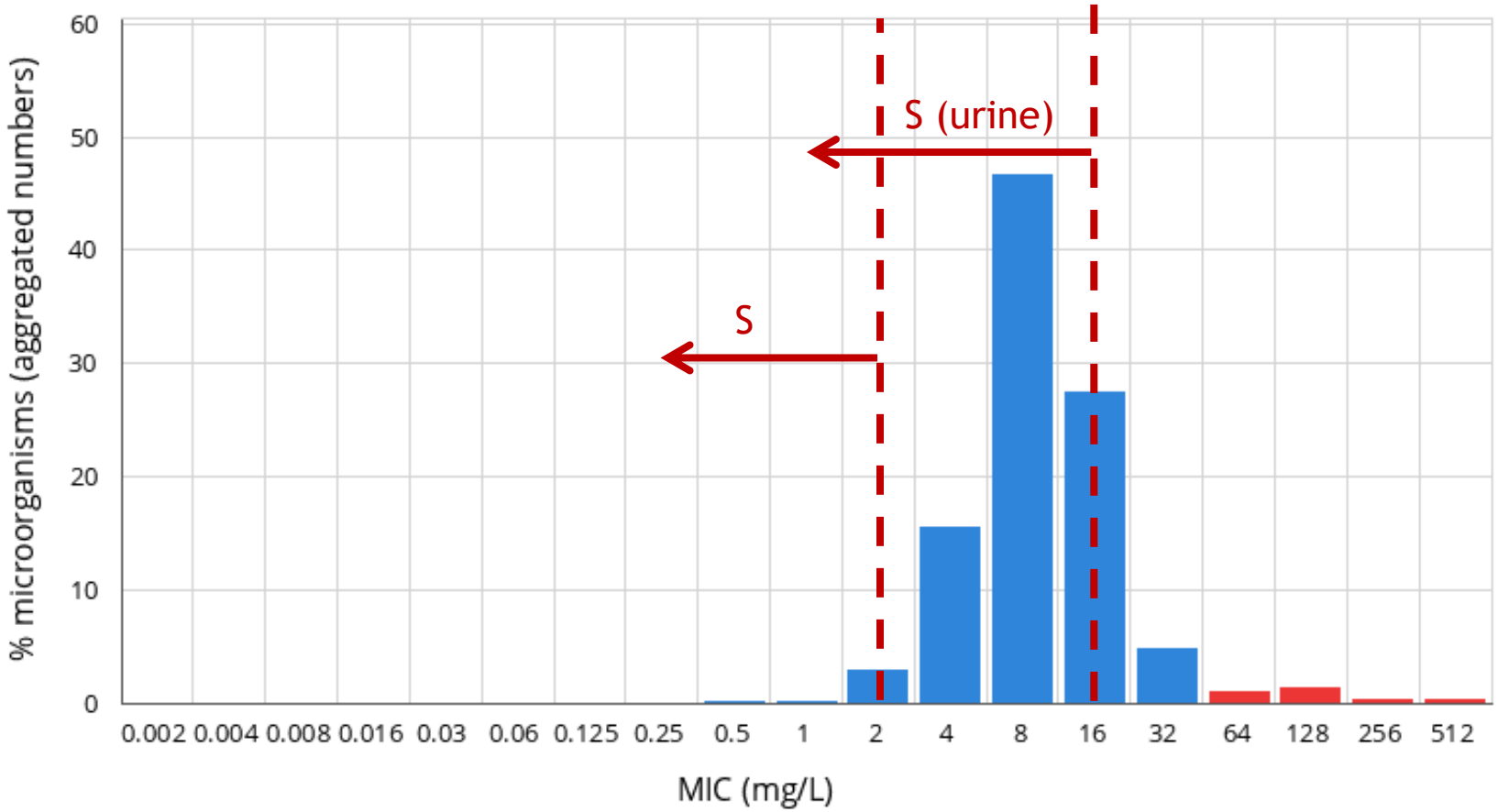
1st-Generation Cephalosporin/*E. coli*

Cefalothin / Escherichia coli

International MIC distribution - Reference database 2021-01-13

Based on aggregated distributions

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



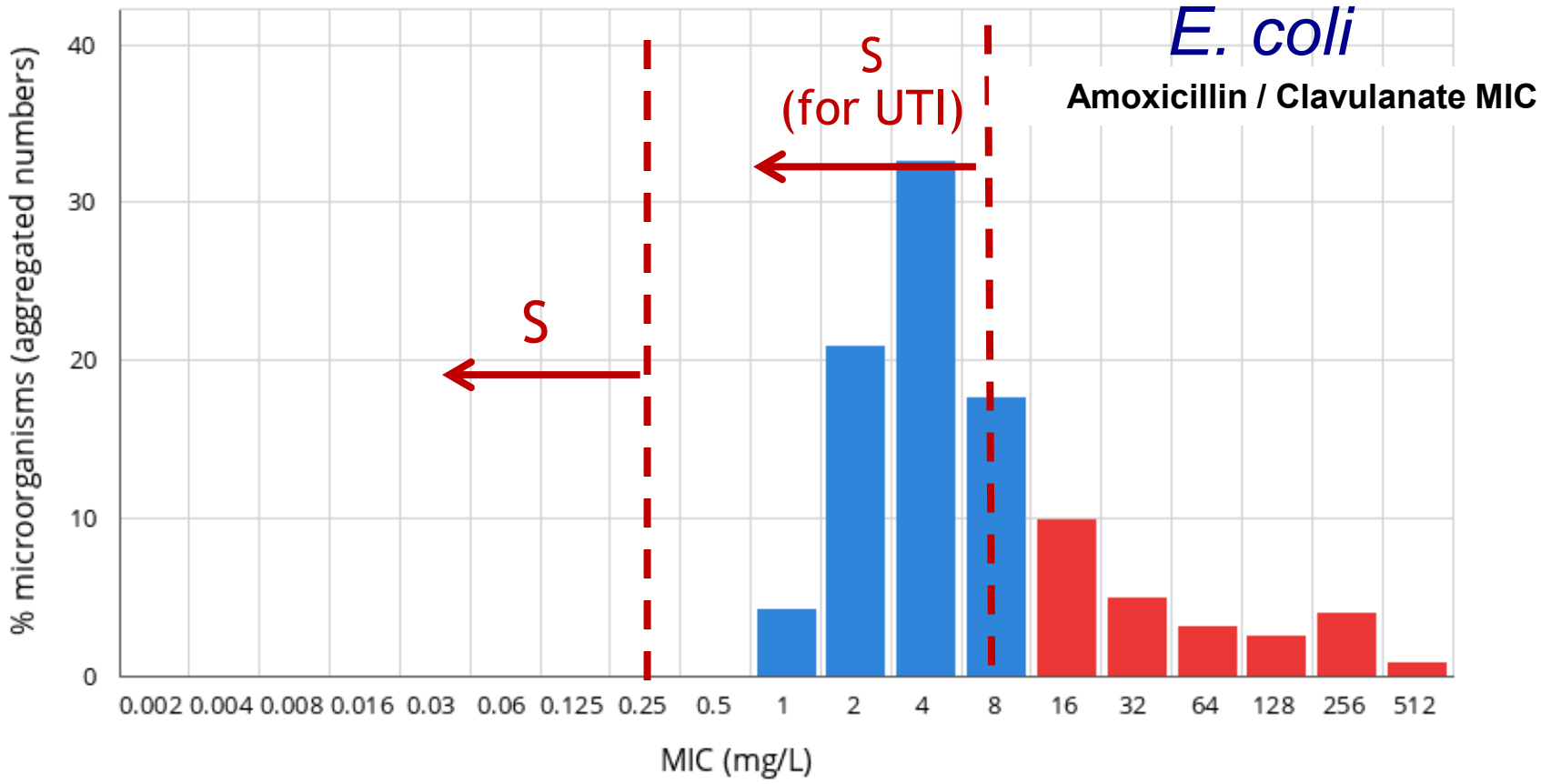
MIC
Epidemiological cut-off (ECOFF): 32 mg/L
Wildtype (WT) organisms: ≤ 32 mg/L

Confidence interval: -
3861 observations (14 data sources)

Amoxicillin-Clavulanate/*E. coli*

Amoxicillin-clavulanic acid (fixed) / Escherichia coli
International MIC distribution - Reference database 2021-01-13
Based on aggregated distributions

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): (8) mg/L
Wildtype (WT) organisms: ≤ 8 mg/L

Confidence interval: -
10159 observations (4 data sources)

CLSI-VAST Urine-Specific Breakpoints

Breakpoints for uncomplicated lower urinary tract infections (UTI)

Drug	Typical Breakpoint	Urine Breakpoint
1st-generation Cephalosporin	≤ 2 µg/mL	≤ 16 µg/mL
Amoxicillin	≤ 0.25 µg/mL	≤ 8 µg/mL
Amoxicillin-Clavulanate	≤ 0.25 µg/mL (dogs, cats)	≤ 8 µg/mL
Cefovecin (Convenia)	≤ 0.5 µg/mL (dogs) ≤ 0.12 µg/mL (cats)	≤ 2 µg/mL
Ceftiofur (Naxcel) injection (proposed)	None	≤ 1 µg/mL

Urinary Tract Infections
Can we rely on high urine concentrations?

- No: if urine is not concentrated

- No: if infection is not confined to urine, but other tissues are involved (prostate, bladder mucosa, kidney)

Conclusions

- This presentation shows how CLSI develops breakpoints for susceptibility testing.

- Part 2 will show some specific examples of updated breakpoints.

Thank you for your attention.

Any questions?