



ANTIMICROBIAL SUSCEPTIBILITY TESTING

of bacteria and fungi



PIONEERING DIAGNOSTICS



INTRODUCTION

QUESTIONS AND ANSWERS

on Antimicrobial Susceptibility Testing of bacteria and fungi

This brochure is intended to provide succinct answers to common questions about the performance of *in vitro* antimicrobial susceptibility testing of bacteria and fungi and the value of the results in guiding antimicrobial therapy.

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Of all the laboratory examinations performed daily by clinical microbiologists, *in vitro* susceptibility testing is of particular clinical importance as an aid for selecting the most appropriate antimicrobial therapy for individual patients, monitoring the evolution of microbial resistance, and updating empirical therapeutic strategies.

The methodology for *in vitro* antibacterial testing and the criteria for interpretation are well established. Antibacterial susceptibility testing is routinely performed in microbiology laboratories worldwide. Methods for *in vitro* antifungal testing and criteria for interpretation have been developed more recently and are similar in concept to antibacterial testing. Susceptibility testing of certain antiviral agents (e.g. anti-HIV agents) is also established but the methods and concepts are quite different. The scope of this brochure will be limited to a discussion of antibacterial and antifungal susceptibility testing.

This brochure explains basic facts concerning the relevance and procedures of *in vitro* susceptibility testing. It provides information on the essential elements required to perform and utilize susceptibility testing as a tool for optimizing anti-infectious therapy.



1 What is *in vitro* susceptibility testing?

Susceptibility testing measures the level at which a particular antimicrobial inhibits the growth of a specific microbial strain. A variety of laboratory methods can be used to measure the *in vitro* susceptibility of microbial pathogens to antimicrobial agents. Methods should be standardized based on international standards such as EUCAST, CLSI and ISO.

“Sensitivity” is a widely used term and is essentially synonymous with susceptibility in the context of susceptibility testing.

2 Why perform *in vitro* susceptibility testing?

The goal of *in vitro* antimicrobial susceptibility testing is to **assess the activity of an antimicrobial agent on a bacterial or fungal strain** in order to predict the likelihood of ***in vivo* efficacy** of antimicrobial therapy when the antimicrobial is administered to patients.

The main purpose of routine *in vitro* susceptibility testing in the clinical microbiology laboratory is to guide physicians in **selecting antimicrobial therapy for treatment of individual patients**. The susceptibility testing is performed on bacterial and fungal strains isolated from individual patients and presumed to be the etiology of their infection. The physician utilizes the susceptibility test result along with other available clinical information (e.g. site of infection, severity of infection, immune status of patient, co-morbidities, etc.) to select the optimal therapeutic agent for that particular patient's infection. Usually the susceptibility test results become available after initiation of empirical antimicrobial therapy. When this occurs, the susceptibility test results serve to confirm the appropriateness of

the empirical therapy and/or indicate appropriate alternative agents for treatment. Alternative agents may be required when resistance is detected or the patient experiences an adverse reaction to the empirical agent. Often, it is possible to identify appropriate agents for oral step-down therapy or narrower spectrum agents likely to be as effective as the broader empirical therapy.

A second important purpose of routine *in vitro* susceptibility testing is to monitor the **evolution of bacterial and fungal resistance**. This role requires periodic statistical analysis of the accumulated levels of resistance per species, type of specimen, and patient, in order to guide the initial empiric choice of antimicrobial therapy while awaiting laboratory test results. The pattern of antimicrobial resistance by ward, healthcare establishment, region or country guides **empiric antibiotic therapy choices** and **antibiotic formulary decisions**. Detailed statistical analysis enables the detection of outbreaks, especially in the hospital or long-term care setting, caused by multi-resistant bacteria, which justify investigation and appropriate infection control intervention. The detection of a new resistance pattern or a large number of patients infected with multi-resistant bacterial strains at one time and in the same place may indicate the need for implementation or change of infection control practices.

Data from routine antimicrobial susceptibility testing performed in clinical microbiology laboratories therefore influences the therapeutic decisions for current and future patients.

DUAL PURPOSE OF SUSCEPTIBILITY TESTING:

> Individual (to guide the selection and modification of antimicrobial therapy) and Epidemiological

3 When should a susceptibility test be performed?

In general, assuming that standardized testing methodologies have been developed, susceptibility testing is indicated for microorganisms causing infections warranting antimicrobial therapy when the susceptibility cannot be reliably predicted based on the known characteristics of the organism.

In vitro susceptibility testing methodology is well established for bacteria and is considered a routine part of the culture process (*Clinical Laboratory Standards Institute (CLSI), 2009 and European Committee for Antimicrobial Susceptibility Testing (EUCAST), 2000, 2003*). *In vitro* susceptibility testing is usually performed each time bacteria considered to be responsible for a patient's infection are isolated from a clinical specimen.

Susceptibility testing for yeast species is less commonly performed and is not available for all species. There are published reference methods (CLSI and EUCAST) and some commercial products are available. Each laboratory will determine the need for routine testing of yeast isolates from clinical specimens based on the need of the patient population. Reference methodology for *in vitro* testing of mould species is under development and currently only available from specialised mycology laboratories.

When the same species is isolated from specimens taken from different body sites (e.g. urine and blood) or from multiple specimens from the same body site (e.g. multiple blood culture bottles are positive), the laboratory may elect not to perform susceptibility testing on all of the isolates from the patient.

Susceptibility testing should not be routinely performed on organisms that are part of the normal bacterial flora and usually not considered pathogenic.

Sometimes microbiologists cannot definitely determine if susceptibility testing is required, without obtaining the clinical information that only a clinician can provide.

For example, a commensal bacterium (e.g. *Staphylococcus epidermidis*) is occasionally isolated from sterile site cultures (e.g. blood, joint fluid, cerebrospinal fluid) due to inadequate decontamination of the skin during specimen collection.

Susceptibility testing should not be performed on probable contaminants. However, the same *S. epidermidis* can cause a true bloodstream infection in an immuno-compromised patient or an infection at a specific body site (e. g. prosthetic joint, cerebrospinal fluid shunt) in which case, susceptibility testing should be performed.

Clinical symptoms can also be a determining factor when deciding whether to perform susceptibility tests (e.g. diagnosis of urinary tract infection with a low bacterial count).

Establishing the need for susceptibility testing requires a close working relationship between microbiologists and clinicians.

4 Can susceptibility and/or resistance of bacteria to an antibiotic be predicted?

Each antibiotic is characterized by a **natural spectrum** of antibacterial activity. This spectrum is the list of bacterial species which, in their naïve (wild-type) state, have their growth inhibited at a concentration known to predict efficacy *in vivo*. These bacterial species are said to be **naturally susceptible** to this antibiotic. Bacterial species which are not included in this spectrum are said to be **naturally** (intrinsically) **resistant**.

→ **Natural resistance** is a **stable** characteristic of all strains of the same bacterial species. It occurs as a result of the microorganism's genetic composition. This intrinsic resistance means that the antimicrobial agent is unlikely to ever be useful to treat an infection due to strains of this species. Knowledge of naturally resistant species enables prediction of the likely inactivity of a molecule in relation to the identified or probable bacterial pathogen. It sometimes constitutes an **identification aid** as some species can be characterized by their natural resistance.

EXAMPLES:

- Natural resistance of *Klebsiella pneumoniae* to aminopenicillins (ampicillin, amoxicillin) due to a β -lactamase (mostly SHV-1).
- Natural resistance of *Proteus mirabilis* to tetracyclines and colistin (due to natural targets with reduced binding ability).

→ **Acquired resistance** is a characteristic specific to some strains, within a naturally susceptible bacterial species, in which the genotype has been modified by **gene mutation or gene acquisition**. Contrary to natural resistance, acquired resistances are evolutionary and their frequency is often dependent on the amount of exposure to antibiotics. Given the evolution of acquired resistances, the natural activity spectrum is no longer sufficient to guide the choice of antibiotic therapy for numerous species. **Acquired resistance** results from a mutation in the microbial chromosome or the acquisition of extra-chromosomal DNA. In bacteria, the spread of resistance mechanisms occurs through vertical transmission (parent to daughter cells) of inherited mutations from previous generations as well as horizontal spread of mobile genetic elements such as plasmids (moving between cells and often between different species of bacteria).

Acquisition of antimicrobial resistance mechanisms can render previously useful antimicrobial agents useless for treating most strains of the species (e.g. penicillin and *Staphylococcus aureus*). Therefore, **susceptibility testing** becomes **essential for the detection of acquired resistance**.

NATURAL RESISTANCE:

permanent characteristic of the species, which is known and predictable.

ACQUIRED RESISTANCE:

characteristic of some bacterial strains, which is evolutionary, unpredictable and justifies the need for susceptibility testing.

→ Examples of natural resistance (adapted from Livermore DM et al., 2001)

Antibiotics	Enterobacteriaceae			<i>Pseudomonas aeruginosa</i>	Streptococci	Enterococci	Staphylococci
	<i>E. coli</i>	<i>Klebsiella</i>	<i>Enterobacter Serratia</i>				
Penicillin G							
Aminopenicillin							
Aminopenicillin + beta-lactamase inhibitor							
Cephalosporins: C1G							
C2G							
C3G							
Aztreonam							
Macrolides							
Glycopeptides							
Colistin							

 = Natural resistance

C1G: 1st generation cephalosporins
 C2G: 2nd generation cephalosporins
 C3G: 3rd generation cephalosporins

5 What is an antibiotic clinical spectrum?

In order to take into account the evolution of acquired resistances and therefore provide clinicians with useful information when choosing empiric antibiotic therapy, the concept of **clinical spectrum** complements that of the **natural spectrum**.

The **clinical spectrum** is defined for each antibiotic and in some jurisdictions is included in the technical package insert which is approved during the registration of antibiotics. This spectrum is initially defined by **clinical breakpoints**, which are devised by integrating **microbiological data** (Minimum Inhibitory Concentrations – MICs, and wild-type MIC distributions), **pharmacokinetics/pharmacodynamics, and clinical outcome data**. Regulators may also define susceptible species as only those species for which the clinical activity of the product has been demonstrated. Strains other than those defined by the regulator may still be susceptible to an antimicrobial agent at the same clinical breakpoints. The treating clinician then takes responsibility when using that agent to treat infections caused by such strains.

The clinical spectrum is regularly revised to take into account the evolution of acquired resistances. The prevalence of resistance may vary geographically and with time for selected species and local information on resistance is desirable, particularly when treating severe infections.

CLINICAL SPECTRUM OF ACTIVITY:

- ■ useful to guide empiric antibiotic therapy
- depends on the clinical breakpoints and frequency of resistance and the *in vivo* activity of the antibiotic

6 How is the susceptibility to an antimicrobial measured?

Most susceptibility testing is growth based. It involves exposing a pure culture of a microorganism to a range of concentrations of an antimicrobial agent and observing the presence or absence of growth after a period of incubation. The results can vary widely depending on the conditions of testing. It is therefore **imperative to use standardized methods**.

When performing *in vitro* susceptibility testing, technical factors must be controlled by rigorous standardization of all the analysis stages (purity and density of the bacterial inoculum, medium composition, reagents, incubation conditions, reading method and biological and clinical criteria for interpretation of these results). Detailed and continuously updated international recommendations are available, such as those compiled by the CLSI and EUCAST. Quality control procedures for evaluating analytical accuracy and precision must also be applied regularly in order to guarantee the quality of the susceptibility test.

Broth dilution was one of the earliest antimicrobial susceptibility testing (AST) methods. Originally performed in test tubes (macrobroth dilution), it has been miniaturized into microtiter plates (microbroth dilution). Two-fold dilutions of the antimicrobial are made in a nutrient broth and then each well is inoculated with a standardized number of microorganisms. As defined by standardized methodology, the plates are incubated at a defined temperature for a defined period of time. The lowest concentration of antimicrobial with no visible growth is the minimum inhibitory concentration (MIC).

Modern technology has allowed miniaturization and automation of broth dilution methodology which has reduced the time to results. In general, for routine bacterial susceptibility testing, results are available in several hours to a day.

➤ Automation increases precision, minimizes operator error, and provides traceability to AST methods.

Antimicrobial gradient diffusion is another form of AST in which a concentration gradient is established in an agar medium onto which a standardized suspension of microorganism is inoculated. This method has the capability to generate a MIC value across an extensive range of dilutions for a wide range of organism/antimicrobial combinations.

The **disk diffusion method** involves placing antimicrobial impregnated disks onto an agar surface that has been inoculated with a standardized suspension of microorganism. After a defined period of incubation, the zone of no growth around each disk is measured and interpreted based on published interpretive criteria, which have been developed by comparison with MIC methods.

7 What is a MIC and how is it used?

The basic measurement of the susceptibility of a microorganism to an antimicrobial agent is based on the determination of the **minimum inhibitory concentration (MIC)**.

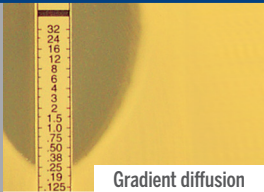

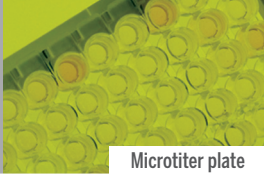
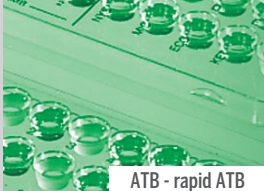
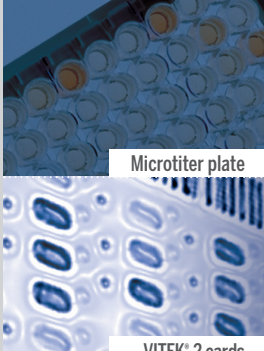
The MIC is defined as the lowest concentration of a range of antibiotic dilutions that inhibits visible growth of bacteria within a defined period of time.

The MIC is a measure of **antimicrobial potency**. It is the fundamental reference value that enables a range of antimicrobial activity to be established for different microbial species, and to which all other testing methods are compared. Each microbial species will have a unique MIC distribution in the naïve or wild-type state (EUCAST website), i.e. not all members of a species will have exactly the same wild-type MIC.

Various laboratory techniques enable the MIC value to be measured or estimated semi-quantitatively in routine use (see opposite).

Using the MIC, a tested strain can then be categorized according to its susceptibility for the antimicrobial being tested. This strain is said to be **Susceptible (S), Intermediate (I) or Resistant (R)** to the antibacterial or antifungal agent.

➔ Routine Laboratory methods for MIC determination

		Principle	Time to results
MANUAL METHODS	 Gradient diffusion	Bacterial growth measured according to an antibiotic concentration gradient	18 hrs
	 ATB™ strip	Bacterial growth measured according to 2 or several antibiotic concentrations	18 hrs
	 Microtiter plate	Bacterial growth measured according to one (4 hrs) or 2 antibiotic concentrations (18 hrs)	4 hrs to 18 hrs
SEMI-AUTOMATED OR AUTOMATED METHODS	 ATB - rapid ATB	Bacterial growth measured according to one (4 hrs) or 2 antibiotic concentrations (18 hrs)	4 hrs to 18 hrs
	 Microtiter plate VITEK® 2 cards	Kinetic analysis of bacterial growth	4 hrs to 18 hrs

8 What are clinical breakpoints?

In general, two antimicrobial concentrations, known as «breakpoints» or interpretive criteria determine three categories: **Susceptible (S), Intermediate (I), and Resistant (R)**. Below (or equal to) one concentration, the clinical isolate is categorized as S, above (or equal to) the second concentration, it is categorized as R, and between these two concentrations, it may be categorized as I.

For some newer antimicrobials, where resistance is very rare or unreported, the categories may be Susceptible and Non-susceptible. Although the term “breakpoint” has been used in a wide range of contexts, it should be reserved for the values determined by the methods described below.

Breakpoints are developed through a detailed examination of MIC data and distributions, resistance data and mechanisms, pharmacokinetic and pharmacodynamic properties of the antimicrobial and available data on clinical outcome (by MIC of the infecting strain if possible). They are regularly reviewed and revised as appropriate when new information becomes available in one or more of these data sources.

Breakpoints are used by clinical microbiology laboratories to **categorize and report clinical isolates as S, I or R**, which will then assist physicians in selection of antimicrobial therapy.

AST interpretive category classifications are based on the *in vitro* response of an organism to an antimicrobial agent at levels correlated to blood or tissue levels attainable with the usually prescribed doses of that agent. The intention is to **correlate clinical breakpoints with the likely performance of the drug** when used to treat an infected patient.

Breakpoints are of two types:

- **MIC breakpoints**, applied to broth or agar-based methods, including the automated methods such as VITEK® 2.
- **zone diameter breakpoints**, used to categorize test results for the disk diffusion method. Disk diffusion breakpoints compare the MICs determined using a reference MIC testing method with the zone diameters generated by standardized disk diffusion on large numbers of relevant strains, using sophisticated statistical techniques.

9 What do S, I, R categories mean?

For a given antimicrobial, a bacterial or yeast strain is classified according to the following criteria.

→ Susceptible (S)

Susceptible means that the infection caused by that strain is highly likely to respond to treatment, at the site from which the strain was isolated, with the usual antimicrobial regimen for that type of infection.

→ Intermediate (I)

Intermediate means that the infection is likely to respond to higher dosing regimens (where possible) or because the antimicrobial is concentrated at the site of infection. It is also a buffer category to ensure day-to-day test variation does not result in a resistant isolate being categorized as susceptible or vice versa.

→ Resistant (R)

Resistant means that the infection caused by that strain is unlikely to respond to treatment with any regimen of the antimicrobial agent.

→ Susceptible-dose dependent (SDD)

The “susceptible-dose dependent” category implies clinical efficacy when a higher than normal dosage of a drug is used and maximal blood level achieved. This is currently reserved for certain antifungal drugs (e.g. fluconazole).

→ Nonsusceptible (NS)

This category is used for organisms that currently have only a susceptible interpretive category, but not an intermediate or resistant interpretive category. It is often given to new antimicrobial agents for which few or no resistant clinical isolates have yet been encountered.

10 What criteria are used to select the antibiotics to be tested?

The selection of the antimicrobial agents to be tested must be carefully determined depending on the microbial species and their natural resistance, local epidemiology of acquired resistances, the site of infection and local therapeutic options (formulary).

Each laboratory selects which antimicrobials are appropriate to test and report for each organism isolated.

- The antibiotics tested are those of **therapeutic value** for the type of infection and the body site from which the specimen has been collected.
- Due to the importance of acquired resistance, it is sometimes necessary to test antibiotics which serve as **resistance «markers»**, i.e. which are useful to detect resistance mechanisms.

EXAMPLE: Ertapenem is an excellent carbapenemase resistance marker for *Enterobacteriaceae*.

➤ The choice of antibiotics to be tested is made in relation to their therapeutic value and their usefulness to detect resistance mechanisms.

11 How are susceptibility results reported?

Susceptibility testing results are usually reported as S, I, or R for each antimicrobial tested for that isolate. Most laboratories will also include the actual MIC value, as it provides critical information that assists clinicians in making therapeutic decisions.

Often laboratories report only a **selection of the antimicrobials tested**, those that are most appropriate or commonly used. This selected reporting is often preferred because it assists in “**antimicrobial stewardship**” by guiding the prescriber to the most

appropriate agents for the treatment of the infection and withholding results for agents which may be effective but are unnecessarily broad or are only used as reserve agents.

For example, the laboratory may choose “cascade reporting”, e.g. to withhold the results of a third-generation cephalosporin and a carbapenem for a strain of *Escherichia coli* which is susceptible to earlier generations of cephalosporins and beta-lactamase inhibitor combinations, or to modify the report to include additional agents when certain resistances are detected.

12 What is antibiotic equivalence?

Equivalence is the **prediction of *in vivo* activity** for one antimicrobial based on results obtained by testing another, related antimicrobial agent. In this case, only a category result (S, I, R) can be reported.

EXAMPLE: Equivalence between erythromycin which is tested and other macrolides (e.g. azithromycin and clarithromycin) which are not tested. The category (S, I, or R) result for the other antimicrobials can be predicted from that obtained for erythromycin.

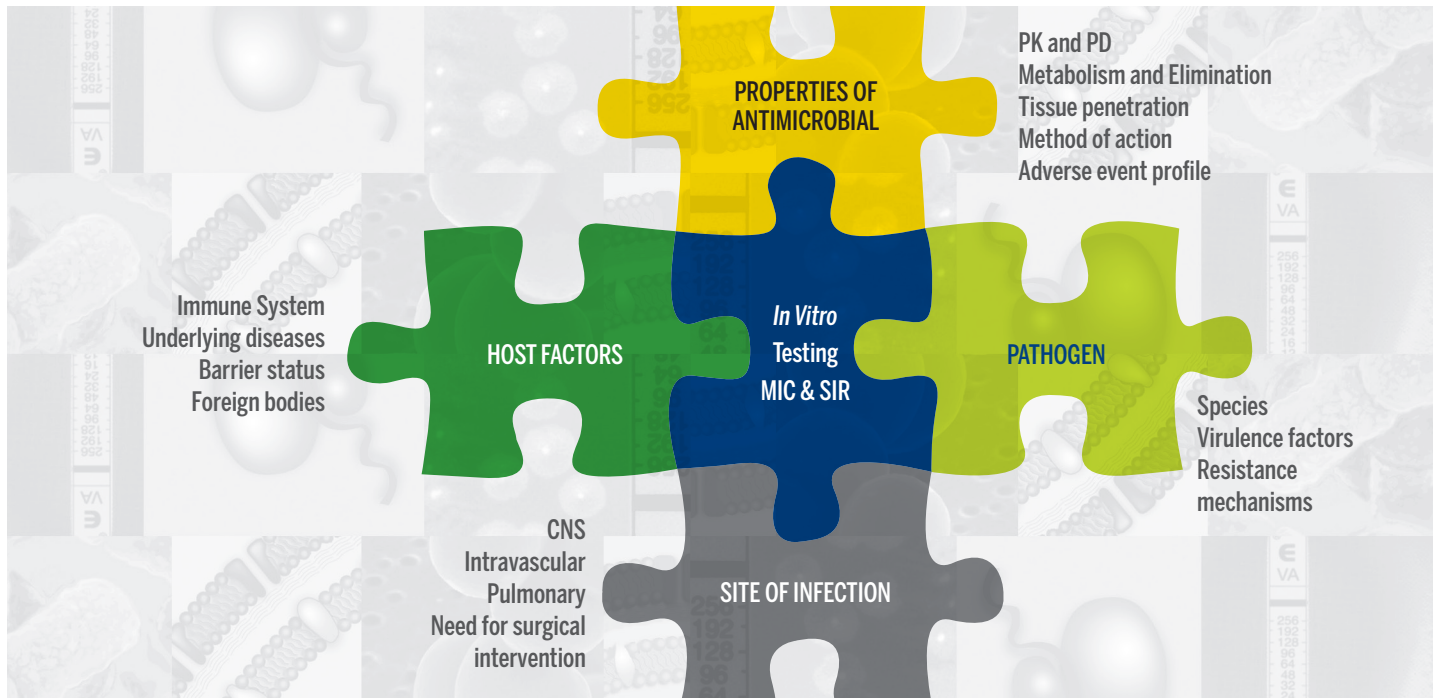
➤ It is possible to test a restricted number of antibiotics without limiting therapeutic possibilities.

13 Why do some patients with susceptible isolates fail therapy?

A MIC is an *in vitro* measurement (a laboratory assay) that provides an estimate of antimicrobial potency. It does not take into account host factors, especially the kinetics of the agent in an individual host, which are just as important as susceptibility test results in determining the outcome of treatment. Therefore, the susceptibility of a microorganism *in vitro* does not always assure successful therapy. As a corollary, resistance defined *in vitro* often, but not always, predicts therapeutic failure.

When multiple reports of the correlation of therapeutic outcome with *in vitro* susceptibility are examined (*Rex J and Pfaller MA 2002*), a pattern referred to as the “**90-60 rule**”, or **natural response rate**, emerges. This 90-60 rule observes that infections due to susceptible isolates respond to appropriate therapy approximately 90% of the time, whereas infections due to resistant isolates (or infections treated with inappropriate antibiotics) respond less than 60% of the time. Although there are some important exceptions, this rule is relatively robust and holds whether the outcome measurement is clinical response, bacteriological response, or mortality.

➔ Antimicrobial Drugs: why do they sometimes fail?



14 How do bacteria acquire resistance?

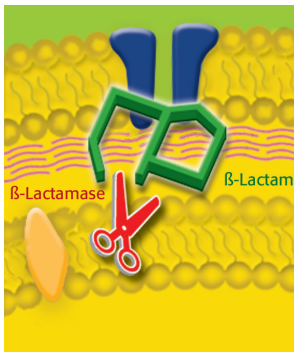
- ➔ The **genetic mechanism** of acquired resistance can be:
 - The **mutation** of a gene involved in the mode of **action** of the antimicrobial that results in an alteration in the molecule that is the target of the antimicrobial. Most often, this type of resistance mutation results in reduced antimicrobial binding to the target. This mechanism is the most commonly observed for the following antibiotics: quinolones, rifampin, fusidic acid, fosfomycin, antituberculosis drugs, and sometimes cephalosporins.

EXAMPLE: Resistance to quinolones by modification of DNA gyrase in *Enterobacteriaceae*.

- **Acquisition of resistance genes** transferred from a strain belonging to an identical or different species, usually on a mobile genetic element such as a plasmid. Some antibiotics are particularly affected by this mechanism: β -lactams, aminoglycosides, tetracyclines, chloramphenicol, sulfonamides.

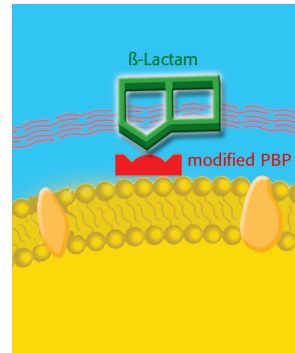
EXAMPLE: Resistance to ampicillin in *E. coli* and *Proteus mirabilis*.

- ➔ The **biochemical mechanism** of resistance can be due to:



Production by the bacteria of enzymes inactivating the antibiotic.

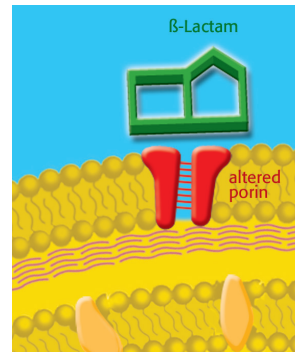
EXAMPLE: Penicillinase in *staphylococci*, extended spectrum β -lactamase (ESBL) in *Enterobacteriaceae*.



Modification of the antimicrobial target resulting in reduced binding.

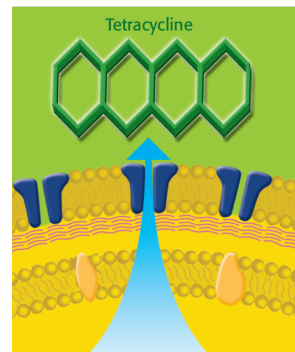
EXAMPLE: Modification of Penicillin-Binding Proteins (PBPs) of:
 - oxacillin-resistant *Staphylococcus aureus* (known as MRSA*).
 - penicillin-resistant *S. pneumoniae*.

* methicillin-resistant *Staphylococcus aureus*



Impermeability of the bacterial outer membrane by alteration or quantitative decrease of porins.

EXAMPLE: Imipenem-resistant *Pseudomonas aeruginosa*.



Efflux mechanism: expulsion of the molecule by active transport.

EXAMPLE: Tetracycline-resistant *staphylococci*.

15 Can antimicrobials «induce» resistance?

Antimicrobials do not cause resistance, but may allow resistant mutants to proliferate by eliminating susceptible microorganisms.

This is known as **selection pressure**.

The increase in the frequency of resistant strains is often linked to increased use of a specific antimicrobial.

16 Which methods enable resistance mechanisms to be demonstrated *in vitro*?

For the moment, only certain techniques enable the direct detection of **biochemical mechanisms** (example: detection of β -lactamase by hydrolysis of the indicator β -lactam nitrocefin) or **genetic determinants** of resistance (example: detection of the *mecA* gene responsible for staphylococcal resistance to oxacillin).

Susceptibility test results can **suggest** the presence of a resistance mechanism.

17 What is cross-resistance or associated resistance?

→ **Cross-resistance** is a resistance mechanism that affects an entire class or subclass of antibiotics.

EXAMPLES:

■ For *Streptococci*, resistance to 14- and 15-membered macrolides can be predicted by testing erythromycin.

■ Resistance to oxacillin in *Staphylococci* confers *in vivo* resistance to almost all β -lactams.

In certain cases, a resistance mechanism can affect antibiotics from different classes.

EXAMPLE: Resistance due to impermeability to tetracyclines also affects chloramphenicol and trimethoprim.

→ **Resistances are said to be associated** when several resistant mechanisms involving different antibiotic classes frequently occur together. Associated resistance is often plasmid-mediated, and in Gram-negative bacteria can often be encoded by genes strung together inside an integron.

EXAMPLE: Resistance to oxacillin in *staphylococci* is often associated with resistance to quinolones, aminoglycosides, macrolides and tetracyclines.

18 Why is it necessary to interpret susceptibility test results?

The rapid evolution of acquired resistance mechanisms by clinically significant bacteria and the sometimes weak expression of these resistance characteristics may require the use of tests in addition to those routinely performed. The goal of the additional tests is to avoid categorizing bacteria as susceptible when they express only **low-level resistance *in vitro*** but are likely to cause **therapeutic failure *in vivo***.

To avoid this, susceptibility testing must be interpreted to discern even a weakly expressed resistance mechanism (by comparing results for each antibiotic). Therefore, with appropriate interpretation, a strain initially testing as susceptible will be categorized as I or R.

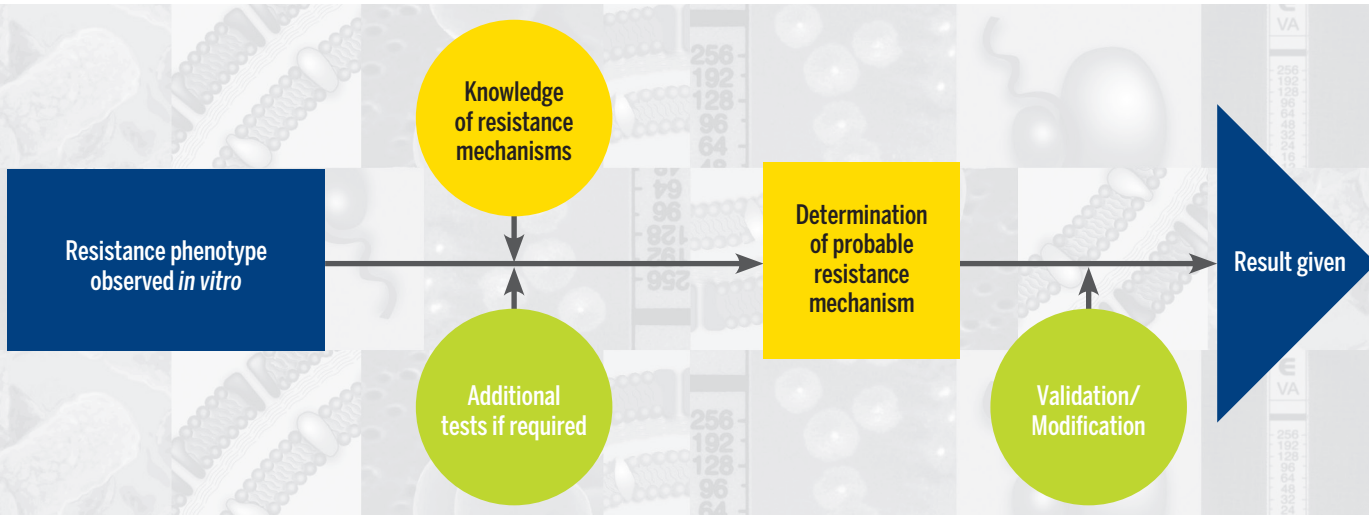
“Interpretive reading” can also be enhanced by the use of expert systems that are capable of examining resistance profiles and making predictions about other agents not tested or their likely therapeutic efficacy (see Question 19).

EXAMPLES:

- Strains of *Staphylococcus aureus* that test Resistant to methicillin or oxacillin (MRSA) may test Susceptible *in vitro* to other beta-lactams, especially cephalosporins. However, *in vivo* data have demonstrated a high level of treatment failures of MRSA infections with beta-lactam therapy. Therefore the interpretation is changed to “Resistant” for all beta-lactams regardless of the actual MIC value.
- A strain of *S. aureus* resistant to erythromycin can test *in vitro* as Susceptible to clindamycin and as positive by an Inducible Clindamycin test. Treatment with clindamycin can result in the selection of resistant mutants that cause inactivation of the antibiotic and treatment failure. For strains of *S. aureus* testing positive with the Inducible Clindamycin test, the Susceptible result for clindamycin must be modified to Resistant or a standard comment must be attached to the report alerting the prescriber of this possibility.

➤ Through the judicious choice of antibiotics tested, the interpretation of susceptibility test results can help detect resistance weakly expressed *in vitro*.

➔ Interpretive procedure



19 What is the role of an Expert System?

Validation of a susceptibility test result requires a comprehensive knowledge of resistance mechanisms and antibiotic activity. An Expert System is a software package designed to help integrate this knowledge and automatically interpret susceptibility tests, check results and suggest the necessary corrections.

The Expert System contributes to the reliability of the result by:

→ **ensuring consistency between the susceptibility test result and bacterial identification**

EXAMPLE: *Klebsiella pneumoniae* - ampicillin S = improbable phenotype.

→ **identifying improbable or impossible resistance phenotypes**

EXAMPLE: *E. coli* - cefazolin S - cefotaxime R = impossible phenotype.

→ **detecting insufficiently expressed resistances**

EXAMPLE: Detection of a third-generation susceptible *Enterobacter cloacae* and correction of S results to R, or appending a standard comment to a report for third-generation cephalosporins.

→ **The Expert System:
a tool for interpretation of susceptibility test results**

→ **indicating a rare phenotype in a given context**

The regular update of information constituting the **knowledge-base** is essential. The genetic make-up of the predominant strains varies over time and from one geographical location to another.

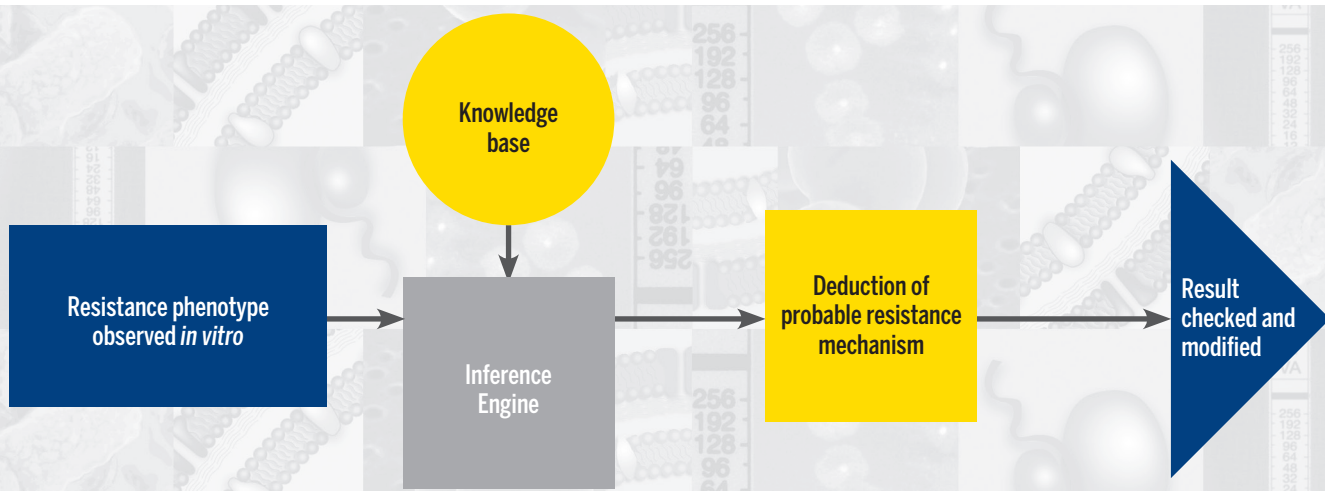
EXAMPLES:

■ MRSA strains have been resistant to gentamicin for a very long time, but this association is less true today due to the emergence of community-associated strains, e.g. USA300, which are gentamicin susceptible.

■ Vancomycin-resistant *enterococci* are frequent in the USA but are uncommon in Europe.

→ **screening for important resistance mechanisms**

EXAMPLE: Detection of carbapenemases in Gram-negative bacteria or heterogeneous vancomycin resistance in *Staphylococcus aureus*.



20 Some specific resistance issues

→ Are bacteria responsible for community-acquired urinary tract infections affected by antibiotic resistance?

Although bacterial resistance is more frequent in hospitals than in the community, bacteria that are most often found in community-acquired pathologies, such as *E. coli*, can acquire antibiotic resistance.

For example, in 2005 the level of acquired resistance of *E. coli* to antibiotics frequently used for the treatment of urinary tract infections were:

Ampicillin	49%
Fluoroquinolones	23%
Trimethoprim-sulfamethoxazole	27%

Source: TRUST 2007

→ What is Community-associated MRSA?

The first reported cases of CA-MRSA began to appear in the mid-1980s in Australia and New Zealand, and in the mid-1990s in the United States, the United Kingdom, Europe and Canada. These cases were notable because they involved people who had **not been exposed to a healthcare setting**.

This increase in the incidence of MRSA infection has been associated with the recognition of **new MRSA clones** known as community-associated MRSA (CA-MRSA). CA-MRSA strains infect a different group of patients, cause different clinical syndromes, and differ in antimicrobial susceptibility patterns compared to healthcare-associated MRSA (HA-MRSA) strains, CA-MRSA strains can **spread rapidly among healthy people** in the community and are now a frequent cause of infections in healthcare environments as well. The clinical spectrum of infectious syndromes associated with CA-MRSA strains ranges from a commensal state to severe, overwhelming infections, especially skin and pulmonary. (*David MZ and Daum RS 2010*).

→ What is the probability of infection by *Enterobacteriaceae* with extended spectrum β -lactamase (ESBL) strains?

In patients from the community, the frequency of this type of multi-resistant bacteria (i.e. with acquired resistance to numerous antibiotics) used to be linked to a previous hospital stay. These bacteria were mainly found in hospitals, where their **multi-resistance** gives them a selective advantage. Generally **transmitted** from one patient to another in the same healthcare unit (hospital, clinic, nursing home, etc.), they were and still are responsible for **nosocomial infections**.

In recent years, however, ESBL-producing *E. coli* have begun to emerge in community strains worldwide (*Oteo J et al., 2010*). These strains carry β -lactamases of the CTX-M type, rather than the more common TEM or SHV type found in hospital-associated strains. One clone in particular, O25:H4-ST131, encoding CTX-M-15, seems to have spread widely across the world (*Nicolas-Chanoine M-H et al., 2008*). Not unexpectedly, they are **most frequently isolated from urine**. Many strains are resistant to multiple anti-microbial agents and are challenging for outpatient management. They are adding to the overall burden of ESBLs in hospitals because they require the same infection control interventions.

→ Why is it essential to check the susceptibility of *S. pneumoniae* to antibiotics and notably to penicillin G?

For over 3 decades, **pneumococcal non-susceptibility to penicillin G** has continuously increased in many countries (less than 1% in 1985, 10-50% in the mid 2000's (*Linares J et al., 2010*)). This resistance is often **associated with resistance to other antibiotics** (e.g. tetracyclines, macrolides). This emerging resistance impacts empirical antibiotic therapy for acute otitis media, sinusitis and bronchopulmonary infections, as well as meningitis which are often caused by *S. pneumoniae*. In some cases, resistance of *S. pneumoniae* to penicillin G may indicate resistance to other β -lactam antibiotics, making determination of susceptibility necessary.

→ Is there any problem with resistance to antifungal agents?

Patient characteristics, antifungal prophylaxis, and other factors appear to have contributed to a **change in the spectrum of invasive fungal pathogens**. Infections with more resistant species of *Candida* (e. g. *C. glabrata*, *C. krusei*), and *Aspergillus* (e.g. *A. terreus*) as well as non-*Aspergillus* moulds appear to be on the rise, at least among certain populations. (Pfaller MA *et al.*, 2006). These species are resistant or less susceptible to some commonly used antifungal agents. With this change in epidemiology as well as an increased choice in available antifungal agents from which to choose, **antifungal susceptibility testing is becoming more of a necessity**, especially for the commonly isolated yeasts.

CONCLUSION

Antibiotic susceptibility testing, at the **interface between the clinical diagnosis and the therapeutic decision**, is a key element essential for guiding both microbiologically-documented and empiric antibiotic therapy. Not only is the susceptibility test result of immediate interest for the clinician to **guide selection of antimicrobial therapy**, but it also plays a role as an **epidemiological surveillance tool** for local bacterial and fungal resistance patterns.

The evolution of resistance, as well as the development of new antibiotics and laboratory techniques make a **close working relationship between the microbiologist and the clinician** more necessary now than ever before.

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