BIOMÉRIEUX

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Clinical and Laboratory Perspectives

PIONEERING DIAGNOSTICS

INTRODUCTION

The World Health Organization (WHO) has declared antimicrobial resistance as a major global health threat due to the misuse and overuse of broad-spectrum antibiotics driving the emergence of multi-drug resistant and pan resistant "superbugs".

The WHO Global Action Plan on Antimicrobial Resistance[1] identifies the need to **increase investment in diagnostic tools** capable of informing healthcare practitioners of the susceptibility of pathogens to available antibiotics. Given this key objective, **antimicrobial susceptibility testing (AST)** clearly plays an important role in not only ascertaining the best possible antimicrobial option for patient treatment but also ensuring the detection of antimicrobial resistance mechanisms in clinical isolates.

Therefore, AST must be:

- **Accurate** – standardized test methods capable of detecting a wide range of different antimicrobial resistance mechanisms need to be used by clinical microbiology laboratories.
- **Timely** – results should be generated within a relevant timeframe to allow optimal antibiotic prescribing practices.
- **Reliably reported** – results reported as susceptible or resistant need to apply international Minimum Inhibitory Concentration (MIC) breakpoints, be selectively reported and include interpretative comments.

AST must also:

- **Promote optimal antibiotic use** – the range of antibiotics reported should allow for optimal treatment in accordance with local antimicrobial stewardship policies.
- **Provide cumulative data** - that is organism and infection site related for the generation of local antibiograms to allow appropriate empirical antibiotic selection.

This practical guide provides a step-by-step approach to AST and antibiotic therapy selection, and will focus on:

- **The role of AST in the clinical setting** and selection of initial empiric therapy based upon clinical and preliminary laboratory test data and cumulative antibiograms.
- **Basic concepts of AST**, and **laboratory methods** for antimicrobial susceptibility and resistance testing.
- **Interpretation of AST results** for clinical treatment and the collection and application of cumulative AST data for wider clinical applications such as antibiograms and Antimicrobial Stewardship Programs (ASPs).

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We wish to thank them for sharing their valuable knowledge and expertise on Antimicrobial Susceptibility Testing from both a laboratory and a clinical perspective, and for their dedicated involvement in this booklet.

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PRE-AST

CLINICAL DECISION-MAKING PROCESS

1 WHAT IS AST?

Antimicrobial susceptibility testing (AST) is an *in vitro* diagnostic test performed in the microbiology laboratory that **measures the ability of an antimicrobial agent to inhibit the growth of a microorganism.**

Standardized test methods using a range of different antimicrobial agents are used depending upon the type of microbe and the site of infection. Additional phenotypic and/or genotypic techniques may be utilized to determine the specific resistance mechanism involved.

A laboratory report listing results for both **susceptible** and **resistant** microorganisms is issued 12-72 hours post collection of the clinical sample, depending on the AST method used.

- A **susceptible result** indicates a high likelihood of **therapeutic success at a standard dosing regimen**.
- Whereas a **resistant result** indicates a high likelihood of **therapeutic failure even at an elevated exposure**.

Although fast susceptibility testing and gene detection may give more rapid results, routine phenotypic AST using conventional methods is usually required to provide a range of possible antibiotic options for treatment.

Should AST be performed on all clinical samples?

Simply put, **there is no need for AST if there is not an infection which needs to be treated**. Unnecessary testing is not just a waste of money but may also "encourage" unnecessary antibiotic prescribing because medical staff are trained to respond to laboratory results. Unnecessary antibiotic prescribing can lead to adverse impacts on the microbiome without providing any clinical benefit, and also contribute to the spread of antimicrobial resistance.

Some examples where samples should not be sent to the microbiology laboratory for processing include:

- Urine from patients without symptoms,
- Central venous line tips, from lines removed because they are not needed any longer,
- Swabs of ulcers without surrounding cellulitis.

Once AST results become available, the clinician is able either to confirm the initial empiric therapy or provide alternate antibiotic options (targeted therapy) **(Figure 1)**. Furthermore, the results can **facilitate antimicrobial stewardship** by directing therapy towards an alternative agent, which may be less toxic or more narrow-spectrum (effective against only a limited range of organisms). More cost-effective antimicrobial options can also be chosen from the list of agents to which the infecting organism is susceptible.

There are also benefits of AST which extend beyond the immediate patient. AST allows detection of clinically significant resistant organisms which are important for **infection control**, e.g., methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), extended spectrum beta-lactamases (ESBL), carbapenemase-producing Enterobacterales (CPE). Other longer-term benefits include **compiling cumulative AST data** for use in local / national / international epidemiological data sources or antibiograms (see page 50 for more details).

2 WHAT ARE THE CLINICAL BENEFITS OF PERFORMING AST?

Without AST, antimicrobial therapy used by clinicians would only be an "educated guess".

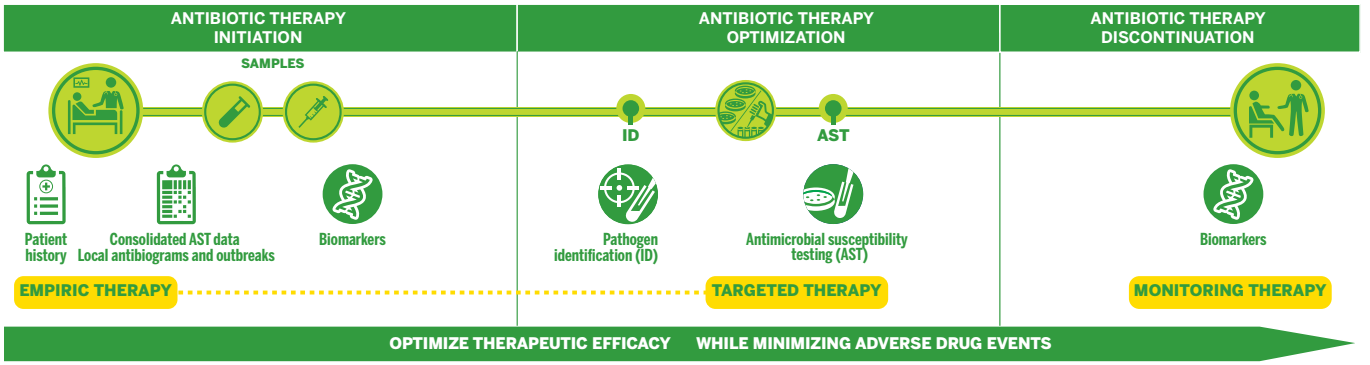
In fact, in seriously ill patients requiring immediate initiation of antimicrobials, the initial doses are always given in the absence of AST results. In this situation, clinicians use their knowledge of several factors in order to determine a rational empiric antimicrobial prescription:

- **The individual's recent history** of antibiotic use and antibiotic allergies,
- **Prior colonization or infection** with antimicrobial resistant organisms,
- **Local cumulative antibiograms** developed at hospital level,
- **Current infection outbreaks** of antimicrobial resistant organisms.

> **AST measures the ability of an antimicrobial to inhibit the growth of a microorganism.**
>
> **The ability of AST methods to detect antimicrobial resistance and reduce the time to test results is critical.**

Figure 1. Place of AST in the patient workflow

Source: bioMerieux



CASE STUDY 1.

A 24-year-old woman returns from a trip to visit family on a Pacific island. She is previously healthy. While visiting, she gets a «band tattoo» around her upper right arm. Unfortunately, the tattoo site becomes infected with redness and discharge of pus. She develops a fever and becomes fatigued. Gram stain of a swab of the pus shows gram-positive cocci in clusters and pairs **(Figure 2)**.

Why is AST important in this case?

Administration of antibiotics is indicated for this wound infection related to the recent tattoo. On the Gram stain, the appearance of the gram-positive cocci in clusters is suggestive evidence that Staphylococci are the primary pathogen causing the infection.

Her general practitioner could use cephalexin because that has been an effective therapy for *Staphylococcus aureus* and *Streptococcus pyogenes* for many years. However, *Staphylococcus aureus* may be resistant to cephalexin. MRSA strains are resistant to beta-lactam antibiotics like anti-staphylococcal penicillins (such as (flu)cloxacillin) and to most cephalosporins (such as cephalexin).

AST is necessary both to detect resistance such as presence of MRSA in which cephalexin would not be effective and also to determine if orally administered options such as clindamycin or trimethoprim-sulfamethoxazole are active.

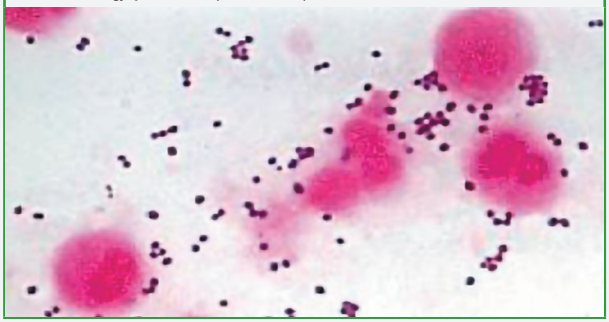
Outcome

The wound swab grew a pure growth of *Staphylococcus aureus*, with resistance to cephalexin and clindamycin but susceptibility to trimethoprim-sulfamethoxazole. The general practitioner recalled the patient who had not improved after 48 hours of cephalexin therapy.

➔ **Based on the AST results, the general practitioner changed the therapy to trimethoprim-sulfamethoxazole with rapid resolution of the infection.**

Figure 2. Gram stain showing gram-positive cocci in clusters and pairs

Source: Pathology Queensland, reproduced with permission.



3 HOW DOES THE CLINICAL LABORATORY SUPPORT ANTIMICROBIAL SELECTION?

To determine the most appropriate antimicrobial for the treatment of a specific clinical infection, isolation and identification (ID) of the suspected pathogen is required followed by performance and interpretation of a range of antimicrobial susceptibility tests (AST).

Clinical samples for ID/AST testing, including positive blood cultures, are routinely processed using traditional *in vitro* **selective and non-selective agar media**, inoculated and incubated aerobically/anaerobically for 24-48 hours. Potential pathogens are identified to the species level and conventional AST is then performed. After a further incubation period of up to 18 hours or more, the AST report is issued. Therefore, AST results may not be available until up to 72 hours post-initiation of empiric therapy.

However, a number of laboratory tests can provide **important preliminary information** to support the selection of the most appropriate antimicrobial therapy until conventional AST results are available. These tests include:

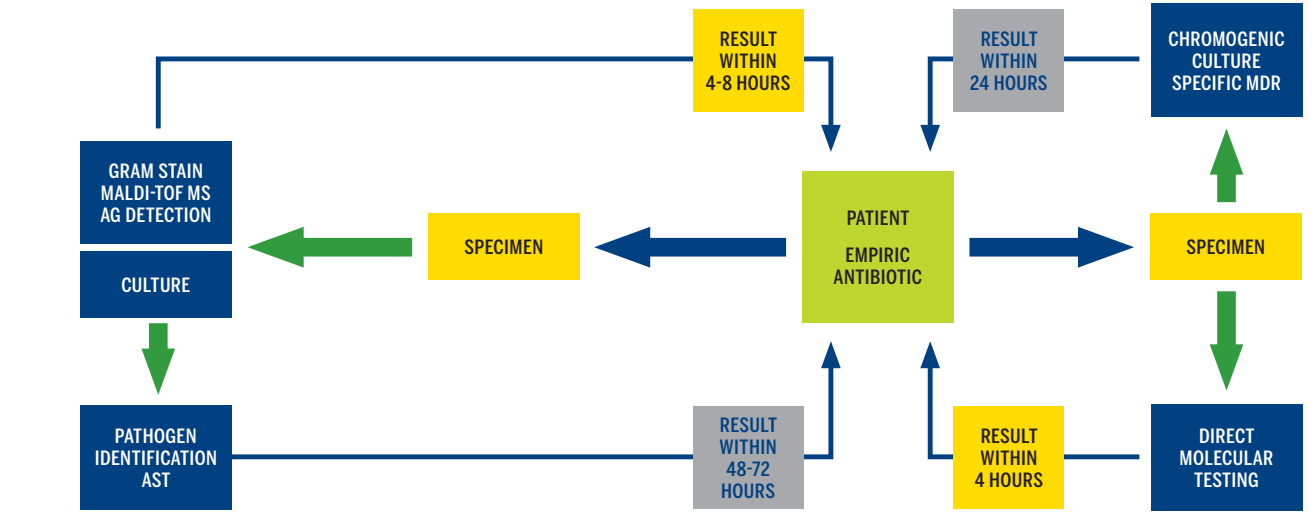
- **Microscopic examination (Gram stain)**: provides information about the types of microorganisms that may be present. The choice of empiric antibiotics will be different depending on the site of infection and whether gram-positive or gram-negative bacteria are seen.
- **Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)**: allows accurate organism identification to the species level within minutes compared to hours using conventional technology.
- **Antigen detection kits**: for direct detection of a specific microorganism in a clinical sample in less than 1 hour (e.g. urinary antigen detection kits for *Legionella pneumophila* and *Streptococcus pneumoniae*).
- **Rapid molecular methods**: for direct detection of **certain organisms and antimicrobial resistance genes** within 1 to 4 hours.
- **Chromogenic culture media**: for direct detection of multi-drug resistant organisms in clinical samples within 18-24 hours.

Figure 3 shows the various laboratory steps in the processing of a clinical specimen for isolation, identification and AST of bacterial pathogens with indication of turn-around times for the generation of clinically useful information that can impact antimicrobial selection.

Rapid laboratory tests such as microscopic appearance, organism identification, detection of specific microbial antigens and antimicrobial resistance genes can support the antibiotic selection process before AST results are available.

Figure 3. Laboratory processing of clinical samples

Source: Pathology Queensland, reproduced with permission



4 WHAT ARE THE TYPES AND MECHANISMS OF ANTIMICROBIAL RESISTANCE?

All antimicrobials have a known **clinical spectrum of activity** that is determined during the initial clinical studies prior to the release of the antimicrobial for clinical use. The product information for antimicrobial agents often lists the genus/species occurring in clinical trials that are susceptible to the specific antimicrobial. However, susceptibility may change over time with the development of resistance against the antimicrobial following its clinical use.

Conversely, organisms against which the antimicrobial has no activity are not included in the list of susceptible organisms. The lack of activity may be due to the presence of **intrinsic resistance mechanisms**.

4.1. WHAT IS INTRINSIC ANTIMICROBIAL RESISTANCE?

Intrinsic (or "natural") resistance genes form part of the genome in all strains belonging to a particular species. AST directed at the detection of this resistance is generally unnecessary. However, **failure to detect resistance** in this group when expected should prompt repeated testing, particularly to confirm the organism identification. Reporting of intrinsic resistance is variable, with some laboratories reporting all agents affected as "R" despite how they "test", others suppressing result reporting, or yet others reporting "as tested" and adding comments, e.g. natural resistance of *Pseudomonas aeruginosa* to beta-lactams due to the presence of beta-lactamases.

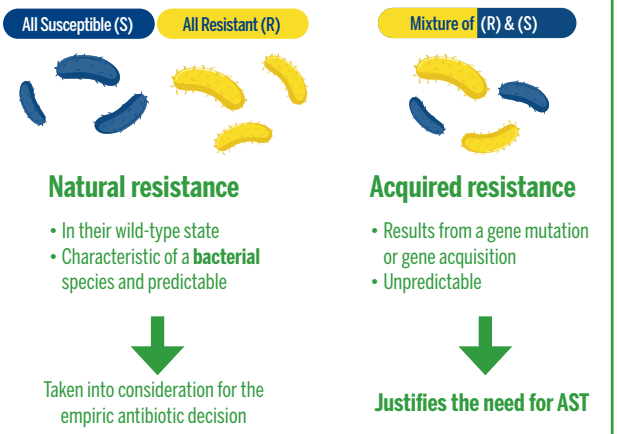
4.2. WHAT IS ACQUIRED ANTIMICROBIAL RESISTANCE?

A more common form of antimicrobial resistance (AMR) occurs via **gene acquisition or gene mutation** where a normally susceptible strain becomes resistant to a particular antimicrobial after initially being susceptible.

➔ **For example**, the acquisition of genes encoding extended-spectrum beta-lactamases (ESBLs, such as CTX-M) by *Klebsiella pneumoniae* or *Escherichia coli*. The ESBLs typically cause resistance to penicillins, first, second and third generation cephalosporins and monobactams.

Figure 4. Intrinsic (Natural) resistance versus Acquired resistance

Source: bioMérieux



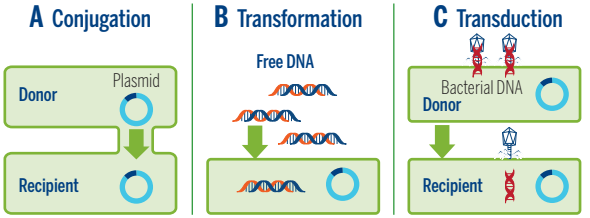
4.3. WHAT ARE THE MAIN MECHANISMS OF ANTIMICROBIAL RESISTANCE ACQUISITION?

Horizontal gene transfer (gene acquisition)

1. **Conjugation** is the main mechanism of horizontal gene transfer. It occurs when genes encoding antibiotic resistance are acquired from other bacteria, usually via plasmids – circular elements of DNA that can be transferred **not just to the same species but also to other genera**. Resistance to almost all classes of antimicrobials can be transferred on plasmids.
2. **Transformation** occurs via direct adsorption of DNA from the external environment. This mechanism is most prevalent in *Streptococcus pneumoniae* with respect to acquisition of beta-lactam resistance genes.
3. **Transduction** occurs where DNA encoding resistance genes (often small plasmids) are incorporated into bacteriophage heads. New cells acquire the DNA through direct injection of genetic material via phage binding. This mechanism is most prevalent in those species with high rates of lysogeny such as staphylococci.

Figure 5. The three mechanisms of horizontal gene acquisition[2]

Reproduced from Liu Y, *et al*. *Microorganisms* 2020;8(8):1211. CC BY 4.0

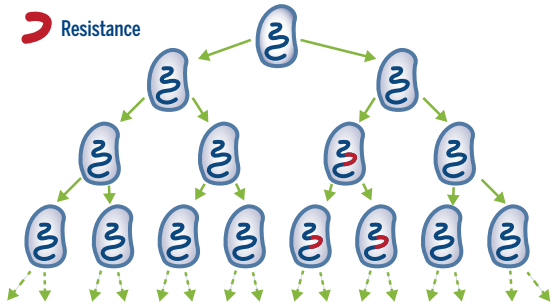


Vertical gene transfer (and gene mutation)

Unlike plasmid acquisition of genes, gene mutation only allows vertical gene transfer **within the population from parent to daughter cells during replication. Spontaneous gene mutations** on the bacterial chromosome can also confer resistance to antimicrobials.

Figure 6. Vertical gene transmission

Source: bioMérieux

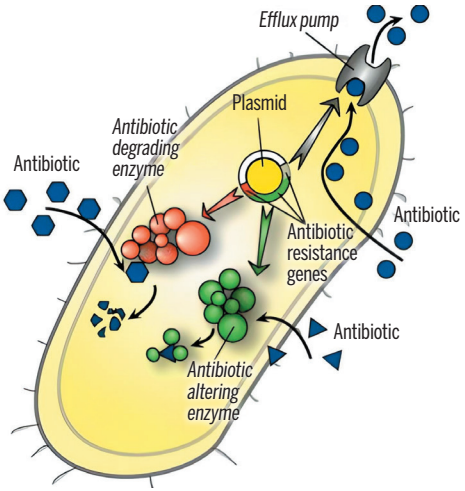


Five main mechanisms of resistance against antimicrobials

- **Enzymatic degradation** of the agent, as in beta-lactamase.
- **Enzymatic alteration** of a side chain of the antimicrobial, as seen with phosphorylation of aminoglycoside antimicrobials.
- **Changes to the structure of outer membrane porins** to restrict entry into the cell (e.g. carbapenem resistance in *Pseudomonas* spp.).
- **Up-regulation of efflux pumps** to remove antimicrobials from inside the cell (e.g. fluoroquinolones, macrolides and other antimicrobial classes as seen in gram-positive organisms including *Streptococcus* spp.).
- **Target site modification**, whereby bacteria alter the antibiotic's binding site, reducing the drug's effectiveness (e.g. *Streptococcus pneumoniae* modifies its penicillin-binding proteins to penicillin).

Figure 7. Three mechanisms of action against antimicrobial encoded by resistance genes[3]

Reproduced with permission from Todars Online Textbook of Bacteriology
https://textbookofbacteriology.net/resantimicrobial_3.html



1. Efflux pumps are high-affinity reverse transport systems located in the membrane that transport the antibiotic out of the cell. This is the mechanism of resistance to tetracycline.

2. Enzymatic alteration of an antibiotic in a way that it loses its activity. In the case of streptomycin, the antibiotic is chemically modified so that it will no longer bind to the ribosome to block protein synthesis.

3. Enzymatic degradation of an antibiotic, thereby inactivating it. For example, the penicillinases are a group of beta-lactamase enzymes that cleave the beta-lactam ring of the penicillin molecule.

Intrinsic antimicrobial resistance is a stable part of the genome in all strains belonging to a particular species. Antimicrobial resistance may also be acquired from other bacteria, usually via plasmids.

Antimicrobial resistance mechanisms include enzymatic degradation, efflux pump to remove antimicrobials and outer membrane porin changes to restrict antimicrobial penetration of the bacterial cells.

AST

BASIC CONCEPTS AND METHODS

1 WHAT ARE THE BASIC CONCEPTS OF AST?

1.1. MINIMUM INHIBITORY CONCENTRATION (MIC)

The basic unit of measure for AST is the MIC or Minimal Inhibitory Concentration.
It is defined as the lowest concentration of an antibiotic that is required to inhibit visible *in vitro* microbial growth after overnight incubation.[4]

MICs are used to measure the susceptibility of a pathogen to an antimicrobial to aid in the selection of the most appropriate antimicrobial therapy.

- A **low MIC** indicates susceptibility to low concentrations of the antimicrobial.
- A **high MIC** indicates potential resistance to the antimicrobial.

To determine the MIC using the Broth Microdilution method (BMD), a series of doubling dilutions of the antibiotic in a chemically defined broth (e.g. cation-adjusted Mueller-Hinton broth [MHB]) is inoculated with a standard dilution of the test organism. After incubation at 35°C for 18-24 hours, the broth is examined for turbidity as an indicator of growth or resistance to the antibiotic.[5] **The MIC is the first concentration that shows no turbidity or growth (Figure 8)**.

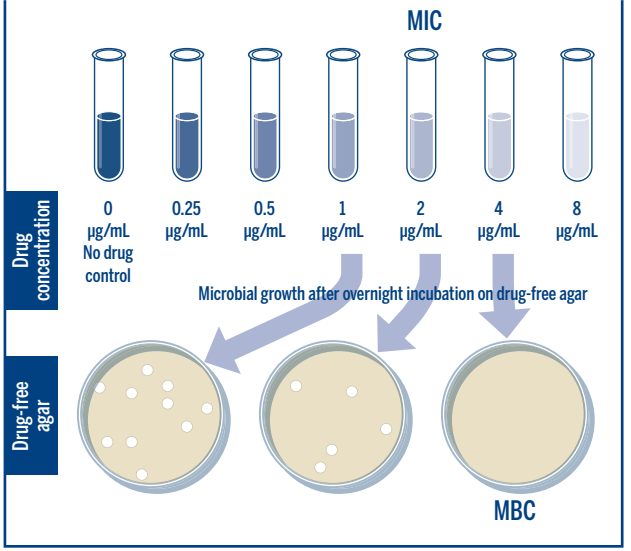
In addition to the MIC, the **Minimum Bactericidal Concentration (MBC)** can also be determined by subculturing each tube in the dilution series onto non-selective agar and examining for microbial growth after overnight incubation. The MBC is defined as the lowest concentration of an antibiotic required to inhibit microbial growth after subculture onto antibiotic-free media[4] (i.e. the minimum concentration required to kill a specific bacterium). Only the **BMD methods** allow both the MIC and MBC of an antimicrobial to be measured in a single test format **(Figure 8)**.

The relationship of MIC to MBC is a reflection of the different modes of antimicrobial activity.

- **For bactericidal antibiotics (e.g. beta-lactams)**, the MIC and MBC will be the same or within one doubling dilution.
- **For bacteriostatic agents (e.g. macrolides)**, the MIC will be lower than the MBC indicating that the antibiotic needs to be given at higher doses to affect microbial viability.

Figure 8. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)[6]

Adapted from Microbe Online



The aim of susceptibility testing and MIC measurement is to predict the likely success or failure of a chosen therapy and determine effective antibiotic dosing.

1.2. CLINICAL BREAKPOINTS

The **exact MIC is not routinely required** to manage the majority of infections except for certain clinical conditions (e.g. meningitis and endocarditis), where precise dosing is required to achieve therapeutic levels, or in the case of multi-drug resistant organisms where the antimicrobial choices are limited. Usually, clinicians only need to know whether the MIC is low enough to render the bacterium susceptible to the antibiotic or high enough to indicate microbial resistance and therefore therapeutic failure, and this is determined using **clinical breakpoints**.

> **Breakpoints are MIC values or zone diameters* that allow the organism to be categorized as susceptible (S), susceptible dose dependent (SDD), intermediate (I), resistant (R) or non-susceptible (NS) to a particular antibiotic.**
>
> **Breakpoints vary according not only to the antimicrobial but also to the organism.**

* A zone diameter refers to the inhibition zone, i.e. the area around an antibiotic disc on an agar plate where bacterial growth has been suppressed (for more details see section 2.1.4).

International organizations such as the Clinical & Laboratory Standards Institute (CLSI)[5] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[7], as well as national organizations such as the U.S. FDA[8], are responsible for the development of **breakpoint tables** that define susceptible and resistant MICs for antibiotics against a range of commonly encountered bacteria of medical importance.

How are breakpoints determined?

To determine clinical breakpoints for a given bug/drug combination, various data are combined:

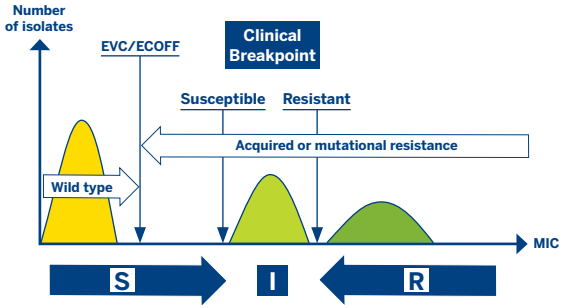
- analysis of **MIC distributions** for a large number of strains of the same species against a single antibiotic. This will include organisms with no known resistance as well as those for which the resistance mechanism is known,
- the **epidemiological cut-off value (ECV for CLSI or ECOFF for EUCAST)** that segregates natural resistance versus acquired resistance,
- **pharmacokinetic** (how the drug moves through the body) and **pharmacodynamic** (how the drug affects the bacteria) data,
- **clinical outcome data** from patients treated for the same drug/bug combination. This helps to ensure that the breakpoints are based on actual therapeutic success rates and not just *in vitro* susceptibility.

Where there are clear differences in the distribution between the two groups then the **susceptible (S)** and **resistant (R)** breakpoints can be set. The breakpoint MIC is the MIC that separates sensitive and resistant strains **(Figure 9)**. Isolates with no known mechanisms that fall between the two lie in the **intermediate (I)** zone.

Another attribute of the breakpoint is correspondence to achievable serum drug concentrations using standard human doses (except for select urine, central nervous system, cerebro-spinal fluid breakpoints).[9]

Where breakpoints are not clear cut, **ECVs/ECOFFs** offer an alternative, until more exact clinical breakpoints can be established. Lack of breakpoints is particularly problematic when the clinician needs to treat with a non-calibrated antimicrobial. The ECV/ECOFF value provides some guidance as to the potential for eradication based on the MIC alone.

Figure 9. Clinical breakpoints and epidemiological cut-off[10]



In certain cases, **breakpoints cannot be set**:

- where they divide a "wild type" distribution,
- where the organism is known to be a poor target for that antibiotic,
- where there is insufficient clinical evidence that the organism is a good target for that antibiotic.

Although CLSI and EUCAST use the same definitions for Susceptible and Resistant, the definition for the Intermediate category is less clear-cut as shown in **Table 1** (page 24).

Table 1. EUCAST and CLSI breakpoint definitions[5,7,11,12]

Adapted from Clinical and Laboratory Standards Institute: Performance standard for Antimicrobial Susceptibility Testing, M100-S34, 2024; EUCAST Breakpoint tables for interpretation of MICs and zone diameters, version 13.1, valid from 2023-06-29: **https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.1_Breakpoint_Tables**; EUCAST New Definitions of S, I and R from 2019: **https://www.eucast.org/newsiand**

EUCAST	CLSI
S - Susceptible, standard dosing regimen: *A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.*	
R - Resistant: *A microorganism is categorised as «Resistant» when there is a high likelihood of therapeutic failure even when there is increased exposure.*	
I - Susceptible, increased exposure*: a microorganism is categorised as «Susceptible, increased exposure*» when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection. *Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection	**I - Intermediate:** a buffer zone for technical uncertainty. **I^ - Intermediate category** was added to highlight those antimicrobial agents that concentrate in urine and the likelihood of treatment success when the agent is prescribed for uncomplicated urinary tract infections. **SDD - Susceptible dose-dependent:** use of a high dosage to treat the site of infection, resulting in higher antibiotic exposure and likelihood of clinical efficacy.
	NS - Non-susceptible: a category used for isolates for which only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains. Isolates with MICs above the susceptible breakpoint are reported as non susceptible.
ATU - Area of Technical Uncertainty: uncertain interpretation of antimicrobial susceptibility testing (AST) results.	

From a clinical point of view, the susceptible and resistant categories can be clearly understood, but reporting of the intermediate category has resulted in clinicians avoiding use of these antibiotics. This prompted EUCAST to amend its intermediate category definition to encourage use of these agents, in order to limit the use of more broad-spectrum agents against a background of increasing multi-drug resistance.[11] Furthermore, in 2019, EUCAST also added a new category for **Area of Technical Uncertainty (ATU)**.[12]

For newer antimicrobial agents or organisms such as *Streptococcus pyogenes*, for which no resistance to penicillin has been described, CLSI recommends that the results be interpreted as S for susceptible or NS for non-susceptible.

Both EUCAST and CLSI provide information relating to the dosing regimens to which their breakpoints are calibrated. EUCAST has added meningitis MIC breakpoints for some drug/bug combinations to ensure accurate reporting by laboratories.[7]

Thus, when interpreting AST results from the laboratory, **clinicians need to be aware of which standard AST method is used by their local microbiology laboratory**.

While most laboratories will standardize on either CLSI or EUCAST breakpoints for susceptibility testing, many will use a combination of both to cover reporting for a wider range of organisms that may not be included in the guideline they normally follow.

N.B. In the US, laboratories may also use FDA breakpoints, and may not be able to use CLSI or EUCAST breakpoints if they are not FDA-cleared on their systems.

- **Breakpoints that allow the organism to be categorized as susceptible, susceptible dose dependent, intermediate, resistant or non-susceptible to a particular antibiotic form the basis of all laboratory AST methods and reporting.**
- **Determination and publication of clinical breakpoints is largely the responsibility of international organizations such as CLSI, EUCAST and the FDA, though local/country-specific guidance may also exist.**

CASE STUDY 2.

A 65-year-old man develops headache and neck stiffness. The emergency room doctor recognizes this as possible meningitis and therefore performs a lumbar puncture. The cerebrospinal fluid has 450 white blood cells per microliter, 95% of which are neutrophils. Gram-positive cocci are seen in the Gram stain.

Why is AST important in this case?

Meningitis is a life-threatening infection. The Gram stain shows numerous neutrophils and gram-positive cocci in pairs or short chains, more characteristic of *Streptococcus pneumoniae*, so bacterial meningitis is likely. High doses of intravenous penicillin or ceftriaxone are the antibiotics of choice for pneumococcal meningitis. However, resistance to penicillin and/or ceftriaxone may occur.

Antibiotics may not penetrate the blood-brain barrier very well so it is important to assist in the optimization of therapy by not just performing disc susceptibility but also performing MICs for penicillin and ceftriaxone. Different breakpoints are applied for susceptibility when *Streptococcus pneumoniae* causes meningitis as compared to when it causes pneumonia. Penicillin MIC results are high **(Figure 10)**. MICs above 0.06 mg/L are interpreted as resistant in cases of meningitis.

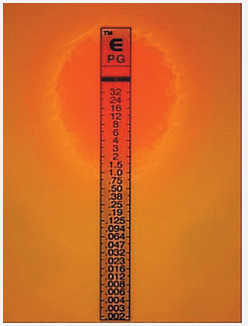
Outcome

The microbiologist calls the intensive care unit doctor caring for the patient and interprets the MIC values - taking care to use the breakpoints for penicillin and ceftriaxone for *Streptococcus pneumoniae* causing meningitis.

➔ **As a result of applying the appropriate breakpoints, the patient is treated with high-dose ceftriaxone and eventually makes a full recovery.**

Figure 10. MIC to penicillin between 1 and 2 mg/L

Source: bioMérieux



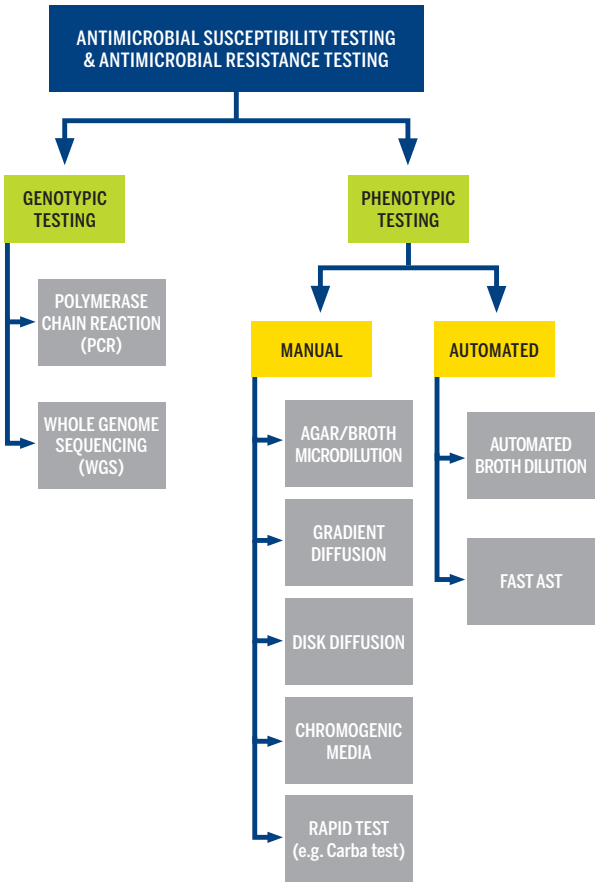
2 WHAT AST METHODS ARE USED IN THE LABORATORY?

A variety of diagnostic methods are available in the laboratory to determine both antimicrobial susceptibility (AST) and antimicrobial resistance (AMR) detection **(Figure 11)**.

- **AST methods are based on detection of the microbial phenotype.**
- **AMR methods are largely based on detection of the genotype.**

Figure 11. Overview of laboratory methods available for antimicrobial susceptibility and antimicrobial resistance testing

Source: bioMérieux



2.1. PHENOTYPIC METHODS - MANUAL

2.1.1 Broth Microdilution (BMD)

Broth dilution techniques are the most widely used AST methods in both Reference Laboratories (e.g. EUCAST, CLSI)[7,13] and in the routine clinical laboratory, because they allow for determination of the MIC.

The original **broth macrodilution tube technique** used as the reference method for routine MIC determinations was tube-based, but for improved ease-of-use has now been miniaturized into the 96-well microtiter plate format (**broth microdilution BMD**). This allows multiple antibiotics to be tested on each row of the plate (e.g. 12 antibiotic MICs using 8-fold dilutions per plate). A number of automated microtiter-based BMD formats are commercialized, which reduce processing and reporting times (see **section 2.2**). However, whilst these provide standardized, ready-to-use panels for AST, laboratories are limited to the range of antibiotics that are included on each panel type.

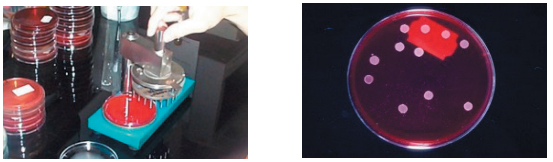
2.1.2 Agar Dilution Methods

Similar to the broth microdilution methods, **agar dilution** involves the addition of antimicrobial dilutions to the AST agar medium (usually **Mueller Hinton agar MHA**). Standardized bacterial inocula are then applied to the agar plate using multipoint application techniques. This test format allows 30-100 organisms to be tested on each agar depending on the plate dimensions **(Figure 12)**. After overnight incubation, plates are examined for the presence of growth. The MIC is determined from the agar plate showing the lowest concentration of antimicrobial that inhibits growth.

While these methods allow multiple organisms to be tested at the same time against a single antimicrobial agent or generation of data that can be used to look at MIC distribution within a population of organisms belonging to the same genus/species, they remain manual and are therefore not widely used today. However, both CLSI and EUCAST recommend use of this technique for testing a limited range of antimicrobials (e.g. fosfomycin MIC breakpoints in *Escherichia coli* are calibrated using agar dilution or with anaerobes using fastidious anaerobe agar (FAA MH) as defined by EUCAST).[14]

Figure 12. Agar dilution techniques

Source: Pathology Queensland, used with permission



2.1.3 MIC Gradient Diffusion Method

The **MIC gradient diffusion method** utilizes both antibiotic dilution and agar diffusion technique in a combined assay to measure the MIC of a clinical isolate to a given antimicrobial. A plastic/paper strip with a single antibiotic at increasing concentration is applied to one side of the strip with a corresponding concentration scale on the upper surface **(Figure 13)**. After overnight incubation, the MIC is determined at the point of intersection between the zone of bacterial inhibition and the MIC scale on the strip. Where the intersection falls between a set of MIC values, the MIC endpoint is reported as the higher of the two. Multiple strips can be tested on a single agar plate depending on size but the cost per strip generally precludes laboratories from testing more than 2-3 antimicrobials per isolate. Commercially available strips include **ETEST® (bioMérieux)** and **MTS (Liofilchem®)**.

Specialized antimicrobial combination strips have been developed to allow detection of specific antimicrobial resistance phenotypes including ESBLs, ampC and carbapenemase production in gram-negative bacteria[15] **(Table 2)**.

These dual strips have a single antimicrobial at one end with the same antimicrobial plus an enzymatic inhibitor at the other end. A positive result is indicated when the MIC of the antimicrobial inhibitor combination is equal to or greater than the antibiotic on its own.

Table 2. Antibiotic and beta-lactamase inhibitor combinations used in MIC gradient diffusion strips (ETEST®) to detect beta-lactamases responsible for antibiotic resistance

Source: bioMérieux

Resistance mechanism	Antibiotic/Antibiotic Inhibitor combination
Extended spectrum beta-lactamases (ESBL)	Cefotaxime / cefotaxime clavulanate Ceftazidime / ceftazidime clavulanate Cefepime / cefepime clavulanate
AmpC cephalosporinase	Cefotetan / cefotetan cloxacillin
Metallo beta-lactamases	Imipenem / Imipenem EDTA Meropenem / Meropenem EDTA

2.1.4 Disc Diffusion

Disc diffusion is an alternative AST method to MIC that uses **Mueller-Hinton agar (MHA)**, the international standard medium for AST. For fastidious organisms that require additional growth factors, supplementation of MHA with blood is required.[15,16] The disc diffusion technique involves placing an antibiotic impregnated disc with a single concentration of the antibiotic onto MHA inoculated with a lawn of the test isolate. Multiple antibiotic discs can be tested on the same agar plate making this a highly inexpensive and flexible test method **(Figure 14)**. After overnight incubation, zones of growth inhibition are measured. Susceptible/Resistant zone diameters are determined based on zone diameters from Disc/MIC calibrations published by Reference Laboratories (i.e. CLSI/EUCAST).[5,7]

2.1.5 Chromogenic Agar

Microbiology laboratories routinely incorporate **chromogenic media** into their routine testing protocols for the detection of bacteria that possess specific antimicrobial resistance mechanisms **(Figure 15)**. The advantage of chromogenic media containing selective agents is that clinical specimens can be plated directly onto the media allowing **detection of significant resistant phenotypes such as MRSA, VRE, ESBLs and CPE within 24 hours**.[16] Although growth on chromogenic media does not replace the need for routine AST on isolates, the ability to rapidly detect resistance within 24 hours is significant for Infection Control allowing **early identification of carrier states and isolation of positive patients**, and thereby restricting potential spread of AMR to other patients.

Figure 13. Gradient diffusion method

Source: bioMérieux

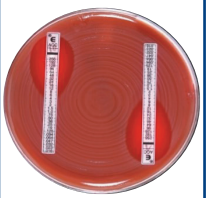
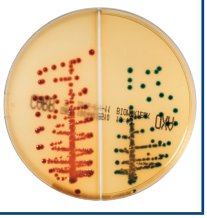


Figure 14. Disk diffusion technique

Source: Queensland Pathology, used with permission

Figure 15. Chromogenic media

Source: bioMérieux



2.2. PHENOTYPIC METHODS - SEMI/FULLY AUTOMATED SYSTEMS

Automated systems for bacterial identification and AST, based on the broth microdilution (BMD) technique, are now widely used in most routine clinical microbiology laboratories, not only to reduce the time to reporting of susceptibility test data, but also to increase efficiency by allowing standardization of the test process.

For the routine microbiology laboratory, testing MICs around the susceptible and resistant breakpoints is more cost-effective than full microbroth dilution, allowing a wider range of antimicrobials to be tested against all isolates. By miniaturizing the test format and utilizing optical arrays to detect subtle changes in growth/color of test panel wells, automated systems such as the **VITEK® 2 (bioMérieux), Phoenix™ (BD), Microscan WalkAwayplus (Beckman Coulter)** and **Sensititre™ (ThermoFisher)** utilize rapid AST technology on defined AST panels to provide susceptibility results between **4 and 18 hours (Figures 16 and 17)**.[16,17]

In addition to streamlining the AST workflow, automated systems also **facilitate an improved data management process**. MICs and interpreted test results can be directly interfaced to the Laboratory Information System (LIS), reducing technical time required for data entry and thus reducing the rate of transcription errors. Additionally, computerized data capture within these systems allows the storage and retrieval of cumulative AST data which can then be linked to local antibiogram and antibiotic stewardship programs.

Whilst panel customization is possible, the number of antibiotics that can be included on each panel is limited. For high throughput laboratories, separate urine and non-urine panels can be used to offset the limited range of antimicrobials per panel. Additionally, when new antimicrobials become available it may be 3 to 5 years before these are available for routine use on the automated systems. In this situation, where the new agents are not available for inclusion on extended AST panels for testing of multi-drug resistant (MDR) gram-negatives, laboratories will augment their automated systems with other methods such as gradient diffusion assays to provide susceptibility data for newer agents.

Figure 16. VITEK® 2 instrument and ID/AST cards

Source: bioMérieux image library

Figure 17. BD Phoenix™ M50 instrument and panels

Used with permission from BD

2.3. PHENOTYPIC METHODS - FAST AST

Antimicrobial susceptibility testing (AST) of bloodstream isolates is critical for optimal management of patients with bacterial infections, and in particular, with sepsis.

While traditional AST results are available only after 48–72 hours, new techniques that enable **rapid AST to be performed directly on positive blood cultures reduce the time-to-result to 4-8 hours** and have significant potential to improve the clinical outcomes for patients with sepsis.[18-20]

2.3.1 Rapid Disc AST

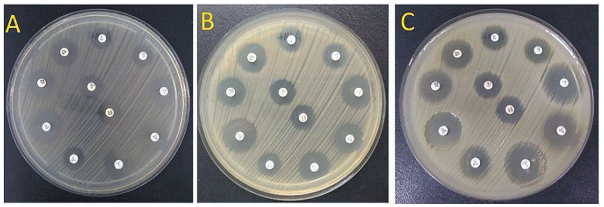
In an effort to further reduce the time from initiation of empiric therapy to AST result, many laboratories perform **direct disc susceptibility testing on positive blood culture broths**. As the ability to control the bacterial inoculum is not possible, the reliability and reproducibility of susceptibility results is variable, however the ability to detect resistance has been shown to have a positive clinical impact when a change in therapy is indicated.

EUCAST has recently developed a **Rapid Antimicrobial Susceptibility Test (RAST)** directly from positive blood cultures against a range of selected antibiotic discs **(Figure 18)**.[21] **Interpretation of susceptibility results is possible within 4-8 hours** reducing the reporting from the usual 18 hours.

However, it is the **direct detection of antimicrobial resistance in positive clinical samples within 1-2 hours** that will provide the greatest impact in the area of RAST.

Figure 18. Rapid Antimicrobial Susceptibility Test (RAST). Readings at 4 (A), 6 (B) and 18 h (C) of the disk diffusion testing directly from blood culture bottles[22]

Reproduced with permission from Martins A, *et al. J Glob Antimicrob Resist.* 2020:22:637–642.



2.3.2 Automated Fast AST

A number of recent developments are providing laboratories with automated options for fast AST results. These have been most frequently developed for use on positive blood culture broths.

One such example **(VITEK® REVEAL™, bioMérieux)** uses **colorimetric sensors** to detect volatile compounds released by bacteria as they grow **(Figure 19)**. By regularly monitoring color changes in the sensors, which correlate with microbial growth in different concentrations of antibiotics, **a MIC can be rapidly determined within 6 hours on average**.[19]

Other methods, for example, **ACCELERATE PHENO® (Accelerate Diagnostics, Inc.)** assess growth and cell morphology changes in the presence or absence of antibiotics using **live-cell imaging** and in this way can also **rapidly determine an MIC from positive blood culture broths (Figure 20)**.[23] Other commercialized Fast AST systems include dRAST™ (Quantamatrix), ASTar® (Q-Linea) and QuickMIC® (Gradientech).

Other technologies are in development which could rapidly perform AST from respiratory samples in patients with pneumonia.

Figure 19. VITEK® REVEAL™

Source: bioMérieux

Figure 20. ACCELERATE PHENO® system

Used with permission from Accelerate Diagnostics, Inc.

2.4. DETECTION OF ANTIMICROBIAL RESISTANCE GENES OR MECHANISMS

Unlike traditional antimicrobial susceptibility methods described above that detect the antimicrobial phenotype, **antimicrobial resistance testing** is based upon the detection of **antimicrobial resistance genes or mechanisms** within the microbial genome.

From a clinical perspective:

- the **phenotypic susceptibility profile** allows the clinician to select the **appropriate antibiotic dosage for treatment**,
- whereas **detection of the antimicrobial resistance** provides guidance on **which antibiotic(s) should not be prescribed**.

Both techniques are useful and important for optimal patient management: while **genotypic methods** are often faster and **important for the first hours of patient management, phenotypic methods** are necessary to select the **long-term targeted therapy**.

2.4.1 Rapid methods for the detection of specific AMR mechanisms

A number of rapid methods are used in the laboratory to confirm the presence of specific resistance genes or mechanisms when these are phenotypically suggested by the AST profile.

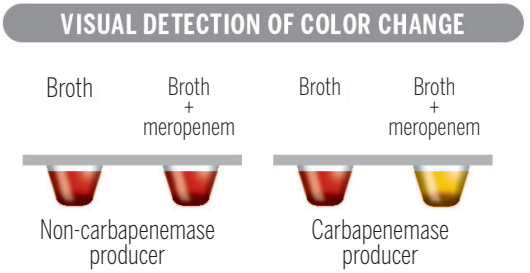
Enzymatic test reaction: antimicrobial resistance derived from enzymic breakdown of the antimicrobial can be detected using colorimetric detection tests.[16] These utilize enzyme substrates with chromogens attached that are released in the presence of the enzyme, resulting in a change in color of the test format.

➔ **For example**, beta-lactamase production in *Staphylococcus aureus*, *Neisseria gonorrhoeae* or *Haemophilus influenzae* can be detected within an hour on a test strip impregnated with a chromogenic cephalosporin.

➔ **Another example** is the the **RAPIDEC® CARBA NP (bioMérieux)** or **Carba-NP** test that utilize a color change to a pH indicator dye, due to acid production arising from carbapenemase degradation of meropenem in CPE **(Figure 21)**.

Figure 21. Example of colorimetric detection test

Source: bioMérieux (extracted from Carbapenem Resistance booklet)



Disc potentiation tests are also widely used for demonstrating the presence of enzymic resistance particularly when the resistance may only be weakly expressed. In these assays, the zones of growth inhibition are measured in the presence or absence of specific enzyme inducers or inhibitors.

➔ **For example**, inducible clindamycin resistance in *Streptococcus spp*. and *Staphylococcus spp*. can be detected by placing an erythromycin disc in close proximity to a clindamycin disc **(Figure 22)**.

Figure 22. Positive D-test for Inducible Clindamycin Resistance in *Staphylococcus aureus*[24]

Reproduced with permission from Prabhu K, Rao S, Rao V. *J Lab Physicians* 2011;3(1):25-27

Disc potentiation tests are also used to detect inducible resistance mechanisms. In this instance, one antimicrobial will induce increased production of a chromosomal enzyme resulting in a flattening of zones of growth inhibition to another antimicrobial at the interface between the two discs (D-test).

2.4.2 Molecular Antimicrobial Resistance Gene Detection

Most methods of detection of antimicrobial resistance genes use **polymerase chain reaction (PCR)**. With some sample types (e.g., respiratory samples, synovial fluid or cerebrospinal fluid), rapid molecular methods can detect certain antimicrobial resistance genes **directly from clinical specimens**. However, where bacterial burden is low (e.g., in blood), sufficient DNA is only available in incubated samples or from isolated colonies.

The **major advantage of rapid molecular tests for clinicians is speed**. However, PCR-based methods cannot indicate whether the gene detected is actually expressed in relevant amounts, nor can they typically link the gene detected and the organism from which it was detected. This is potentially relevant when the *mecA* gene is detected and it is unclear if it arose from *Staphylococcus aureus* or a coagulase-negative Staphylococcus.

In addition, with rapid molecular methods, **it is important to be able to "translate" the results into information that can be readily used by prescribers**. **Table 3** shows an example of how this could be utilized.

Table 3. Knowledge of the presence of beta-lactamase genes can be useful in determining which beta-lactam antibiotics could be used for therapy and which should be avoided[25]

Adapted from Wright H, Bonomo RA, Paterson DL. *Clin Microbiol Infect.* 2017;23(10):704-712

Resistance Gene	Antibiotics that should not be used	Antibiotics that can be used
CTX-M	Cefazolin, ceftriaxone, ceftazidime	Meropenem, ertapenem
KPC	Meropenem, piperacillin-tazobactam, ceftriaxone, cefazolin, ceftazidime	Ceftazidime-avibactam, imipenem-relebactam, meropenem-vaborbactam
OXA-48	Imipenem-relebactam, meropenem, piperacillin-tazobactam, ceftriaxone, cefazolin, ceftazidime	Ceftazidime-avibactam
NDM	Ceftazidime-avibactam, imipenem-relebactam, meropenem-vaborbactam, meropenem, piperacillin-tazobactam, ceftriaxone, cefazolin, ceftazidime	Ceftazidime-avibactam combined with aztreonam, cefiderocol, Aztreonam-avibactam (mainly in Europe)

As shown in **Table 3**, the beta-lactamase gene markers only allow "translation" to beta-lactam antibiotics that are potential substrates for the enzymes encoded. No comment can be specifically made about non-beta-lactam antibiotic classes such as polymyxins, fluoroquinolones, tetracyclines or aminoglycosides.

2.4.3 Whole Genome Sequencing

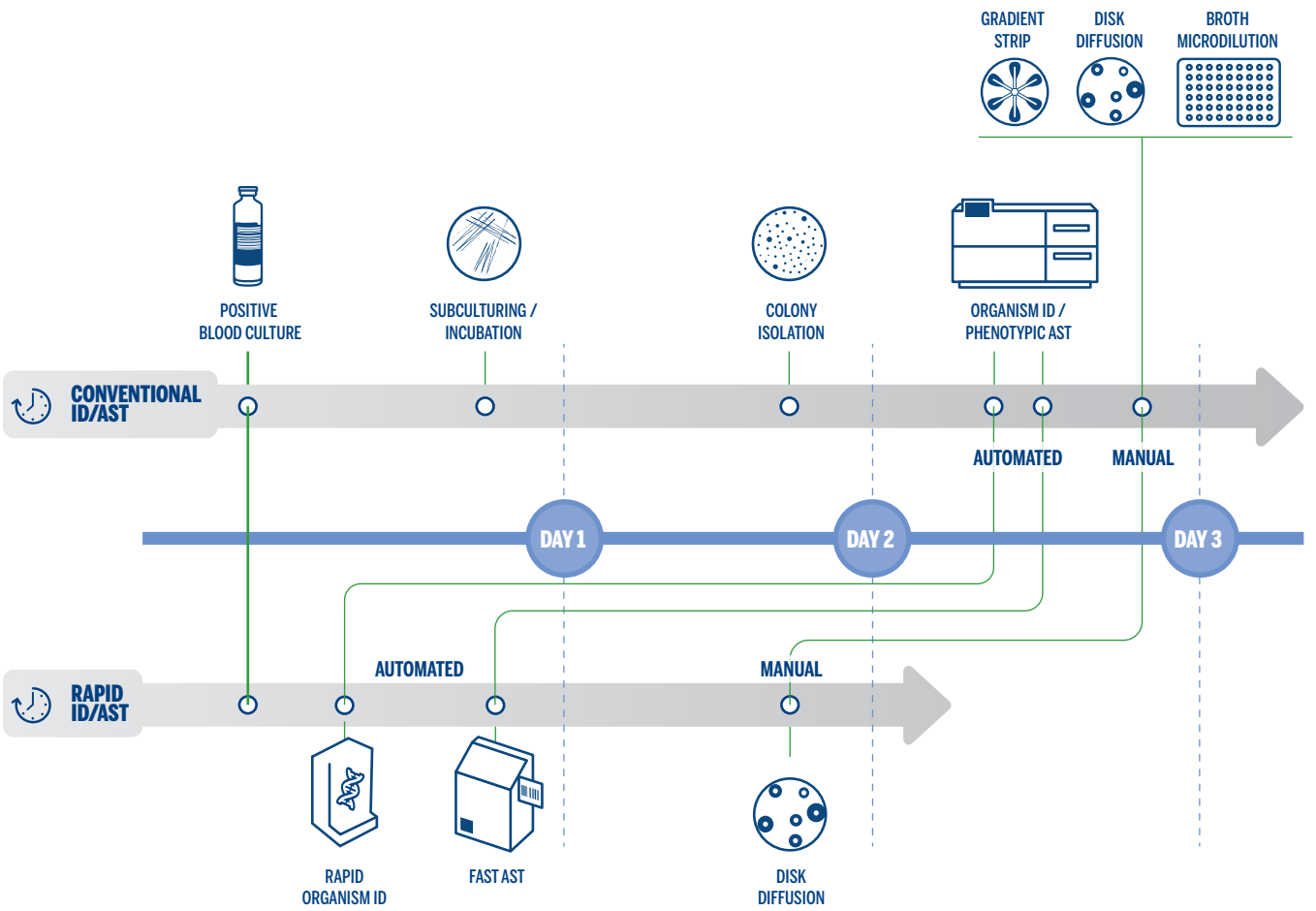
Whole genome sequencing holds the promise of providing much more information than is available from PCR-based methods.

➔ **For example**, organism identity, clonal relationship with other bacteria previously isolated from the same facility, presence of virulence genes and detection of the full range of resistance genes are all possible.

The cost of genome sequencing has also reduced dramatically compared to prior decades making it more attractive as a practical consideration. However, timeliness remains an issue given that specialized bioinformatic resources are still needed in most circumstances. Rapid, low-read sequencing is becoming closer to clinical utility, but issues remain, such as reproducibility and ability to detect low numbers of organisms, for example in patients with bloodstream infections.

Figure 23. Microbiology laboratory workflow and timeline from positive blood culture to bacterial identification (ID) and antimicrobial susceptibility testing (AST)[18,19,26]

Adapted from Banerjee R, *et al. Front Med*. 2021;8:63531; Tibbetts R, *et al. J Clin Microbiol*. 2022;60(6): e0009822; Wenzler EE, *et al. Pharmacotherapy* 2023;43(4):264-278



POST-AST

CLINICAL AND LABORATORY PERSPECTIVES

1 INTERPRETATION OF AST RESULTS AND REPORTS

Selection of antibiotics for testing and reporting

Each clinical laboratory needs to test antibiotics that are the most relevant to the range of clinical pathogens isolated, the site of infection and the local formulary of the healthcare facility.

CLSI produces tables of recommended antimicrobials to be tested against gram-positive and gram-negative pathogens as well as fastidious microorganisms **(Table 4)**.[5] Additionally, urine-only drugs such as nitrofurantoin, trimethoprim and fosfomycin are selectively applied only for AST testing of urinary isolates. Not all antibiotics listed as suitable for testing by CLSI will be tested. The selection of antibiotics will be determined by the antibiotics available locally for clinical use, the range of antimicrobials provided on commercial AST systems and antibiotic combinations that allow detection of AMR phenotypes of relevance to local Infection Control guidelines.

Each laboratory will have a **defined set of antimicrobials** that they test and report for all isolates, followed by a range of **second tier agents** that are reported if resistance to the initial set of agents is detected. This is known as **selective antibiotic reporting**.

➔ **Selective antibiotic reporting or suppression of AST results** is common practice (i.e. not all the antibiotics tested against a particular bacterial pathogen are reported). Only those antimicrobials relevant to the organism and the site of infection are reported. For fully susceptible microorganisms, only the first-line narrow-spectrum antibiotics will be reported. Where resistance is detected, additional second tier agents will then be released. The latter is often referred to as **cascade reporting** (e.g. where isolates are resistant to first/second generation cephalosporins, then third generation cephalosporins such as ceftriaxone or ceftazidime will be reported instead if they test susceptible).

Table 4. Example of CLSI testing recommendations for antimicrobial testing and reporting in *Staphylococcus* species[5]

Adapted from Clinical and Laboratory Standards Institute: Performance Standard for Antimicrobial Susceptibility Testing, M100-Ed 34, 2024, CLSI

Antimicrobials	Group A Test and Report routinely on all isolates	Group B Test and report selectively when resistant to same Antimicrobial class as Group A	Group C Test and report selectively for multidrug resistant strains	Group U Test and report for urine isolates only
Penicillin				
Flucloxacillin / Cefoxitin				
Erythromycin				
Clindamycin				
Trimethoprim sulphameth-oxazole				
Vancomycin				
Tetracycline				
Rifamicin				
Linezolid				
Daptomycin				
Ciprofloxacin				
Nitrofurantoin				
Trimethoprim				

> The aim of selective antimicrobial reporting is to ensure good antibiotic stewardship, whereby the broad-spectrum agents are reported only for more resistant microorganisms.

> Additionally, the range of antimicrobials reported should aim to include narrow-spectrum agents if susceptible, at least one oral and one intravenous option, and at least one agent for patients that may have penicillin allergy.

- **Comments** are frequently applied to reports to **guide the clinician in the interpretation of AST results**.
  This is particularly important where MIC results fall between susceptible and resistant reporting categories. Guidance in the interpretation of intermediate results is required particularly where the intermediate MIC reflects a susceptible increased exposure result as defined by EUCAST.
  The comment needs to indicate that this drug can potentially be used as long as adequate dosing regimens are applied and concentration at the site of infection is likely to occur.
- **Test-related comments** are also added to provide clarity around the potential accuracy of test results. This may be necessary when an MIC result is reported for a drug/bug combination for which neither EUCAST nor CLSI provide clinical breakpoints or in situations where the test method recommended in the standard is not available/used by the testing laboratory (e.g. agar dilution for fosfomycin testing).
- **Additional comments** that provide treatment information are included to assist the clinical team in ongoing case management (e.g. with *Staphylococcus aureus*, use of a single oral agent such as ciprofloxacin, rifampicin or fusidic acid may result in development of resistance).

Role of Expert Systems in AST Platforms

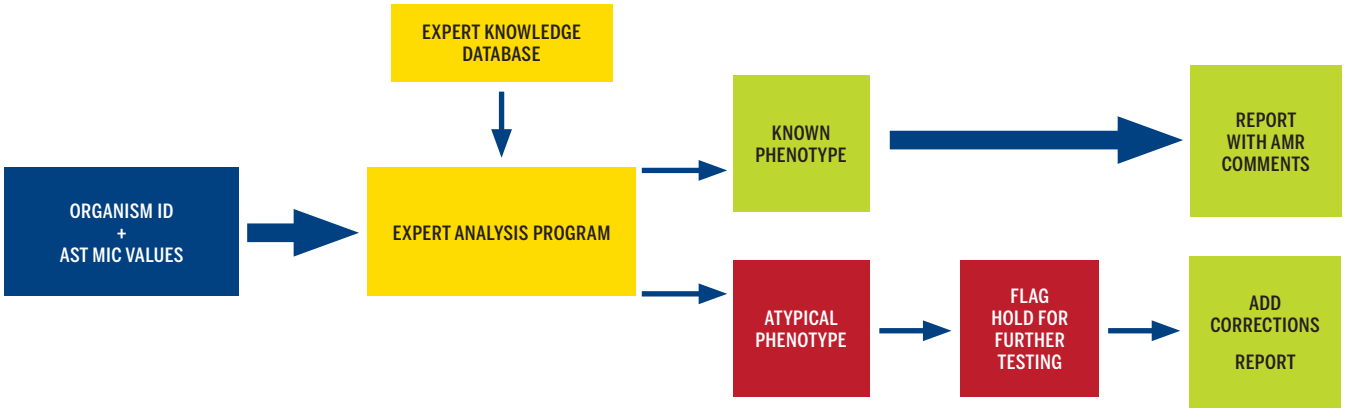
Manual scientific review of AST results prior to reporting is an essential quality activity for all clinical microbiology laboratories. This process can be labor intensive and subject to human error.

In addition to reduced turn-around times to AST results, systems such as the **VITEK® 2** and **Phoenix™** have **computerized Expert Systems** which can rapidly screen susceptibility profiles for typical and atypical results. This process involves the analysis of AST profiles against comprehensive databases consisting of phenotypes generated by microbes with known antimicrobial resistance mechanisms **(Figure 24)**. This allows the AST results for different organisms to be validated for accuracy ensuring that potential errors in test results, important resistance phenotypes or unusual MIC results are detected consistently.[27]

The **VITEK® 2 Advanced Expert System (AES)** has an extensive knowledge base that compares the AST MIC profile (phenotype) of the test isolate against a wide range of MIC distributions for different AMR phenotypes (over 3,000 individual profiles are available for screening). In this phenotype mapping process, MIC results within a specific class of antimicrobial (e.g. cephalosporins) are reviewed to screen for the presence of potential resistance mechanisms. AMR associated with different classes of antimicrobials (e.g. beta-lactams and aminoglycosides) can be detected within the same isolate. This process is referred to as **Biological Validation**.

Figure 24. Workflow of an Expert system in AST context

Source: Pathology Queensland, reproduced with permission



The Biological Validation process can also flag **unusual or impossible phenotypes**.

➔ **For example**, for *Staphylococcus aureus* that show resistance to the glycopeptide antimicrobial teicoplanin but susceptibility to vancomycin.

Additionally, Expert Systems contribute to the validation of AST results by identifying the need for **Therapeutic Corrections** where antimicrobial resistance for a given organism may be insufficiently expressed or incorrectly reported as susceptible.

➔ **For example**, the AES will recommend a change to MIC interpretation from susceptible to resistant where intrinsic resistance has not been detected to ensure consistency in reporting between organism identification and AST result.

e.g. *Enterobacter cloacae* where ampicillin is reported as susceptible when it should be resistant.

Both Biological Validation and Therapeutic Corrections can be applied to the phenotype of a single test isolate. The AES will recommend a change to MIC interpretations from susceptible to resistant where a specific resistance phenotype is detected.

➔ **For example**, ESBL-producing *Escherichia coli* where changes in the interpretation of the third generation cephalosporins including cefotaxime/ceftazidime from susceptible to resistant will be proposed even though the MICs may fall within the susceptible category.

For laboratories which do not have access to computerized Expert Systems, EUCAST has published a series of **Expert rules** that can be applied. These are divided into various categories based on intrinsic resistances by organism, exceptional phenotypes that require additional testing and interpretative rules that cover inferred resistance mechanisms from AST results.[28,29]

The ability of Expert Systems to accurately detect clinically relevant resistance mechanisms particularly beta-lactamase resistance (e.g. ESBL, carbapenemase) ensures not only that the appropriate antibiotic therapy can be prescribed, but also that **appropriate Infection Control** can be initiated via identification and isolation of patients to **minimize nosocomial spread**.

There are however limitations to Expert Systems. Of most significance is the quality of the knowledge databases used and the need to keep these up-to-date with newly and rapidly emerging resistance phenotypes. Additionally, clinically relevant antibiotics for the detection of specific resistance phenotypes may not be on the test panel and differentiation of phenotypes generated by mixed genotypes (e.g. ESBL plus ampC) may result in incorrect analysis.

Expert Systems ensure detection of typical and unusual AMR phenotypes.

Expert System flag results for technical review thereby facilitating the work required of skilled laboratory staff.

However, Expert System databases must be kept up-to-date with newly/rapidly emerging AMR mechanisms.

2 USE OF CUMULATIVE LABORATORY AST DATA – THE ANTIBIOGRAM

Cumulative antibiograms are useful tools for detecting and monitoring local trends in the prevalence of antimicrobial resistance. They help guide clinicians and pharmacists in selecting the most appropriate empiric antimicrobial treatment while pending microbiology culture and susceptibility results. They can be developed at hospital level, or for specific units (e.g. burn units) or specimen sources (e.g. urine cultures), where local organism epidemiology/resistance can differ significantly from the overall hospital data.

> **A cumulative antibiogram is a table summarizing the percent of individual bacterial pathogens susceptible to different antimicrobial agents for a specific setting and time period.**[30]

A cumulative antibiogram is guided by specific rules (CLSI M39[30] or local recommendations) including deduplication of data, minimum number of isolates, and can be filtered according to location, specimen or patient criteria.

One of the goals is to use antibiograms to guide empirical therapy of initial infections whether the causative organism is unknown or known but susceptibility is unknown.

Local cumulative antibiograms are used by antimicrobial stewardship programs to develop local empirical antimicrobial treatment guidelines and to guide decisions regarding empirical antimicrobial treatment and antimicrobial formulary.

➔ For example, a **hospital microbiology laboratory** may produce an antibiogram on an annual basis for the entire hospital or for a specific intensive care unit.

On the other hand, a **community-based microbiology laboratory** may produce an antibiogram for general practitioners comprising cumulative results from outpatient urine samples or from a specific nursing home.

Cumulative antibiograms can be a useful aid to prescribers, pharmacists and infection control teams, who can use this epidemiologic data to "rule out" certain antibiotics for certain conditions. This may change over time. The actual antibiotic prescribed to a patient should take into consideration individual factors such as allergies, renal function, pregnancy or breast-feeding and known colonization or prior infection with resistant organisms.

Antibiogram results with an "*" on the report should be interpreted with caution, particularly when insufficient data is available to allow accurate interpretation. Clinicians should be encouraged to discuss such results with the microbiologist to determine the optimal interpretation and appropriate antibiotic therapy.

Figure 25. Example of a cumulative antibiogram

Source: bioMérieux

ABC HOSPITAL ADULT INPATIENTS ANTIMICROBIAL SUSCEPTIBILITIES (%) 1st ISOLATE ONLY CALENDAR YEAR: 2021		Urine isolates															
		# urine isolates	Ampicillin	Amoxicillin/ Clavulanate	Nafcillin	Piperacillin/ Tazobactam	Cefazolin- Uncomplicated	Cefazolin- complicated	Ceftriaxone	Cefepime	Meropenem	Ciprofloxacin	Levofloxacin	Trimeth/Sulfa	Nitrofurantoin	Linezolid	Vancomycin
Gram-Positive	*Enterococcus faecalis*	168	100				0	0	0	0				0	100	100	99
	Enterococcus faecium	44	7				0	0	0	0				0	7	93	20
	Staphylococcus aureus	39	0		49		49	49						95	100	100	100
Gram-Negative	*Citrobacter species*	25*	0	0		85	0	0	82	100	100	100	100	93	70		
	Enterobacter cloacae	41	0	0		67	0	0	57	89	100	100	100	87	25		
	Escherichia coli	575	49	82		96	86	67	90	92	100	74	75	74	98		
	Klebsiella aerogenes	31	0	0		71	0	0	68	100	100	100	100	100	21		
	Klebsiella oxytoca	16*	0	80		73	13	13	80	87	100	93	100	87	67		
	Klebsiella pneumoniae	222	0	87		88	86	74	88	88	100	88	95	83	38		
	Proteus mirabilis	88	86	98		99	93	2	95	98	100	82	82	83	0		
	Pseudomonas aeruginosa	61	0	0		83	0	0	0	88	86	64	58	0	0		

≥ 90%
70-90%
≤ 70%
≤ 30 isolates

3 ANTIMICROBIAL STEWARDSHIP AND DIAGNOSTIC STEWARDSHIP

Antimicrobial stewardship (AMS) is now commonplace in recognition of its role in optimizing clinical outcomes related to use of antimicrobials.

While the core members of an antimicrobial stewardship team are typically physicians and pharmacists trained in infectious diseases, microbiologists play a key role in these programs.

Antimicrobial stewardship programs vary in their scope, but typically utilize institutional guidelines for antimicrobial use. The inclusion of antimicrobials in guidelines depends on a variety of factors such as clinical trial data, cost, cumulative susceptibilities (such as obtained from an antibiogram) and potential for "collateral damage" whether that be predilection for *Clostridioides difficile* infection or potential for selection of antimicrobial resistance.

Most antimicrobial stewardship programs undertake some review of antimicrobial prescriptions and correlate these with microbiology reports, including AST reports.

This may encourage prescribers to "streamline" antibiotic therapy from a broad-spectrum empiric choice to a targeted choice based on AST results.

➔ **For example**, vancomycin and piperacillin-tazobactam may have been commenced empirically for a seriously ill individual with sepsis, but when microbiology results show *Streptococcus pyogenes*, the antibiotic choice may be de-escalated to penicillin alone.

Conversely, antimicrobial stewardship review may show a need to escalate antibiotic therapy.

➔ **For example**, microbiology reports may reveal the presence of *Klebsiella pneumoniae* resistant to meropenem, necessitating a change in empiric therapy from meropenem to ceftazidime-avibactam.

Diagnostic stewardship is performed in the microbiology laboratory ("the right test") and is a complementary concept to antimicrobial stewardship on the clinical side ("the right interpretation").[31-33] Diagnostic stewardship promotes the judicious use of diagnostic tests to initiate appropriate antibiotic therapy, and aims to avoid the excessive use of broad-spectrum antibiotics. To avoid overdiagnosis and excessive healthcare costs, correct interpretation of test results is essential. Clinical assessment of signs and symptoms combined with knowledge of the local epidemiology are critical for diagnostic stewardship, and enable correct interpretation of microbiological results.

Current major barriers to the implementation of diagnostic stewardship are a lack of resources, a lack of trained personnel and, most importantly, a lack of knowledge. The lack of resources allocated to diagnostic stewardship is often due to the lack of awareness of the impact that diagnostic tests can have on clinical decision-making and the optimization of appropriate antimicrobial prescribing.

Diagnostic stewardship means ordering the right tests for the right patient at the right time to inform optimal clinical care and positively impact patient outcomes.[32]

The full potential of diagnostic stewardship cannot be reached unless it is integrated in an AMS approach.

Both antimicrobial stewardship and diagnostic stewardship require the constitution and collaboration of **multidisciplinary teams** who can help establish clear criteria for ordering diagnostic tests and acquiring the appropriate technologies. Clinical microbiology laboratories should integrate diagnostic stewardship as a **core activity to ensure successful AMS and infection control (Figure 26)**. Moreover, **information technology (IT) systems** now play a crucial role in antimicrobial stewardship programs, enabling timely and accurate data tracking, monitoring, and analysis. These systems help healthcare facilities to identify patterns of antibiotic use and track resistance trends. **Clinical decision support systems (CDSS)** facilitate decision-making by healthcare professionals regarding appropriate antimicrobial therapy.

Both antimicrobial stewardship and diagnostic stewardship have demonstrated their clinical utility in optimizing patient outcomes, and microbiology laboratories play a key role in both endeavors.

Figure 26. The continuum from diagnostic stewardship to antimicrobial stewardship across the diagnostic pathway[33]

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1 - Pre-analytical phase

Physicians	Microbiologists	Nurses/Adjunct staff
Perform thorough history and clinical exam	Recommend diagnostic tests	Ensure adequate sampling
Determine optimal sample source	Counsel on novel diagnostics	Ensure proper sample labelling and transportation
Determine pre-test probability	Assess sample appropriateness	Report additional clinical data
Choose adequate test		

2 - Analytical phase

Microbiologists	Laboratory staff	Physicians
Determine sample adequacy for testing	Ensure proper processing and preservation of samples	Provide real-time clinical feedback to guide sample processing and testing
Recommend alternative/additional diagnostic tools	Avoid contamination of samples	Establish institutional criteria for sample appropriateness
Reject damaged/ unsealed samples		

DIAGNOSTIC STEWARDSHIP

ANTIMICROBIAL STEWARDSHIP

3 - Post-analytical phase

Laboratory staff	Microbiologists	Physicians
Ensure timely reporting of results	Modify reporting to indicate colonization	Collaborate with microbiologists to ensure proper analysis of results
Integrate Electronic Medical Record (EMR) into result reporting	Selectively report susceptibility results	Correlate results to clinical data to ensure a proper diagnosis
	Properly analyze results and correlate to pretest probability and clinical input	
	Recommend additional testing/novel diagnostics if possible	

4 - Antimicrobial stewardship

Physicians	Pharmacists	Nurses
Choose appropriate treatment while considering local susceptibility patterns	Determine appropriateness of provided treatment	Ensure proper and timely administration of treatment
Reassess regularly and correlate to clinical input	Provide adequate dose adjustment and adverse events surveillance	Provide additional clinical input
De-escalate to narrow spectrum or oral therapy when possible		Report possible adverse events
Discontinue therapy when infectious etiology is unlikely		

CONCLUSIONS AND FUTURE PERSPECTIVES

AST is an essential tool in the fight against multi-drug resistant bacteria. Conventional phenotypic methods remain the mainstay of current AST in the clinical setting. Rapid disc and automated broth microdilution (BMD) methods can generate useful AST data within 4-8 hours and 4-18 hours respectively, but time-to-reporting of AST results needs to be further reduced.

Although **rapid molecular technology** is capable of detecting antimicrobial resistance in bacteria within a short time period, resistance *in vivo* can only be inferred from the presence of resistance genes, and additional phenotypic testing is required to confirm this *in vitro* and to provide possible alternative therapeutic options.

Newer technologies, e.g. microfluidics, fluorescence-activated cell sorting (FACS), adenosine triphosphate (ATP) bioluminescence testing, that allow the **real-time assessment of drug/bug interactions** are under investigation but are not at the stage of commercialization.[34] Nevertheless, a better understanding of antimicrobial usage and its impact on bacterial resistance rates based on **local cumulative AST data** will help to ensure antimicrobial therapy is directed to the best possible patient outcomes, whilst reducing selection pressure for development of multi-drug resistance.

LIST OF ABBREVIATIONS

AES	Advanced Expert system
AMR	Antimicrobial resistance
AMS	Antimicrobial stewardship
ASP	Antimicrobial stewardship program
AST	Antimicrobial susceptibility testing
ATP	Adenosine triphosphate
ATU	Area of technical uncertainty
BMD	Broth microdilution
CDSS	Clinical decision support systems
CLSI	Clinical and Laboratory Standards Institute
CPE	Carbapenemase-producing Enterobacterales
CTX-M	Cefotaximase M type
DNA	Desoxyribose nucleic acid
ECOFF/ECV	Epidemiological cut-off value
EDTA	Ethylenediaminetetraacetic acid
ESBL	Extended spectrum beta-lactamase
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FAA	Fastidious anaerobe agar
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
I	Intermediate
ID	Identification
IT	Information technology
KPC	*Klebsiella pneumoniae* carbapenemase
LIS	Laboratory information system
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MBC	Minimal bactericidal concentration
MDR	Multidrug-resistant
MIC	Minimal inhibitory concentration
MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
MRSA	Methicillin-resistant *Staphylococcus aureus*
NDM	New Delhi metallo-beta-lactamase
NS	Non-susceptible
OXA-48	Oxacillinase-48
PCR	Polymerase chain reaction
R	Resistant
RAST	Rapid antimicrobial susceptibility testing
S	Susceptible
SDD	Susceptible dose-dependent
VRE	Vancomycin-resistant Enterococcus
WHO	World Health Organization

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