SITA

Società Italiana di Terapia Antinfettiva
Antibatterica Antivirale Antifungina

12° CONGRESSO NAZIONALE

CATANIA | 17-18 novembre 2022

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Diagnostica rapida

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Conflict of Interest declaration

Consultant and board of speakers:

- *Pfizer Italy and Europe, MSD, Angelini, Nordic Pharma, Accelerate, Biomerieux, Cepheid, Shionogi, Gilead, BD, Menarini*

Research grants

- *DMG, Biotest, Zambon Italia, Correvio, Nordic Pharma, IHMA – Europe and USA, Liofilchem, Shionogi, Biosynth*

In this 15’ will go through….

- **Role of the clinical micro lab in the AST**
- **Fast is not only molecular**
- **Red phone- Fast track – smart reporting**
- **Towards a precision medicine**

In the clinical microbiology laboratory, AST represent the finalization of a diagnostic path that originates outside the laboratory

- They represent:
- Most of the communication activities with users (clinicians / patients / company management / pharmacy / Regional Agency)
- Are highly time-consuming

- All managerial and technical staff in the bacteriology sector are involved in carrying out, interpreting and communicating the in vitro sensitivity tests.

The goal of the diagnostic stewardship

- To select the right test for the right patient, generating accurate, clinically relevant results at the right time to optimally influence clinical care and to conserve health care resources.
- The laboratory is at the forefront of this mission, functioning as stewards to maximize clinical excellence.
- Clinical microbiology laboratory directors must determine the added value of the panel compared with existing standard of care tests, answering some questions:
- Will the test provide highly accurate, actionable results that can improve patient outcomes?
- Does the test potentially improve the workload in the clinical laboratory by replacing a laborious test?

- <u>This decision should be often made in collaboration with our infectious disease colleagues and/or antimicrobial stewardship team</u>.

Bard Jd Clin Lab Med 40 (2020) 393–420 https://doi.org/10.1016/j.cll.2020.08.001

1. Fast processes

2. Possibility to identify the main aetiological agents of severe infections

3. Availability of an acceptable antibiotics panel

4. Agreement between new technologies and official categorization expert rules

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Narrative review

How to accelerate antimicrobial susceptibility testing

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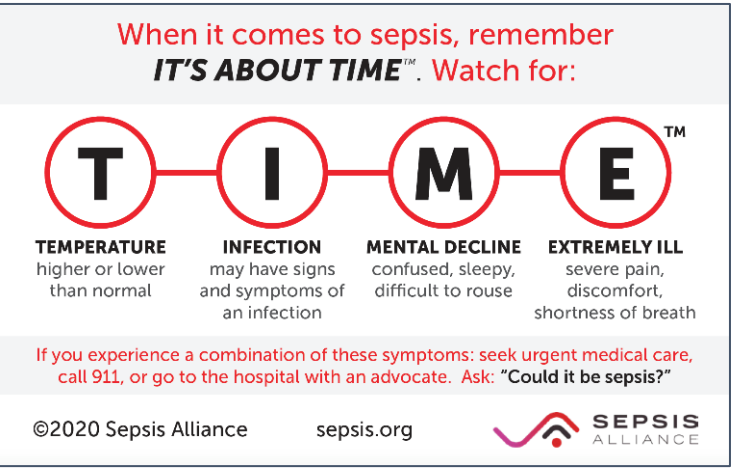
According to the time to result, rAST has been defined as technologies yielding results in ≤8 hr [19], whereas ultra-rapid methods provide results in ≤4 hr [96]. This classification appears reasonable, because in addition to time and technological capabilities it reflects clinical purpose (see below) and laboratory workflow.

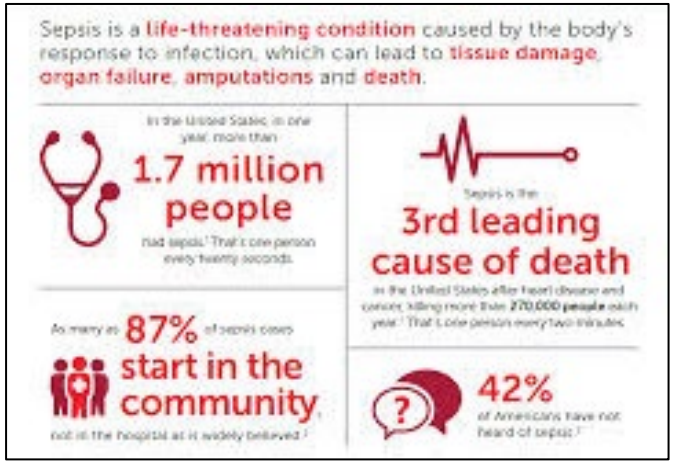
IDEAL DIAGNOSTIC TIMELINE

1 hour

4 hours

8 hours





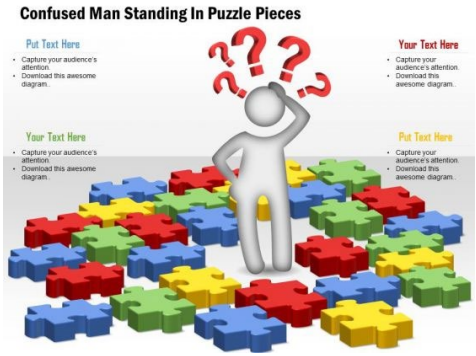
What is going on in the clinical microbiology lab? **The problem of GNB**

- Great array of pathogens (Enterobacterales, Acinetobacter, MRSA, Enterococci)
- Emerging, re-emerging, MDR, XDR
- Diagnostic questions requiring increased complexity in answering
- Request to improve quality and productivity while reducing TAT in an era of personnel shortages and limited funding

Antibiotic	MIC mg/L(S/I/R)
Pip/Tazo	>128 R
Ceftriaxone	>64 R
Ceftazidime	>64 R
Cefepime	>64 R
Ertapenem	>32 R
Imipenem	>32 R
Meropenem	>32 R
Aztreonam	>64 R
Amikacin	>64 R
Gentamicin	2 S
Tobramycin	>16 R
Ciprofloxacin	>4 R
Levofloxacin	>8 R
Tigecycline	1.5 I
Colistin	>32

Data courtesy of speaker.

...and from the site of the patient? The complexity



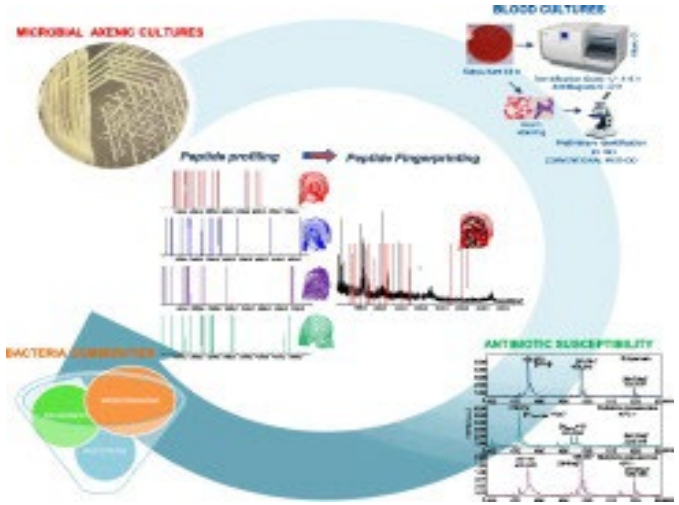
Complexity of patients

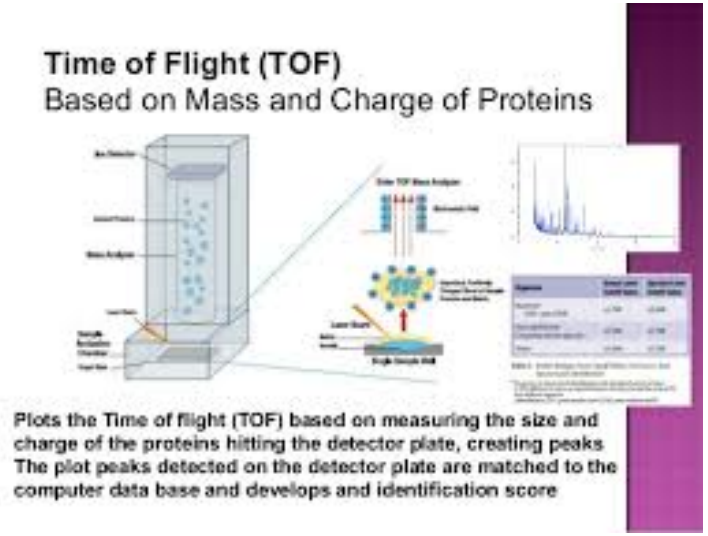
Complexity to be appropriate in antibiotic therapy

What a clinician is waiting for?

MALDI-TOF

Rapid ID





HOW DOES MALDI-TOF COMPARE TO BIOCHEMICAL METHODS OF IDENTIFICATION?

Biochemical Method	MALDI-TOF
Lactose fermenting GNR on MacConkey agar	Lactose fermenting GNR on MacConkey agar
Vitek AST test card	MALDI-TOF
TAT @ **6-8 hours**	TAT @ **10 min**
Materials cost @ **$4.00**	Cost @ $ 0.**50**
Labor neutral	Labor comparable

	TTR (hrs)	Targets	Specimens
FilmArray® (bioMérieux)	1 h	Bla_{kpc} vanA, vanB,mecA	Positive blood culture
Verigene® (Nanosphere)	2 h	mecA, vanA,vanB bla_{CTX-M}, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla-$_{oxa48}$, bla_{VIM}	Positive blood culture
GeneXpert® (Cepheid)	1 h	mecA, vanA, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, blaVIM	Rectal swab
Unyvero® (Curetis)	5h	bla_{CTX-M}, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-58}	positive blood culture, respiratory samples, implant and tissue samples
Eazyplex® (Amplex)	0.5 h	mecA, mecC, bla_{KPC}, bla_{NDM}, bla lik$_{OXA-48like}$,blaVIM	colony, positive blood culture, rectal swab
Check-Direct CPE (Check-Points)	2 h	bla_{KPC}, $bla_{OXA-48like}$, bla_{VIM}, bla_{NDM}	colony, rectal swab

Search of resistance genes, two options:

1. resistance genes detected
2. when is negative?

Search for resistance genes	Species	Report
KPC- **NDM+** **VIM-** **OXA-** **CTX-M-**	**Enterobacterales**	**NDM carbapeneamase:** **high probability of resistance to penicillin, cephalosporins and carbapenems, including combination of con ß-lactamase inhibitors**

Detection of resistance genes

CTX	Non rilevato
KPC	Non rilevato
VIM	Non rilevato
IMP	Non rilevato
NDM	**Rilevato**
OXA-48	Non rilevato

Figura 10. *Klebsiella pneumoniae NDM; antibiogramma molecolare.*

CTX-M	**Rilevato**
KPC	**Rilevato**
VIM	Non rilevato
IMP	Non rilevato
NDM	Non rilevato
OXA-48	Non rilevato

Figura 8. *Klebsiella pneumoniae KPC; antibiogramma molecolare.*

CTX-M	Non rilevato
KPC	Non rilevato
VIM	Non rilevato
IMP	Non rilevato
NDM	Non rilevato
OXA-48	Non rilevato

Figura 24. *Pseudomonas aeruginosa; antibiogramma molecolare.*

A «resistant gene negative» example…

PCR multiplex	
Enterobacter spp	ND
Proteus spp	ND
Escherichia coli	Detected
Klebsiella oxytoca	ND
Klebsiella pneumonia	ND
Serratia marcescens	ND
Acinetobacter spp	ND
Citrobacter spp	ND
Pseudomonas aeruginosa	ND
Resistence genes	
CTX-M	ND
KPC	ND
VIM	ND
NDM	ND
IMP	ND
OXA (48/23/24/58)	ND

Absence of ESBL and most common carbapenemases;

Possible susceptibility to 3d and 4th GC possibile If combined with BLI

Possible susceptibility to carbapenems

ANTIBIOTICI	Ceppo1	MIC
Amikacina	S	2
Amoxicillina/A.CLAV.	S	<=2
Cefotaxima	S	<=0.25
Ceftazidima	S	<=0.12
Ciprofloxacina	S	<=0.06
Ertapenem	S	<=0.12
Gentamicina	S	<=1
Meropenem	S	<=0.25
Piperacillina/tazobactam	S	<=4
Trimethoprim/Sulfam.	S	<=20

Amoxicillina/A.CLAV.	S	8
Cefotaxima	R	>=64
Ceftazidima	R	8
Ciprofloxacina	R	>=4
Ertapenem	S	<=0.12
Gentamicina	S	<=1
Meropenem	S	<=0.25
Piperacillina/tazobactam	S	<=4
Trimethoprim/Sulfam.	R	>=320

INSTRUMENT FEATURES

- Identification in < 90 minutes
- AST with MIC in < 7 hours
- Easy three-step workflow
- Monomicrobial definitive alert
- Polymicrobial capability
- Resistance phenotypes

EU: CE Marked for in-vitro diagnostic use.
US: FDA *de novo* clearance for ID and antimicrobial susceptibilities on positive blood cultures.

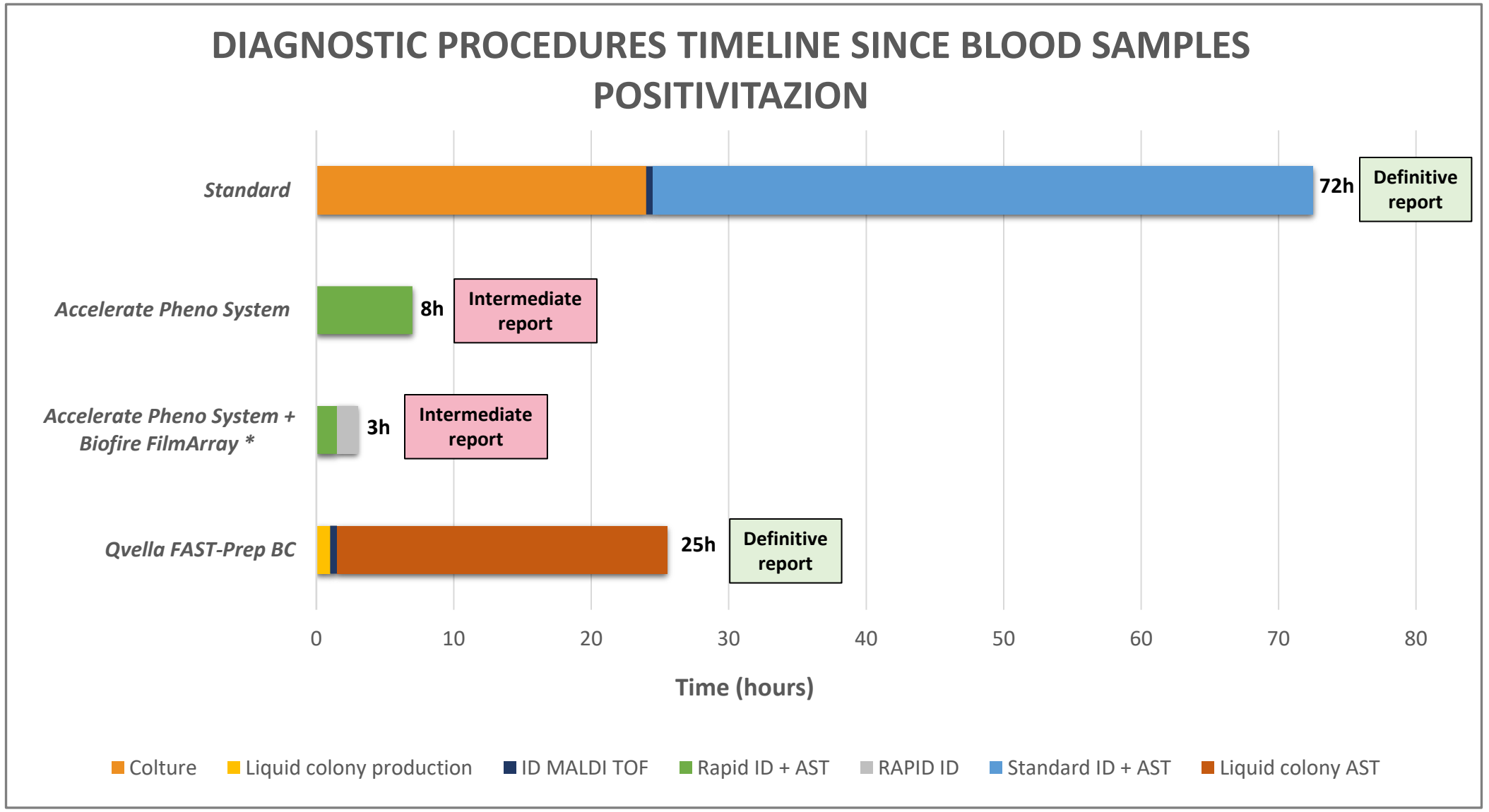
FROM A POSITIVE BLOOD CULTURE TO A LIQUID COLONY

A liquid colony shows the same potential of a solid colony

Possibility to perform fast identification (1 hour) through spectrometric technologies

Possibility to perform a definitive and complete susceptibility profile through conventional technologies

Stefani S et al –personal unpublished data 2022



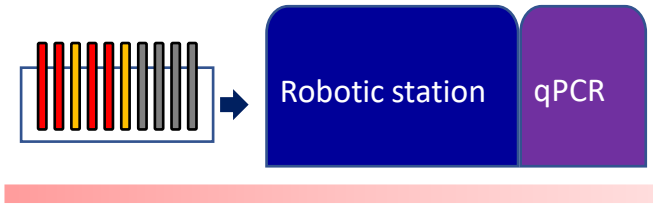
Graph 1. Comparison between standard procedure and two different fast protocols for sepsis diagnosis. Every procedure implies a time to positivity before processing blood samples. *Identification protocols were extended to Biofire FilmArray in case of failed identification using Accelerate Pheno System.

Diagnostic stewardship - Laboratory stewardship – some applications

- Respiratory panel (upper and lower)
- Bloodstream infection panel
- Gastroenteritis panel
- Meningitis panel
- Encephalitis panel

Sepsis and LRTs panels

SEPSIS PANEL	LRTIS PANEL
Staphylococcus spp	*Staphylococcus aureus*
Staphylococcus aureus	*Streptococcus pneumoniae*
Streptococcus spp	*Streptococcus pyogenes*
Streptococcus pneumoniae	*Streptococcus agalactiae*
Streptococcus pyogenes	*Enterococcus faecium*
Streptococcus agalactiae	*Enterococcus gallinarum*
Enterococcus faecium	*Enterococcus faecalis*
Enterococcus gallinarum	*Escherichia coli*
Enterococcus faecalis	*Pseudomonas aeruginosa*
Escherichia coli	*Proteus mirabilis*
Pseudomonas aeruginosa	*Acinetobacter baumannii*
Proteus mirabilis	*Klebsiella pneumoniae*
Acinetobacter baumannii	*Serratia marcesens*
Klebsiella spp	*Moraxella catarrhalis*
Enterobacter cloacae	*Haemophilus influenzae*
Enterobacter aerogenes	*Neisseria meningitidis*
Candida spp	*Bordetella pertussis*
Candida albicans	*Bordetella parapertussis*
Candida glabrata	*Legionella pneumophila*
Candida krusei	*Chlamydophila pneumoniae*
	Mycoplasma pneumoniae
ANTIBIOTICO-RESISTENZE SEPSI/RESPIRATORI	*Candida spp*
MRSA	*Candida albicans*
BLA KPC	*Candida glabrata*
Van A/B	*Aspergillus fumigatus*
	Pneumocystis jiroveci



Bar code reading
Inserimento di 1-24 campioni
Scelta di 1-4 pannelli diagnostici
Inserimento guidato dei reattivi

15’

Purificazione DNA
Preparazione Pannelli diagnostici

3h

Analisi
Refertazione risultati

1h 30’

Nosocomial panel

PANNELLO RESISTENZE	PANNELLO NOSOCOMIALI
MRSA	*Staphylococcus aureus*
VanA, VanB	*Streptococcus pneumoniae*
KPC	*Enterococcus faecium*
NDM	*Enterococcus gallinarum*
IMP	*Enterococcus faecalis*
VIM	*Clostridium difficile*
OXA-48	*Pseudomonas aeruginosa*
CTX-M-1, CTX-M-9	*Proteus mirabilis*
CMY-2	*Serratia marcescens*
	Enterobacter cloacae
	Enterobacter aerogenes
	Acinetobacter baumanni
	Klebsiella pneumoniae
	Legionella pneumophila
	Stenotrophomonas maltophilia
	Candida albicans
	Aspergillus spp.
	MRSA
	BLA KPC
	Van A/B

CLINICAL APPLICATION OF WGS:

DIAGNOSIS
AST
EPIDEMIOLOGY

TAKE HOME MESSAGES

The crucial importance of the diagnostic/antimicrobial stewardship

A «thoughtful» interpretation of a rapid anti-susceptibility test

The correct collocation of any technology within a (shared) diagnostic path for a critical patient