# Il paziente con infezioni del torrente circolatorio: come approcciarlo in termini di inquadramento diagnostico/microbiologico e terapia antibiotica empirico-ragionata

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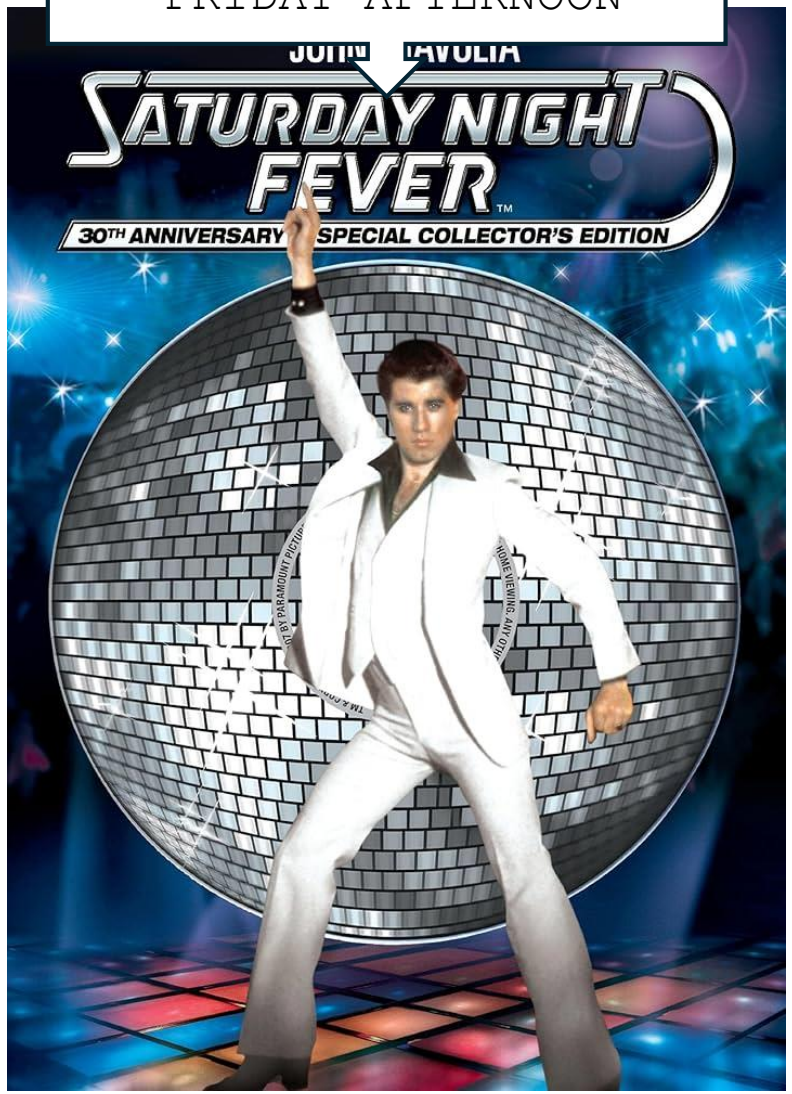
UNIVERSITA' DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE BIOMEDICHE E CLINICHE

# Case

FRIDAY AFTERNOON




F, 70 yrs

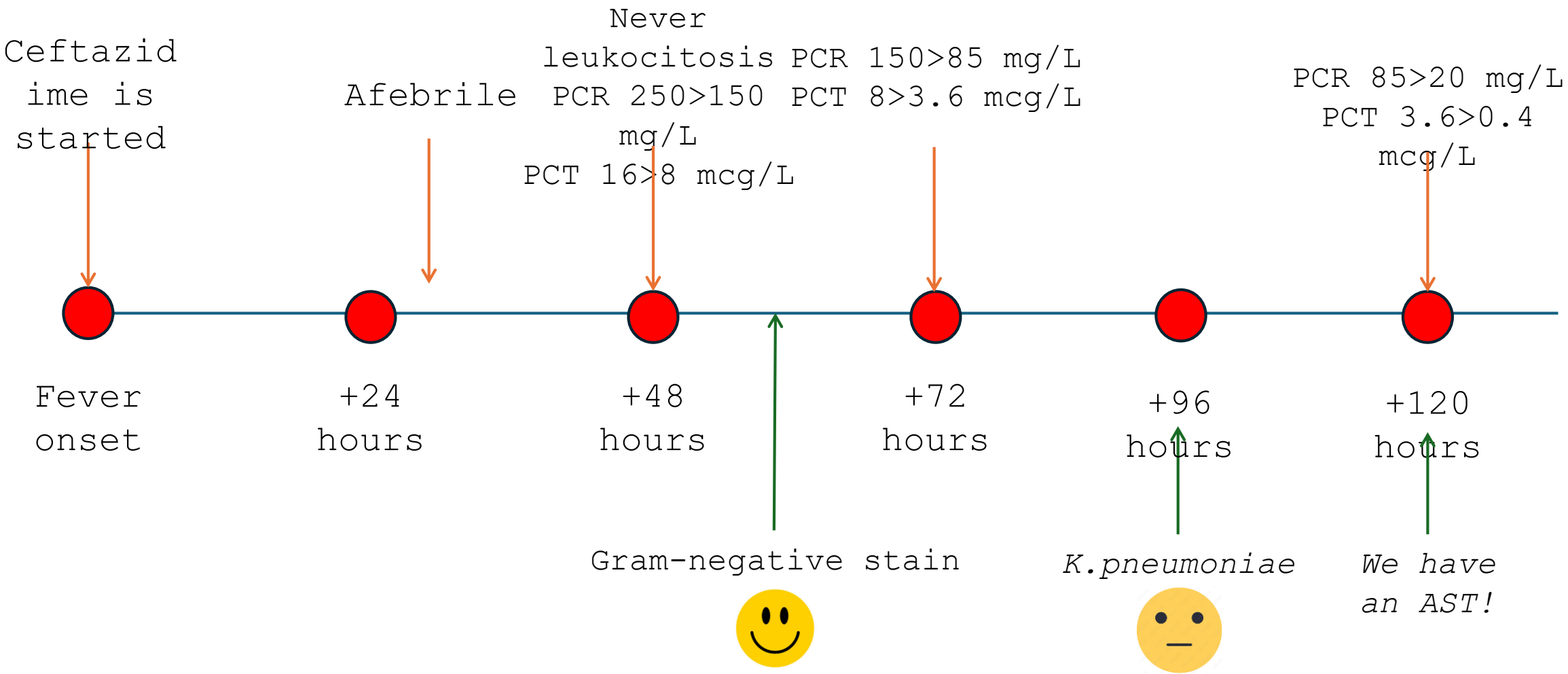
Neurorehabilitation for sequelae of cerebral hemorrhage and hydrocephalus, needed MV (now tracheostomy)

Previous two months in neuro-ICU and neurosurgery ward

Colonisations:

- Respiratory (ETA): CRAB; *P.aeruginosa* (TZP R, MEM 2, CAZ/FEP 8/8)
- Urinary CR-*K.pneumoniae*
- Rectal: CR-*K.pneumoniae*; *P.aeruginosa* (TZP R, MEM 8, CAZ/FEP 2/4)

# Case vignette



Esame Colturale Emocoltura Anaerobi II **Positivo**
Campione

1 Klebsiella pneumoniae

| | Interpret. | MIC µg/mL |
|---|---|---|
| Ciprofloxacina | R | >2 |
| Levofloxacina | R | >4 |
| Amoxicillina/A.Clavulanico | R | >32 |
| Ampicillina | | |
| Ampicillina/Sulbactam | | |
| Aztreonam | | |
| Cefepime | R | >16 |
| Cefotaxime | R | >32 |
| Ceftazidime | R | >32 |
| Ceftazidime/Avibactam | | |
| Ceftolozano/tazobactam | | |
| Ceftriaxone | | |
| Cefuroxime (acetil-) | | |
| Cefuroxime (sodio-) | | |
| Ertapenem | R | >4 |
| Gentamicina | R | 8 |
| Imipenem | | |
| Imipenem/Relebactam | | |
| Meropenem | R | >8 |
| Meropenem/Vaborbactam | | |
| Moxifloxacina | | |
| Piperacillina/Tazobactam | R | >64 |
| Tobramicina | | |
| Trimetoprim/Sulfametossazolo | S | <=20 |

Legenda: S - Sensibile a dosaggio standard; I - Sensibile ad aumentata esposizione; R - Resistente.
Criteri interpretativi secondo linee guida Eucast 2024

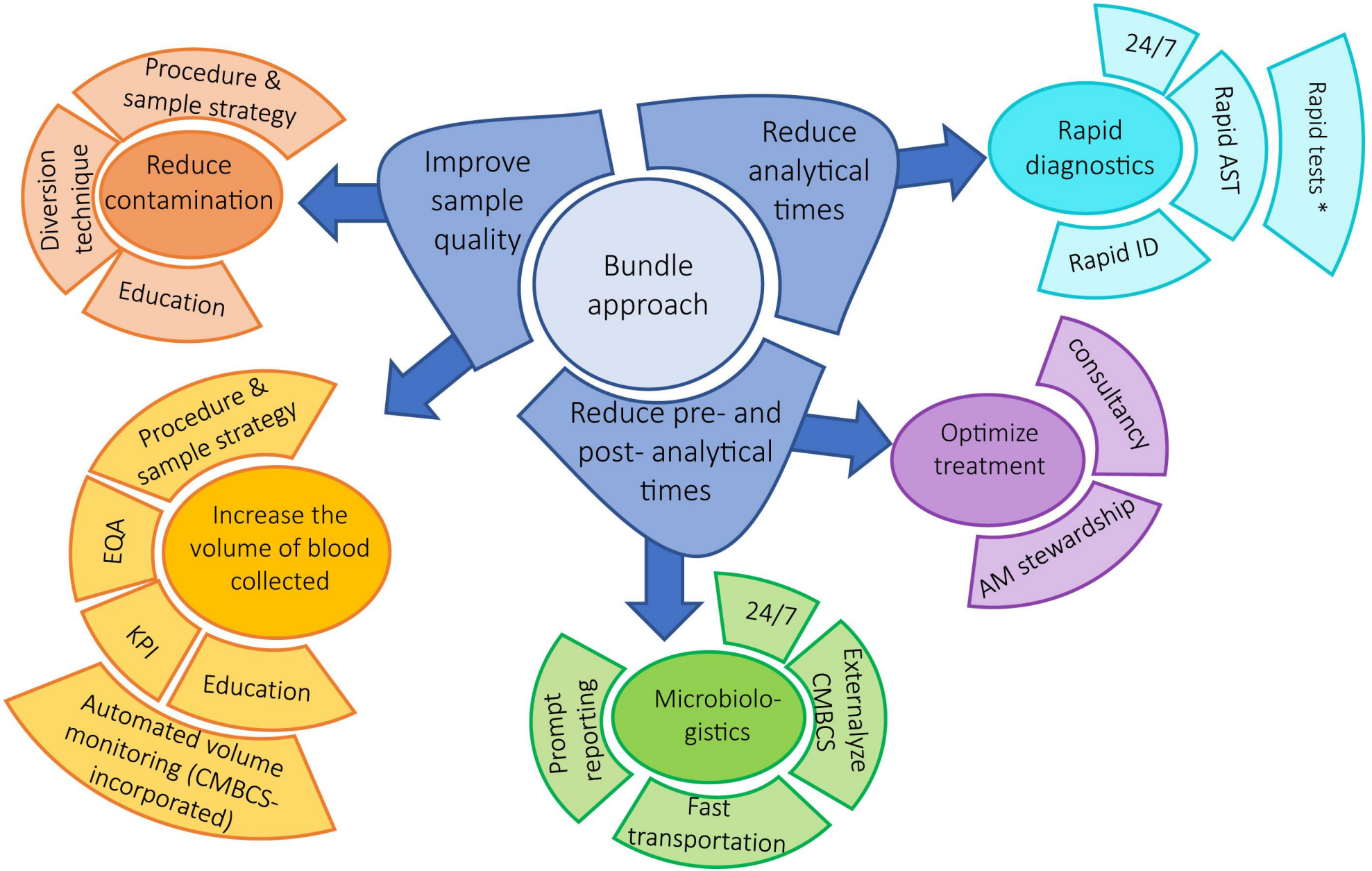
Sito di prelievo: periferico dx.
Ceppo produttore di carbapenemasi

# Global burden of BSIs

- 575,000-677,000 / year with 79,000-94  0 deaths
- 1.2 million / year with 157  0 deaths

- Up to 30% of empirical regimens are inappropriate
- 50% on empirical therapy at 72 hours despite no confirmed infection

Kumar et al., Chest, 2009
Klevens et al., CID, 2008
Marquet et al., Crit care 2015
Buehler et al., CMI, 2016




Lamy et al., CMI, 2020

# Why *appropriate* matters - 1

| Number of studies | Subgroup | Appropriate Events | Appropriate Total | Inappropriate Events | Inappropriate Total | Weight | Odds ratio (95% CI) | Overall effect (Z) | Heterogeneity ($I^2$) |
|---|---|---|---|---|---|---|---|---|---|
| 101 | Overall | 7,054 | 53,565 | 4,052 | 15,599 | 100.0% | 0.4 4 (0.3 8, 0.50) | 11.75 (P < .00001) | 80% |
| 6 | 2–7 days | 76 | 928 | 58 | 471 | 5.4% | 0.65 (0.27, 1.57) | 0.95 ( P = .34) | 78% |
| 12 | 14–15 days | 232 | 1,328 | 371 | 1,061 | 13.1% | 0.45 (0.29, 0.70) | 3.51 ( P = .0004) | 74% |
| 45 | 21–30 days | 2,319 | 12,407 | 1,937 | 5,522 | 50.9% | 0.40 (0.33, 0.50) | 0.53 ( P <0.00001) | 81% |
| 6 | During ICU stay | 249 | 810 | 113 | 194 | 5.5% | 0.27 (0.15, 0.50) | 4.20 ( P < .0001) | 57% |
| 24 | During hospital stay | 3,977 | 37, 541 | 1,443 | 8,0 93 | 25.1% | 0.4 7 (0.36, 0. 61) | 5.63 (P < 0.00001) | 81% |
| 3 | Acute pyelonephritis/UTI | 50 | 939 | 49 | 521 | 3.0% | 0.46 (0.17, 1.23) | 1.54 ( P = .12) | 72% |
| 19 | Pneumonia | 1,333 | 6,618 | 696 | 1,813 | 18.4 % | 0.35 (0.2 4, 0. 51) | 5.43 (P < 0.00001) | 83% |
| 63 | Bact eraemi a, sepsis or septic shock | 5,497 | 44,291 | 3,039 | 12,084 | 68.4% | 0.44 (0.37, 0.52) | 9.54 ( P < 0.00001) | 82% |

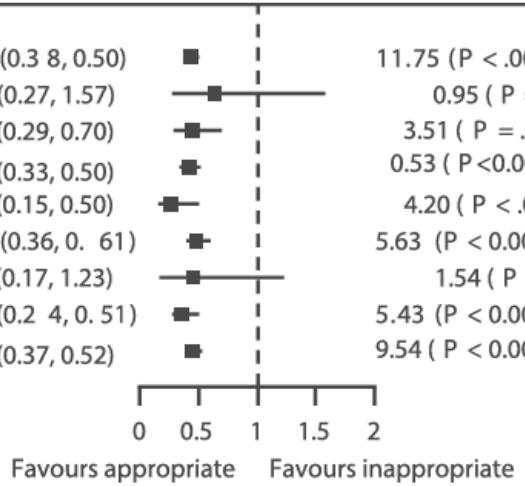



**Fig. 2.** Summary of effect of appropriate versus inappropriate antibiotic therapy on mortality. CI, confidence interval; ICU, intensive care unit; UTI, urinary tract infection.

| Number of studies | Subgroup | Appropriate (n) | Inappropriate (n) | Weight | Mean difference in days (95% CI) | Overall effect (Z) | Heterogeneity ($I^2$) |
|---|---|---|---|---|---|---|---|
| 8 | Overall | 33,889 | 5,343 | 100.0% | –2.54 (–5.30, 0.23) | 1.80 (*P* = .07) | 86% |
| 3 | ICU stay | 230 | 70 | 100.0% | 0.39 (–2.19, 2.98) | 0.30 (*P* = 0.77) | 22% |
| 4 | Bacteraemia/sepsis | 32,988 | 5,064 | 42.9% | –5.04 (–8.31, –1.77) | 3.02 (*P* = 0.003) | 45% |
| 3 | Pneumonia | 535 | 196 | 38.8% | –1.43 (–3.99, 1.13) | 1.10 (*P* = 0.27) | 31% |
| 1 | Skin and soft tissue | 366 | 83 | 18.4% | –0.50 (–2.31, 1.31) | 0.54 (*P* = .59) | NA |

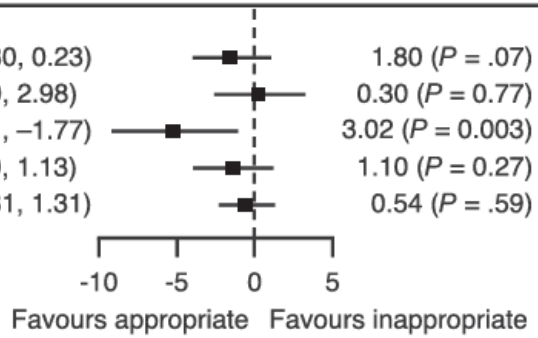



**Fig. 3.** Summary of effect of appropriate versus inappropriate therapy on length of hospital stay. CI, confidence interval; ICU, intensive care unit; NA, not applicable.

Bassetti et al, Int J Antimicrob Agents, 2020

# Why *appropriate* matters - 2

| Number of study | Subgroup | Appropriate Events | Appropriate Total | Inappropriate Events | Inappropriate Total | Weight (%) | Odds ratio (95% CI) | Overall effect (Z) | Heterogeneity ($I^2$) |
|---|---|---|---|---|---|---|---|---|---|
| 6 | Overall | 322 | 1,195 | 330 | 745 | 100 | 0.33 (0.16, 0.66) | 3.10 ( P = .002) | 89% |
| 4 | 3–7 days | 212 | 705 | 198 | 334 | 65.5 | 0.25 (0.08, 0.80) | 2.33 ( P = .02) | 93% |
| 1 | 30 days | 97 | 429 | 114 | 377 | 19.4 | 0.67 (0.49, 0.92) | 2.45 ( P = .01) | NA |
| 1 | During hospital stay | 13 | 61 | 18 | 34 | 15.1 | 0.24 (0.10, 0.60) | 3.07 ( P = .002) | NA |
| 2 | Acute pyelonephritis/UTI | 152 | 750 | 139 | 444 | 37.2 | 0.51 (0.27, 0.96) | 2.07 ( P = .04) | 75% |
| 4 | Bacteraemia/sepsis | 170 | 445 | 191 | 301 | 62.8 | 0.22 (0.06, 0.83) | 2.24 ( P = .03) | 93% |

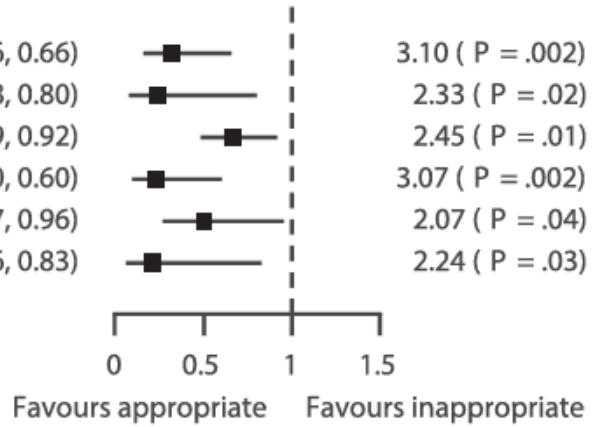



**Fig. 4.** Summary of effect of appropriate versus inappropriate therapy on treatment failure. CI, confidence interval; NA, not applicable; UTI, urinary tract infection.

| Number of studies | Subgroup | Appropriate (n) | Inappropriate (n) | Weight (%) | Mean difference in thousand $ or thousand Euro (95% CI) | Overall effect (Z) | Heterogeneity ($I^2$) |
|---|---|---|---|---|---|---|---|
| 3 | Overall | 32,876 | 5,028 | 100 | −6.99 (−15.27, 1.29) | 1.66 ( P = 0.10) | 98% |
| 2 | Bacteraemia | 32,817 | 5,0 11 | 93.8 | −8.64 (−17.13, −0.15) | 1.99 ( P = .05) | 99% |
| 1 | Pneumonia | 59 | 17 | 6.2 | 18.05 (−13.03, 49.12) | 1.14 ( P = 0.25) | NA |

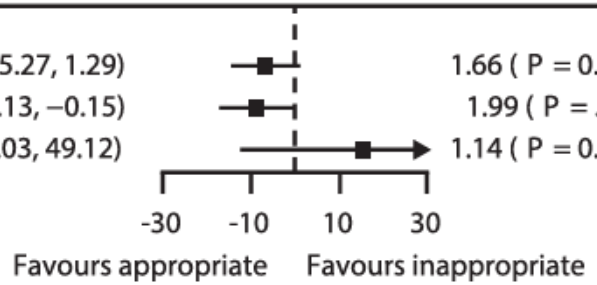



**Fig. 5.** Summary of impact of appropriate versus inappropriate therapy on costs. CI, confidence interval.

Bassetti et al, Int J Antimicrob Agents, 2020

# Why *appropriate & timely* matters

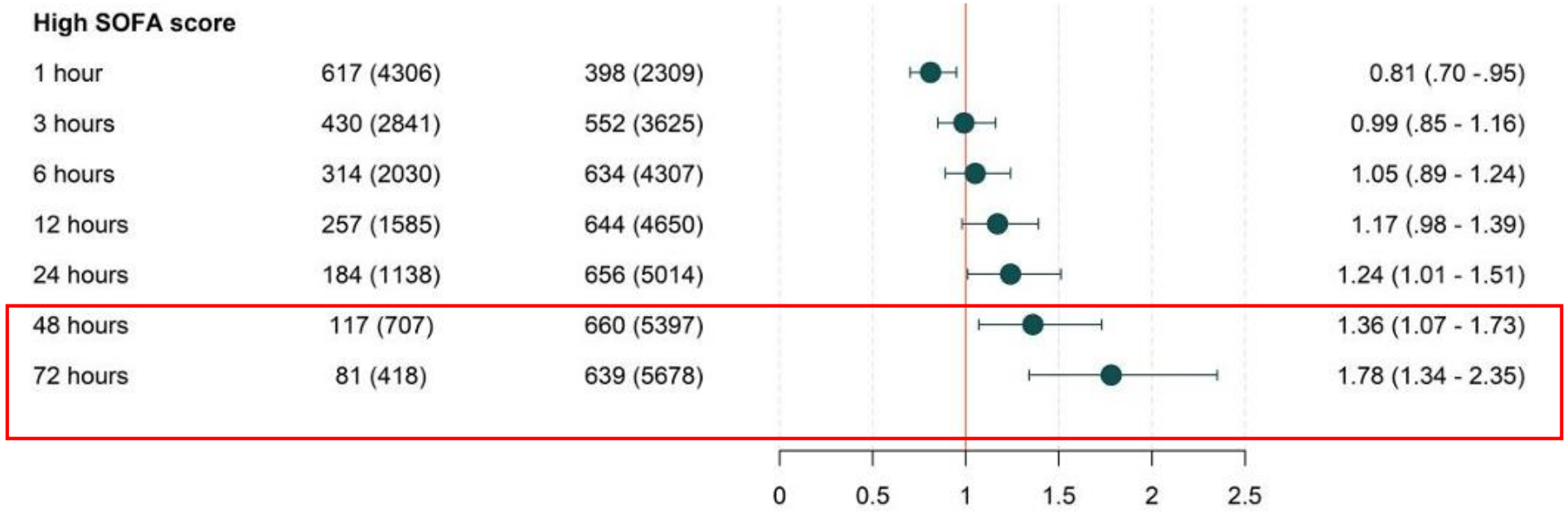
*Clinical Infectious Diseases*

MAJOR ARTICLE

## Association Between Time to Appropriate Antimicrobial Treatment and 30-day Mortality in Patients With Bloodstream Infections: A Retrospective Cohort Study

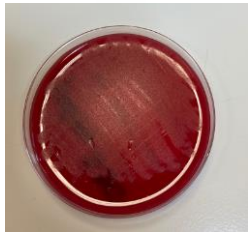
**Jasper Van Heuverswyn,[1,a] John Karlsson Valik,[1,2,a] Suzanne Desirée van der Werff,[1,2] Pontus Hedberg,[1,2] Christian Giske,[3,4] and Pontus Nauclér[1,2]**

[1]Department of Medicine, Solna, Division of Infectious Diseases, Karolinska Institutet, Stockholm, Sweden; [2]Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden; [3]Clinical microbiology, Karolinska University Hospital, Stockholm, Sweden; and [4]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden



# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?



**Short term incubation**

**Blood culture collected**

**Gram strain**

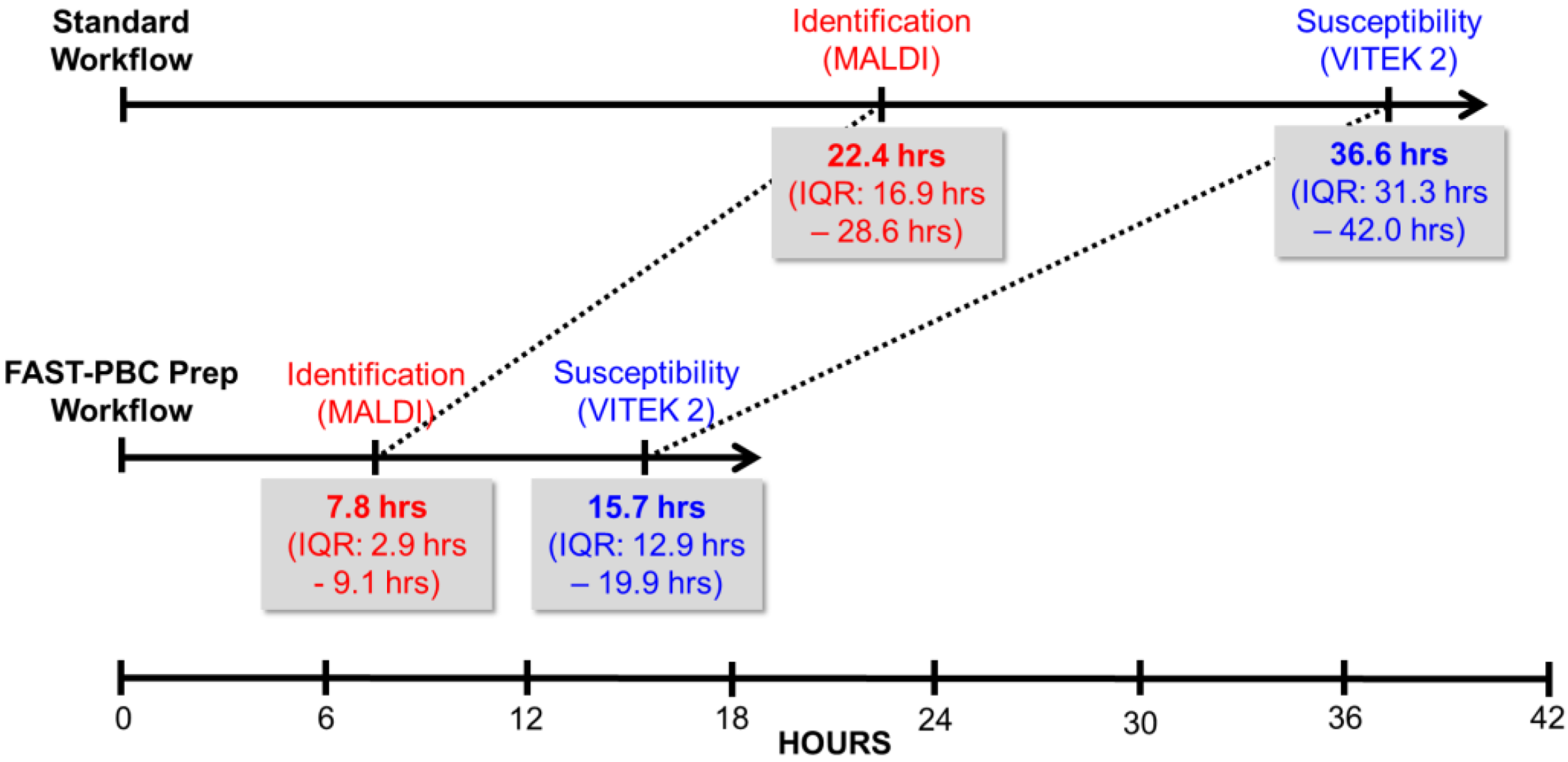
**Growth on agar**

**ID & AST**
(e.g. Vitek2, disk diffusion)

**Additional Susceptibility testing**
(e.g. MICs)

**Additional analysis**
(e.g. MLST, PCR)

# Short term incubation AST




Ugaban et al, Microorganisms, 2022

# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?

**Syndromic panels**

**Blood culture collected** → **Gram strain** → **Growth on agar** → **ID & AST** (e.g. Vitek2, disk diffusion) → **Additional Susceptibility testing** (e.g. MICs) → **Additional analysis** (e.g. MLST, PCR)

# Syndromic panels

**Table 1: BCID2 Pathogen and Resistance Gene Panel**

| Gram-Positive Bacteria | Gram-Negative Bacteria | Yeast | Resistance Genes |
|---|---|---|---|
| *Enterococcus faecalis*<br>*Enterococcus faecium*<br>*Listeria monocytogenes*<br>*Staphylococcus* genus<br>- *Staphylococcus aureus*<br>- *Staphylococcus epidermidis*<br>- *Staphylococcus lugdunensis*<br>*Streptococcus* genus<br>- *Streptococcus agalactiae*<br>- *Streptococcus pneumoniae*<br>- *Streptococcus pyogenes* | *Acinetobacter baumannii complex*<br>*Bacteroides fragilis*<br>*Enterobacterales* Order<br>- *Enterobacter cloacae complex*<br>- *Escherichia coli*<br>- *Klebsiella aerogenes*<br>- *Klebsiella oxytoca*<br>- *Klebsiella pneumoniae* group<br>- *Proteus spp.*<br>- *Salmonella spp.*<br>- *Serratia marcescens*<br>*Haemophilus influenzae*<br>*Neisseria meningitidis*<br>*Pseudomonas aeruginosa*<br>*Stenotrophomonas maltophilia* | *Candida albicans*<br>*Candida auris*<br>*Candida glabrata*<br>*Candida krusei*<br>*Candida parapsilosis*<br>*Candida tropicalis*<br>*Cryptococcus neoformans/gatti* | Carbapenemases<br>- IMP<br>- KPC<br>- OXA-48-like<br>- NDM<br>- VIM<br>Colistin Resistance<br>- mcr-1<br>ESBL<br>- CTX-M<br>Methicillin-resistance<br>- mecA/C<br>- mecA/C and MREJ (MRSA)<br>Vancomycin Resistance<br>- vanA/B |

# Syndromic panels: accuracy

## Performance of BioFire Blood Culture Identification 2 Panel (BCID2) for the detection of bloodstream pathogens and their associated resistance markers: a systematic review and meta-analysis of diagnostic test accuracy studies

Anna Maria Peri[1*], Weiping Ling[1], Luis Furuya-Kanamori[1], Patrick N. A. Harris[1,2] and David L. Paterson[1,3]

- Meta-analysis of 9 studies > 2,000 BCs
- High pooled sensitivity (92-98%) and specificity (>97%) for common BSI pathogens
  - Enterobacterales
  - *S.aureus*
  - *Streptococcus* spp.
  - *P.aeruginosa*
  - *E.faecalis*
- >94% pooled sensitivity for blaCTX-M, carbapenemases and mecA/C & MREJ
- Limited assessment of polymicrobial BCs

Peri et al, BMC Inf Dis, 2022

**Pros**

- ✓ Simultaneous ID and AST gene screening from few bacteria **shortens turnaround time**.
- ✓ It also detects **fastidious organisms** missed by blood culture.

**Cons**

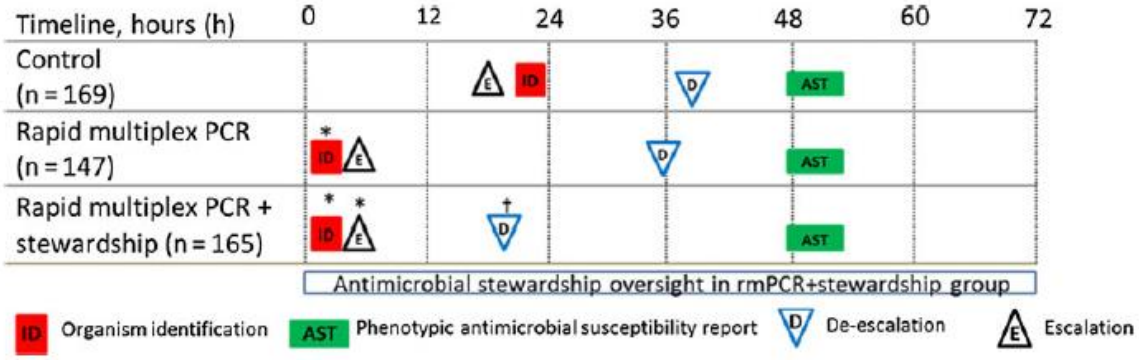
- ✓ Currently known genes **do not fully explain** the AMR mechanisms.
- ✓ Resistance genes often **fail to predict actual resistance** in pathogens.
- ✓ **The effective dose cannot be guided** since MIC cannot be determined.
- ✓ It can detect microbial DNA even from **dead organisms**.
- ✓ **Profiling and preservation of clinical isolate is impossible** since all pathogens are lysed during the assay.

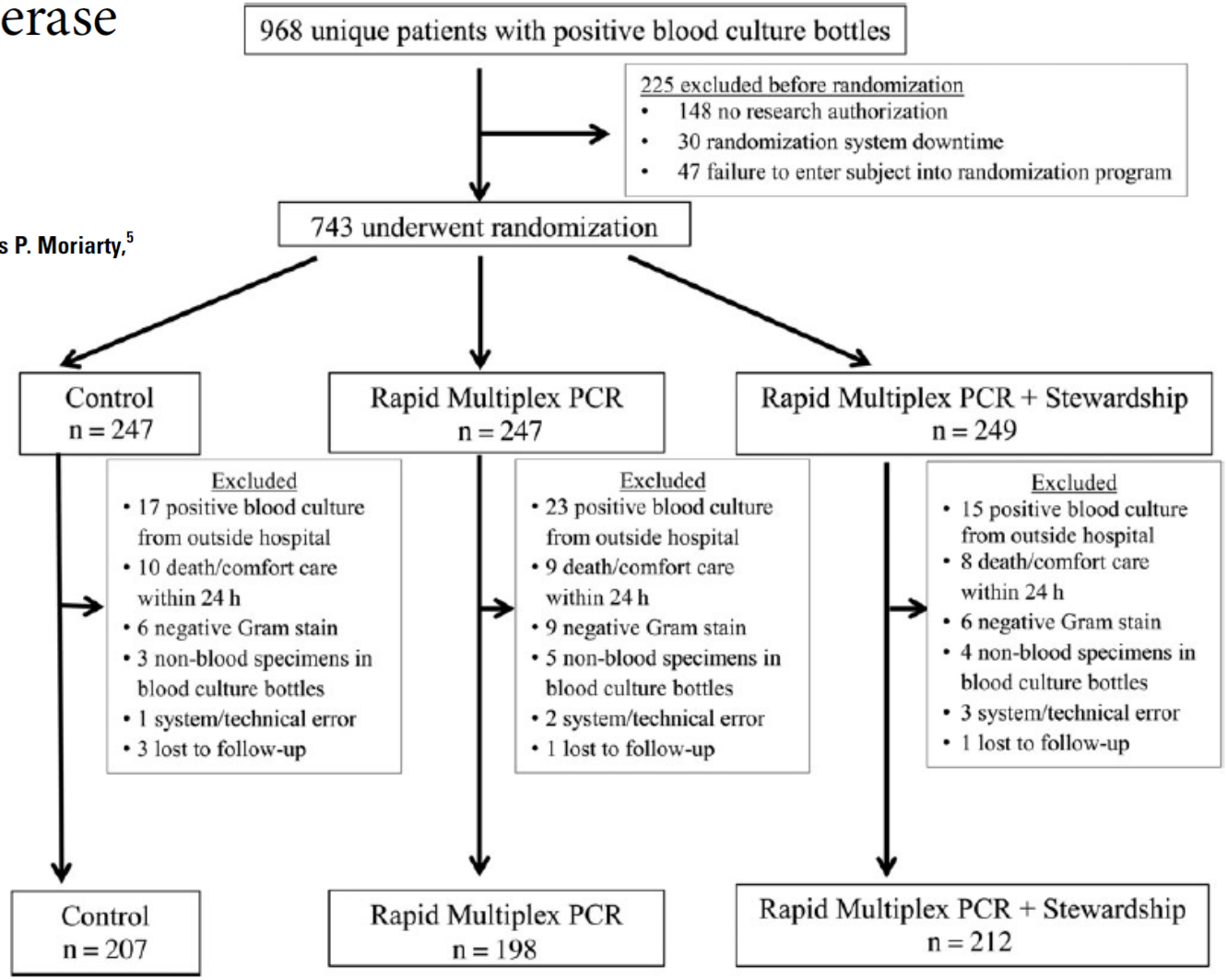
And… you'll only find what you are looking for!

# Syndromic panels and outcomes

## Randomized Trial of Rapid Multiplex Polymerase Chain Reaction–Based Blood Culture Identification and Susceptibility Testing

Ritu Banerjee,[1,a] Christine B. Teng,[2,a] Scott A. Cunningham,[3] Sherry M. Ihde,[3] James M. Steckelberg,[4] James P. Moriarty,[5] Nilay D. Shah,[5] Jayawant N. Mandrekar,[6] and Robin Patel[3,4]




- Clear benefit for de-escalation & appropriate therapy > enhanced by ASP
- Harder to demonstrate improved clinical outcome

Banerjee et al, CID, 2015

# Syndromic panels and outcomes in GN-MDR

*Clinical Infectious Diseases*

MAJOR ARTICLE

## Impact of a Rapid Molecular Test for *Klebsiella pneumoniae* Carbapenemase and Ceftazidime-Avibactam Use on Outcomes After Bacteremia Caused by Carbapenem-Resistant Enterobacterales

Michael J. Satlin,[1,2] Liang Chen,[3,4] Angela Gomez-Simmonds,[5] Jamie Marino,[2] Gregory Weston,[6] Tanaya Bhowmick,[7] Susan K. Seo,[8] Steven J. Sperber,[9,10] Angela C. Kim,[11] Brandon Eilertson,[12] Sierra Derti,[1] Stephen G. Jenkins,[1,2] Michael H. Levi,[13] Melvin P. Weinstein,[7,14] Yi-Wei Tang,[15] Tao Hong,[16] Stefan Juretschko,[17] Katherine L. Hoffman,[18] Thomas J. Walsh,[1] Lars F. Westblade,[1,2] Anne-Catrin Uhlemann,[5] and Barry N. Kreiswirth[3,4]

| Bloodstream Pathogen | No. (% of Total Cohort) | No. (%) of Patients Who Died Within 30 Days, per Pathogen |
|---|---|---|
| *Klebsiella pneumoniae* | 88 (64) | 38 (43) |
| ST258 | 51 (58)[a] | 22 (43) |
| *Escherichia coli* | 20 (15) | 8 (40) |
| ST131 | 7 (35)[a] | 3 (43) |
| *Enterobacter cloacae* complex | 15 (11) | 4 (27) |
| ST171 | 5 (33)[a] | 3 (60) |
| *Klebsiella oxytoca* | 5 (4) | 1 (20) |
| *Serratia marcescens* | 3 (2) | 0 |
| Multiple CRE | 4 (3) | 1 (25) |
| Other | 2 (2) | 2 (100) |
| Meropenem-resistant | 103 (75) | 41 (40) |
| Difficult-to-treat [31] | 89 (65) | 37 (42) |
| Carbapenemase-producer[b] | 106 (77) | 40 (38) |
| KPC-producer[c] | 89 (65) | 34 (38) |
| OXA-48–like-producer[d] | 8 (6) | 4 (50) |
| NDM-producer[e] | 7 (5) | 2 (29) |
| Non–carbapenemase-producer | 31 (23) | 12 (39) |
| Polymicrobial bacteremia[f] | 44 (32) | 18 (41) |

- 137 patients with CRE BSIs, 65% KPC-Kp
- Shorter time to appropriate therapy
- Decreased 14-days and 30-days mortality

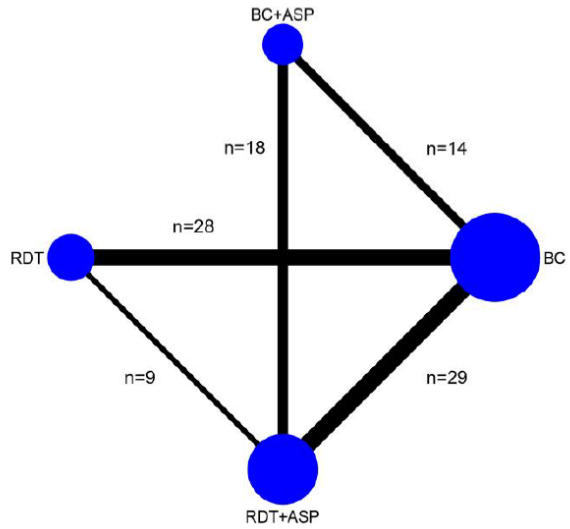
Satlin et al, CID, 2022

# Syndromic panels and stewarc

Clinical Infectious Diseases
MAJOR ARTICLE

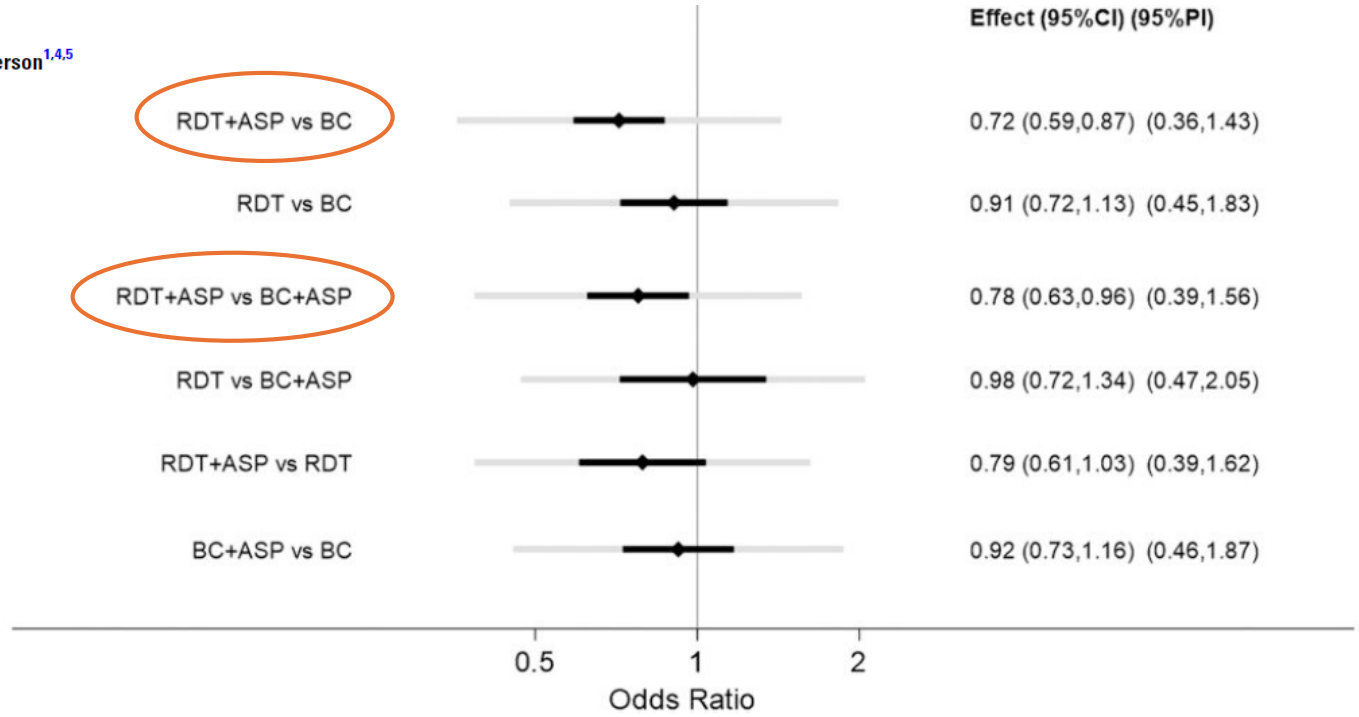
## Rapid Diagnostic Tests and Antimicrobial Stewardship Programs for the Management of Bloodstream Infection: What Is Their Relative Contribution to Improving Clinical Outcomes? A Systematic Review and Network Meta-analysis

Anna Maria Peri,[1] Mark D. Chatfield,[1] Weiping Ling,[1] Luis Furuya-Kanamori,[1] Patrick N. A. Harris,[1,2,3] and David L. Paterson[1,4,5]




- 88 studies, 25’682 patients
- No survival benefit with RDT alone or BC+ASP
- Reduction in LOS in RDT + ASP vs BC alone
- Reduced time to optimal therapy RDT + ASP vs BC alone

Mortality




Peri et al, CID, 2024

# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?

**T2 bacteria**

**Blood culture collected**

**Gram strain**

**Growth on agar**

**ID & AST**
(e.g. Vitek2, disk diffusion)

**Additional Susceptibility testing**
(e.g. MICs)

**Additional analysis**
(e.g. MLST, PCR)

# T2 bacteria

## T2BACTERIA PANEL

Sensitivity: 90%[7]

Specificity: 98%[7]

*E. faecium*
*S. aureus*
*K. pneumoniae*
*A. baumannii*
*P. aeruginosa*
*E. coli*

T2 Positive – BC negative:

- Closed space infections
- Recent use of antibiotics *(Kalligeros et al 2020)*

Better performance when:

- Probable and possible BSI included as comparators with proven BSI *(Nguyen et al 2019; De Angelis et al 2018)*

## CLINICAL IMPACT

ED: theoretical potential to influence time to effective therapy and antibiotic de-escalation

*Kalligeros M, et al BMC Infect Dis 2020;20:326.; Voigt et al. J Emerg Med 2020;58:785e96.*

## T2RESISTANCE PANEL

**Gram-negative marker**

*KPC*
*OXA-48 Group*
*NDM /VIM/IMP*
*CTX-M 14/15*
*AmpC(CMY/DHA)*

**Gram-positive marker**

*vanA/B*
*mecA/C*

~~T2 bacteria~~

# T2 Biosystems, Inc.

Thank you for visiting the website for T2 Biosystems, Inc. ("Company"), an in vitro diagnostics company formerly based in Lexington, MA. The Company is no longer operating. Any questions may be submitted by email to: T2@vlpc.com

Correspondence can also be sent to:
T2 Biosystems, Inc., 124 Washington Street, Ste. 101, Foxboro, MA 02035

# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?

**VOCs & RAPID-AST**

**Blood culture collected**

**Gram strain**

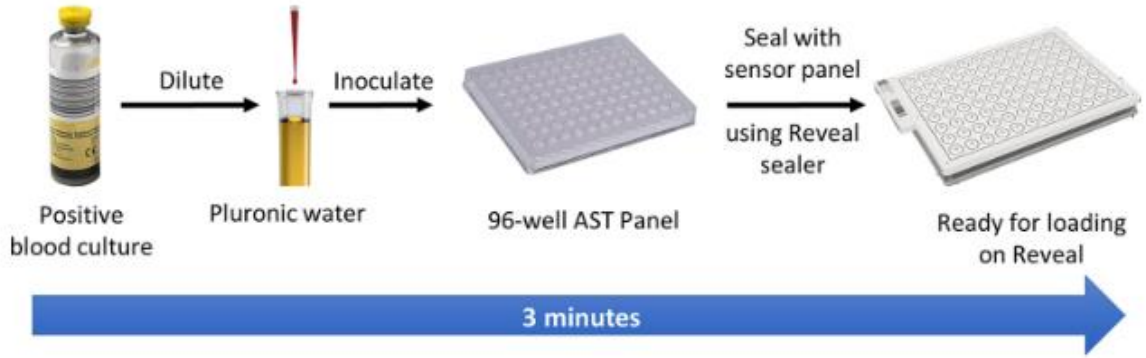
**Growth on agar**

**ID & AST** (e.g. Vitek2, disk diffusion)

**Additional Susceptibility testing** (e.g. MICs)

**Additional analysis** (e.g. MLST, PCR)

# VOCs and Reveal rapid-AST


- Reaction to bacterial metabolic volatiles (VOCs)
- AST results in about 5 hours

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY
June 2022 Volume 60 Issue 6 e00098-22
https://doi.org/10.1128/jcm.00098-22

## Performance of the Reveal Rapid Antibiotic Susceptibility Testing System on Gram-Negative Blood Cultures at a Large Urban Hospital

Robert Tibbetts [a], Sheeja George[a], Reece Burwell[b], Lara Rajeev[b], Paul A. Rhodes[b], Pragya Singh[b], Linoj Samuel [a]

- Mean TTR 4.6 hours
- Compared to Vitek 2 and

| | Performance of Reveal AST against: | |
|---|---|---|
| Parameter or detail | Sensititre | Vitek 2 |
| Parameter, % (no. positive/total no.) | | |
| EA[c] | 98.0 (2,129/2,173) | 97.0 (1,482/1,528) |
| CA[c] | 96.3 (2,174/2,258) | 96.2 (1,554/1,615) |
| mE | 3.5 (78/2,258) | 3.3 (54/1,615) |
| ME | 0.3 (5/1,889) | 0.3 (4/1,342) |
| VME | 1.3 (4/313) | 1.3 (3/232) |

Tibbets et al, J Clin Mic, 2022

# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?

**WGS/NGS**

**Blood culture collected**

**Gram strain**

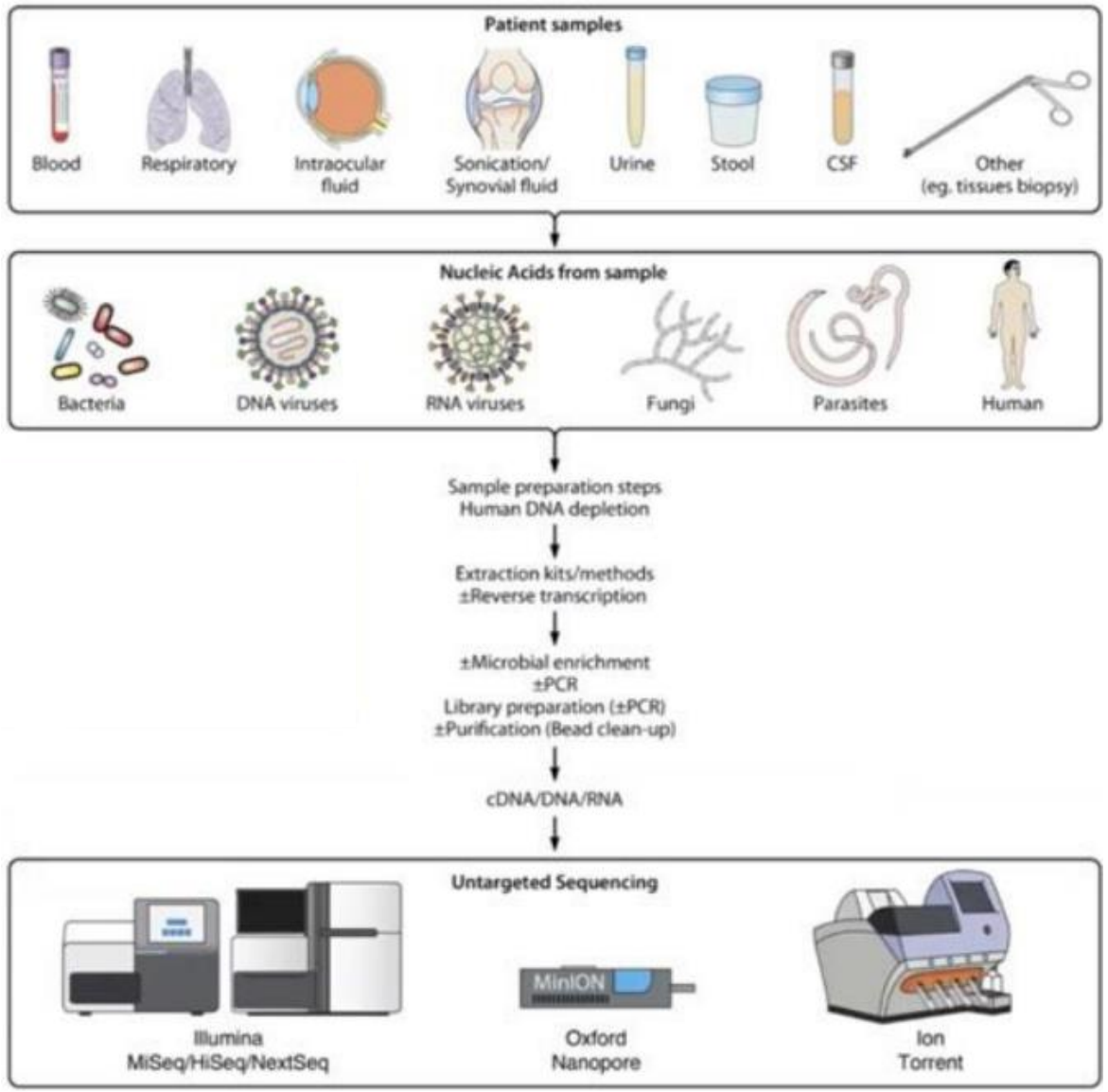
**Growth on agar**

**ID & AST**
(e.g. Vitek2, disk diffusion)

**Additional Susceptibility testing**
(e.g. MICs)

**Additional analysis**
(e.g. MLST, PCR)

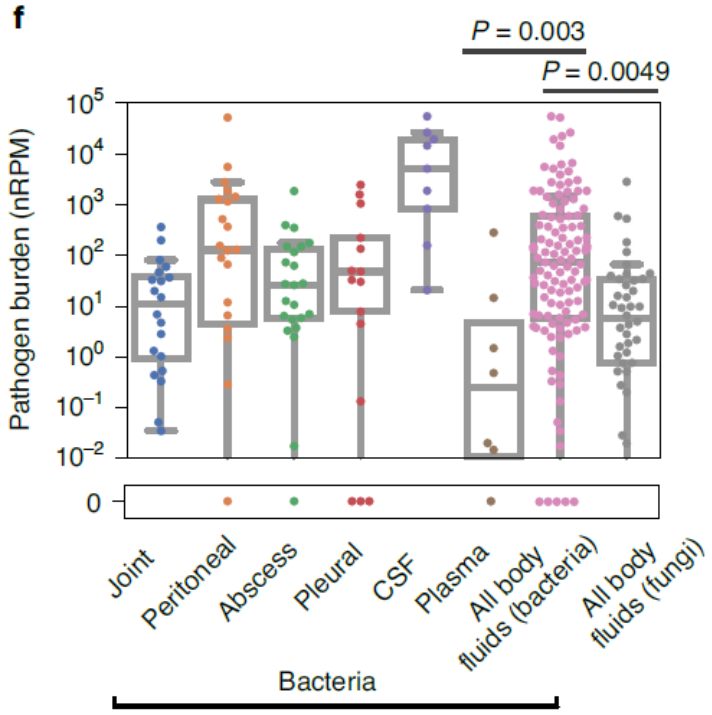
# Clinical metagenomics and WGS




nature medicine
ARTICLES
https://doi.org/10.1038/s41591-020-1105-z
Check for updates

## Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids

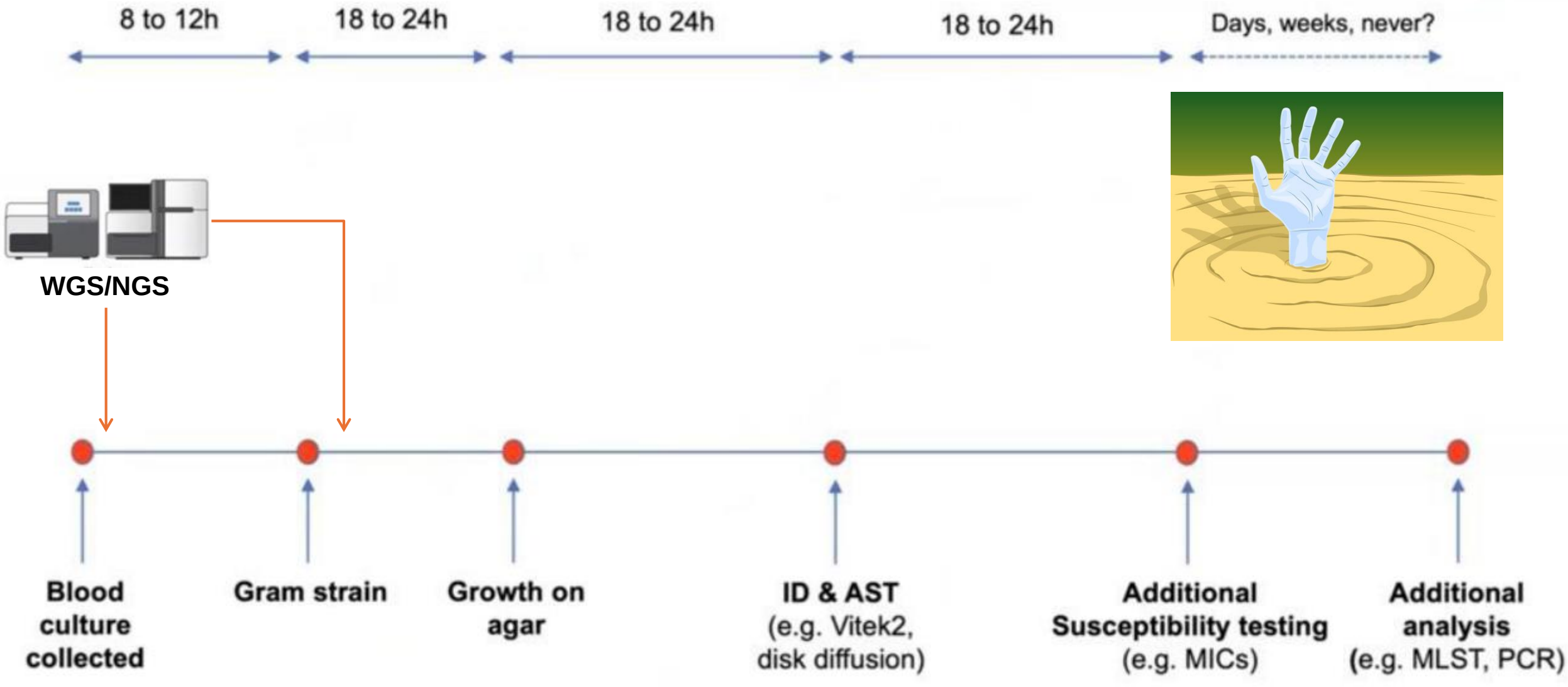
Wei Gu[1,2,10], Xianding Deng[1,2,10], Marco Lee[3,10], Yasemin D. Sucu[1,2], Shaun Arevalo[1,2], Doug Stryke[1,2], Scot Federman[1,2], Allan Gopez[1,2], Kevin Reyes[1,2], Kelsey Zorn[4], Hannah Sample[4], Guixia Yu[1,2], Gurpreet Ishpuniani[1,2], Benjamin Briggs[1,2], Eric D. Chow[4], Amy Berger[5], Michael R. Wilson[6,7], Candace Wang[1,2], Elaine Hsu[1], Steve Miller[1,2], Joseph L. DeRisi[4,8] and Charles Y. Chiu[1,2,9]




Lower DNA burden in plasma compared to other body fluids!

Chiu et al, Nat Rev Gen, 2019; Gu et al, Nat Med, 2020

# The quicksand of empirical therapy



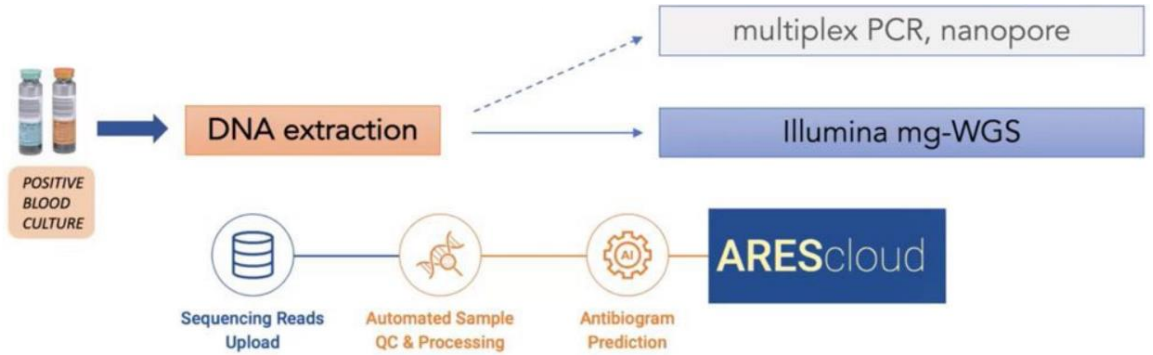
# WGS and AI-AST on positive cultures

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY
November 2022 Volume 60 Issue 11 e01012-22
https://doi.org/10.1128/jcm.01012-22

**Optimized Method for Bacterial Nucleic Acid Extraction from Positive Blood Culture Broth for Whole-Genome Sequencing, Resistance Phenotype Prediction, and Downstream Molecular Applications**

Michelle J. Bauer[a], Anna Maria Peri[a], Lukas Lüftinger[b], Stephan Beisken[b], Haakon Bergh[c], Brian M. Forde[a], Cameron Buckley[a], Thom Cuddihy[a], Patrice Tan[a], David L. Paterson[a], David M. Whiley[a], Patrick N. A. Harris[a,c]




**Matched phenotypic species ID in 38/40 (95%) samples (100% if monomicrobial)**

- Species identification via metagenomic sequencing
- Susceptibility prediction via a machine-learning algorithm

Contamination!!

| Taxon | Categorical Agreement | Sensitivity | Specificity | VME | ME |
|---|---|---|---|---|---|
| All | 0.95 | 0.89 | 0.96 | 0.11 | 0.04 |
| *Escherichia coli* | 0.96 | 1.00 | 0.95 | 0.00 | 0.05 |
| *Klebsiella pneumoniae* | 0.96 | | 0.96 | | 0.04 |
| *Pseudomonas aeruginosa* | 0.97 | 1.00 | 0.97 | 0.00 | 0.03 |
| *Proteus mirabilis* | 1.00 | 1.00 | 1.00 | 0.00 | 0.00 |
| *Klebsiella oxytoca* | 0.67 | 0.33 | 1.00 | 0.67 | 0.00 |
| *Campylobacter jejuni* | 1.00 | | 1.00 | | 0.00 |

Overall good agreement with conventional susceptibility testing (Vitek 2) for common GN pathogens, generally low error rates

- *K. oxytoca* – chromosomal inducible beta-lactamases confuse the system!
- Less reliable for uncommon species (e.g. *Elizabethkingia*)

Bauer et al, J Clin Microbio, 2022

# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?

**Microbial cell-free DNA**

**Blood culture collected**

**Gram strain**

**Growth on agar**

**ID & AST**
(e.g. Vitek2, disk diffusion)

**Additional Susceptibility testing**
(e.g. MICs)

**Additional analysis**
(e.g. MLST, PCR)

# Microbial cell-free DNA sequencing

nature microbiology

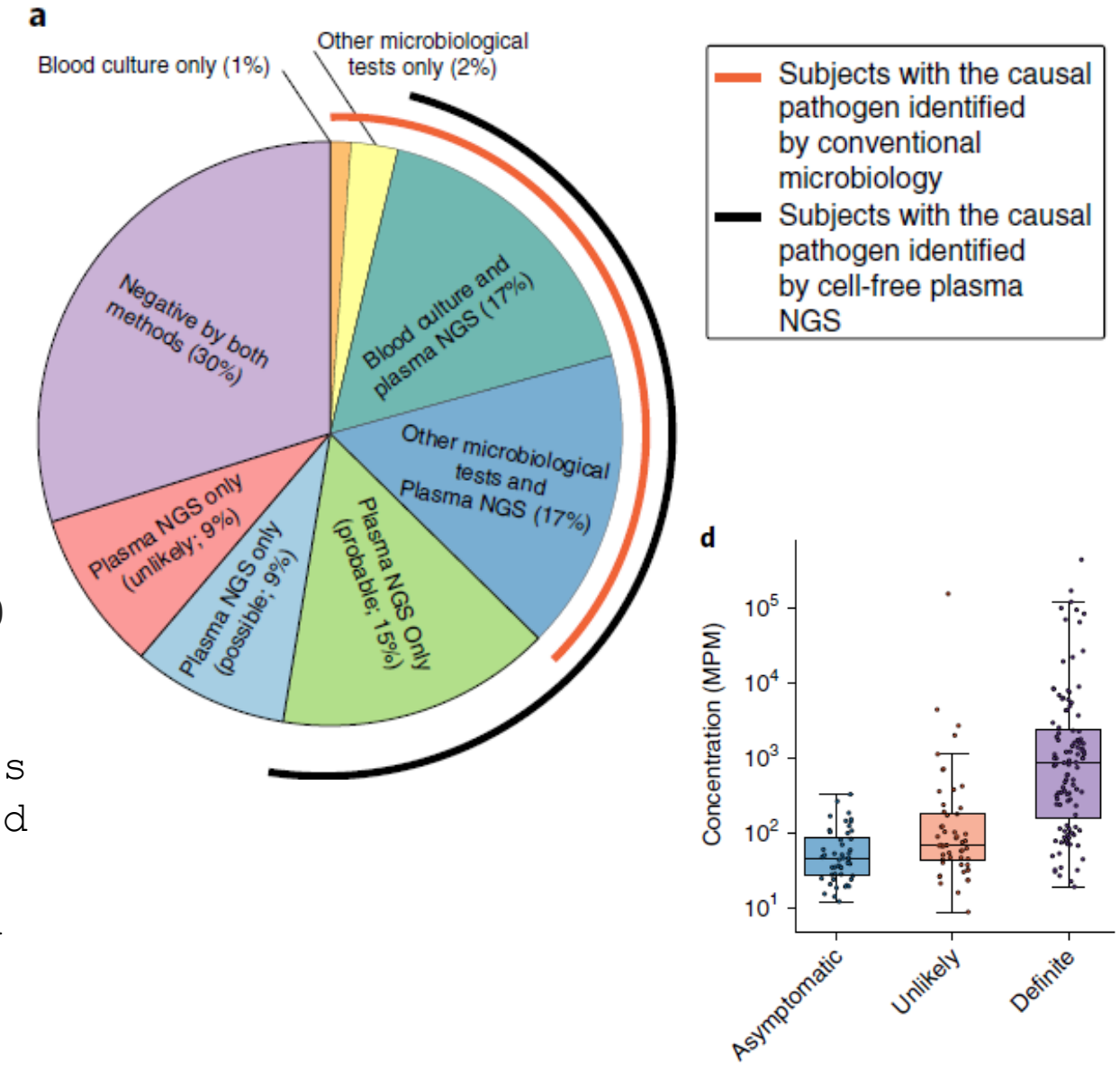
ARTICLES

https://doi.org/10.1038/s41564-018-0349-6

## Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease

Timothy A. Blauwkamp[1,3*], Simone Thair[2,3], Michael J. Rosen[1], Lily Blair[1], Martin S. Lindner[1], Igor D. Vilfan[1], Trupti Kawli[1], Fred C. Christians[1], Shivkumar Venkatasubrahmanyam[1], Gregory D. Wall[1], Anita Cheung[1], Zoë N. Rogers[1], Galit Meshulam-Simon[1], Liza Huijse[1], Sanjeev Balakrishnan[1], James V. Quinn[2], Desiree Hollemon[1], David K. Hong[1], Marla Lay Vaughn[1], Mickey Kertesz[1], Sivan Bercovici[1], Judith C. Wilber[1,3] and Samuel Yang[2,3]

- 93.7% agreement with blood culture in a cohort of 350 patients with a sepsis alert and identified
- 166 samples adjudicated to have no sepsis aetiology identified by any of the tested methods, sequencing identified microbial cell-free DNA in 62, likely derived from commensal organisms and incidental findings unrelated to the sepsis alert




Blaukwamp et al, Nat Microbiology, 2019

# Microbial cell-free DNA and clinical decision

| Category of Clinical Impact | Change in Management | Clinical Impact Type |
|---|---|---|
| Positive | Yes | New diagnosis based on Karius result and not confirmed by conventional microbiological methods |
| | | Earlier diagnosis based on Karius result and later confirmed by conventional microbiological methods |
| | | Karius result enabled avoidance of invasive surgical biopsy |
| | | Karius result enabled initiation of appropriate therapy |
| | | Karius result enabled de-escalation of therapy |
| | | Karius result enabled escalation of therapy |
| | No | Karius result confirmed clinical diagnosis |
| Negative | Yes | Karius result led to unnecessary treatment |
| | | Karius result led to additional unnecessary diagnostic investigations |
| | | Karius result led to longer length of stay |
| None | No | Karius result showed new organism but result not acted upon |
| | | Karius result confirmed conventional microbiological diagnosis and not acted upon |
| | | Karius test result was negative and not acted upon |
| | | Patient died before Karius result available |

Hogan et al, CID, 2021

# Microbial cell-free DNA and clinical decision

Clinical Infectious Diseases

MAJOR ARTICLE

IDSA hivma

Clinical Impact of Metagenomic Next-Generation Sequencing of Plasma Cell-Free DNA for the Diagnosis of Infectious Diseases: A Multicenter Retrospective Cohort Study

Catherine A. Hogan, Shangxin Yang, Omai B. Garner, Daniel A. Green, Carlos A. Gomez, Jennifer Dien Bard, Benjamin A. Pinsky, and Niaz Banaei

82 Karius tests - positivity rate: 50/82 (61%)

- Positive impact in 6 (7%) cases
- Negative impact in 3 (4%) cases
- No impact in 71 (87%) cases

SHEA

Original Article

Real-world clinical impact of plasma cell-free DNA metagenomic next-generation sequencing assay

Ishminder Kaur MD, Bennett Shaw, Ashrit Multani MBBS, Christine Pham PharmD

Research

JAMA Oncology | Brief Report

Evaluation of Plasma Microbial Cell-Free DNA Sequencing to Predict Bloodstream Infection in Pediatric Patients With Relapsed or Refractory Cancer

Kathryn P. Goggin, MD; Veronica Gonzalez-Pena, PhD; Yuki Inaba, BS; Kim J. Allison, BSN; David K. Hong, MD; Asim A. Ahmed, MD; Desiree Hollemon, MSN, MPH; Sivaraman Natarajan, PhD; Ousman Mahmud, PhD; William Kuenzinger, BS; Sarah Youssef, MSc; Abigail Brenner, BS; Gabriela Maron, MD; John Choi, MD, PhD; Jeffrey E. Rubnitz, MD, PhD; Yilun Sun, MS; Li Tang, PhD; Joshua Wolf, MBBS, PhD, FRACP; Charles Gawad, MD, PhD

1000 Karius tests from 941 patients.

- Positive impact in 162 (16%) cases
- Negative impact in 16 (2%) cases
- No impact in 822 (82%) cases

Multivariable logistic regression (adult patients)

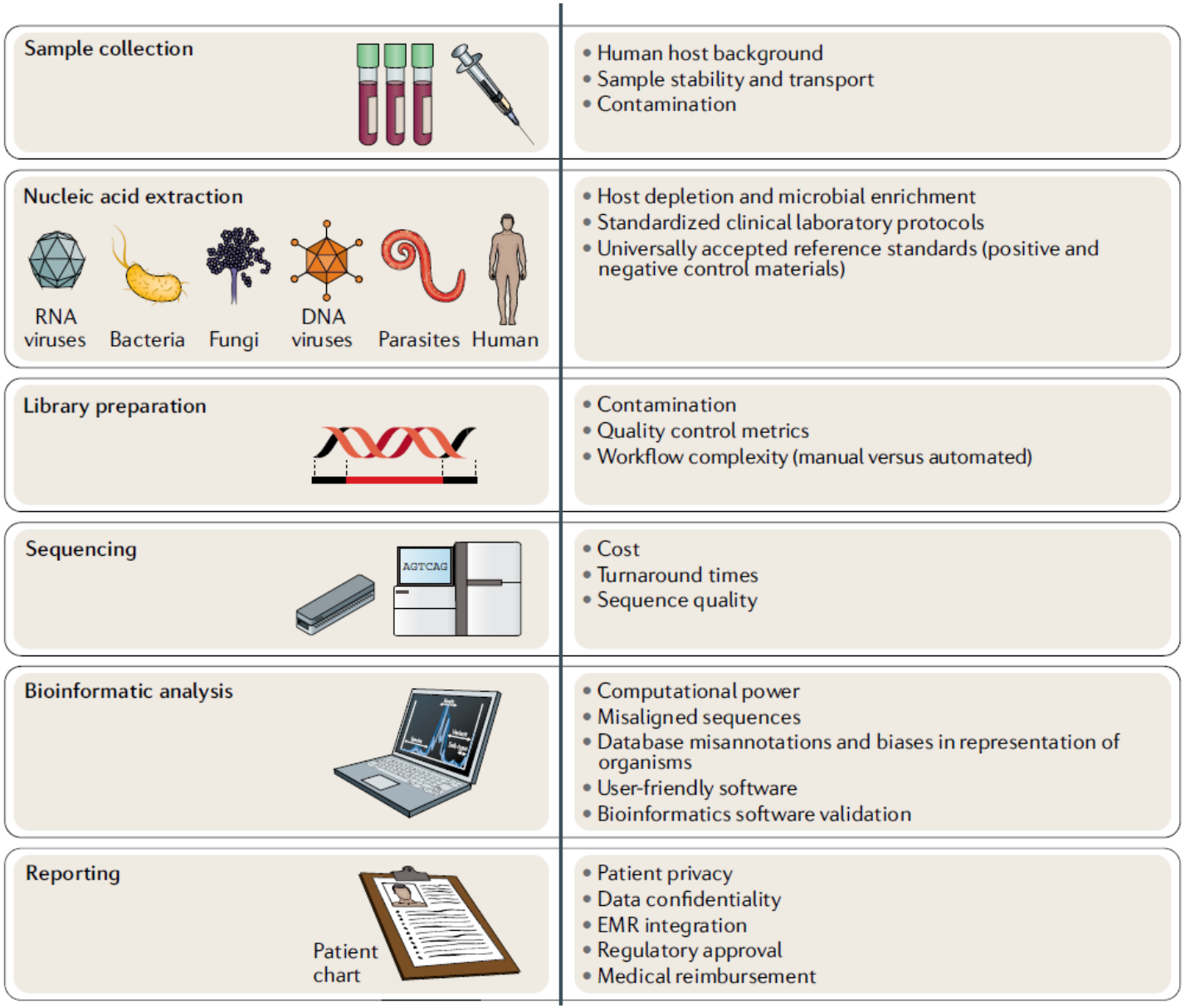
- **Culture-negative endocarditis** (OR 2.3, 95%CI 1.11-4.53)
- Concern for **fastidious/zoonotic/vector-borne pathogens** (OR 2.1, 95%CI 1.11-3.76)

associated with positive clinical impact

- Leftover blood samples were collected during neutropenia due to chemotherapy and BMT
- mcfDNA identified clinically relevant pathogens in blood days before onset of attributable symptoms
- In the 3 days before the onset of BSI: mcfDNA predictive sensitivity of 75%; specificity of 91%
- Tool to **guide pre-emptive therapy**

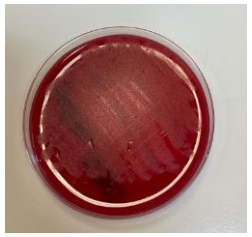
Hogan et al, CID, 2021; Kaur et al, Infect Control Hosp, 2025; Goggin et al, JAMA Oncology, 2020

# Clinical metagenomics feasability



# The quicksand of empirical therapy

8 to 12h | 18 to 24h | 18 to 24h | 18 to 24h | Days, weeks, never?



**Short term incubation + syndromic panels**

**Blood culture collected**

**Gram strain**

**Growth on agar**

**ID & AST**
(e.g. Vitek2, disk diffusion)

**Additional Susceptibility testing**
(e.g. MICs)

**Additional analysis**
(e.g. MLST, PCR)

# How to implement RDT: flowcharts and ASP

## PERCORSO PZ CRITICO – DIAGNOSTICA RAPIDA

Inserimento emocolture nel Sistema Informatico
con richiesta dedicata: Emocoltura paziente critico
Prelevare almeno 2 set

↓ Invio entro 2 ore al LAB di MICRO o CORELAB per l'incubazione

Positivizzazione del flacone (media <24ore)

↓

1. Esecuzione esame batterioscopico (1 ora)
2. Test molecolare rapido: identificazione di specie e meccanismi di resistenza (2ore)
3. Fascia oraria di positivizzazione 8-12: Semina del flacone per identificazione da coltura giovane (4-5 ore) e antibiogramma
4. Fascia oraria di positivizzazione 12-20: Semina del flacone *overnight*

↓

Inserimento dei risultati nel Sistema Informatico
e comunicazione all'infettivologo di guardia (tel.7304)

## PERCORSO ORDINARIO

Inserimento emocolture nel Sistema Informatico
Prelevare almeno 2 set

↓ Invio entro 2 ore al LAB di MICRO o CORELAB per l'incubazione

Positivizzazione del flacone (media <24ore)

↓

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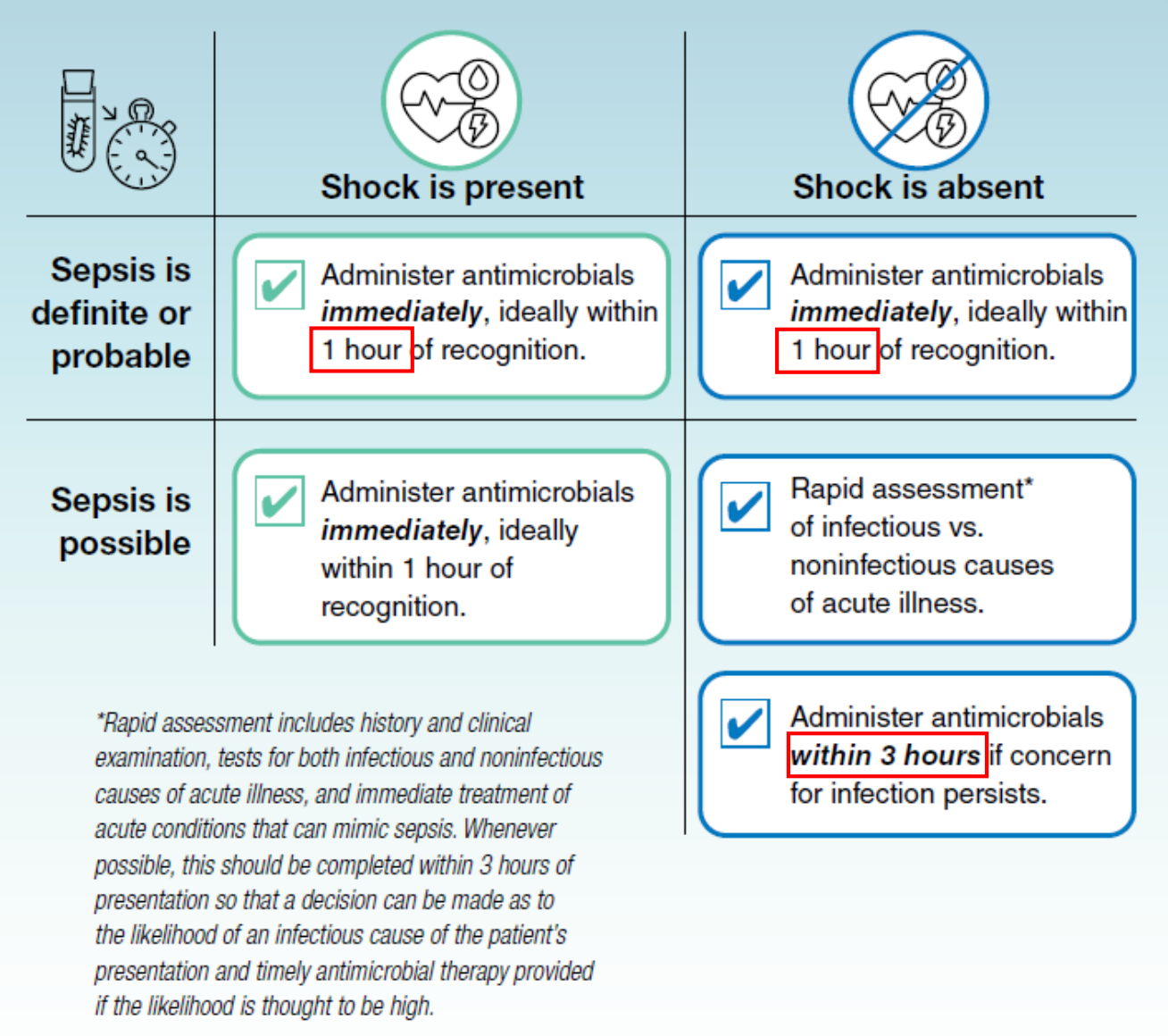
2. Fascia oraria di positivizzazione 8-12: Semina del flacone per identificazione da coltura giovane (4-5 ore) e antibiogramma
3. Fascia oraria di positivizzazione 12-20: Semina del flacone *overnight*

↓

Inserimento dei risultati nel Sistema informatico

# Yes, but what should I administer, as I wait?



| | Shock is present | Shock is absent |
|---|---|---|
| **Sepsis is definite or probable** | ✔ Administer antimicrobials ***immediately***, ideally within 1 hour of recognition. | ✔ Administer antimicrobials ***immediately***, ideally within 1 hour of recognition. |
| **Sepsis is possible** | ✔ Administer antimicrobials ***immediately***, ideally within 1 hour of recognition. | ✔ Rapid assessment* of infectious vs. noninfectious causes of acute illness.<br>✔ Administer antimicrobials ***within 3 hours*** if concern for infection persists. |

**Rapid assessment includes history and clinical examination, tests for both infectious and noninfectious causes of acute illness, and immediate treatment of acute conditions that can mimic sepsis. Whenever possible, this should be completed within 3 hours of presentation so that a decision can be made as to the likelihood of an infectious cause of the patient's presentation and timely antimicrobial therapy provided if the likelihood is thought to be high.*

Evans et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock (2021)

# A never-ending puzzle

| **Clinica riferita** | **Opzioni terapeutiche** |
|---|---|
| Effetti collaterali: nausea, diarrea, pirosi gastrica, ipoacusia, dolori addominali, etc.<br>Familiarità per allergia alla penicillina.<br>→ **Paziente NON allergico** | Penicillina o derivati<br>(cercando di evitare gli effetti collaterali) |
| Eruzione maculo-papulare con/senza prurito<br>Anamnesi positiva di allergia alla penicillina senza ricordo del paziente<br>→ **Reazioni lievi con caratteristiche IgE-mediate** | Cefalosporina di 3^, 4^ o 5^ generazione, Aztreonam, carbapenemi o antibiotici non beta-lattamici<br><br>Penicillina o cefalosporina di 1^ o 2^ generazione **somministrate con test dose** |
| SJS, TEN, DRESS, DiHS, anemia emolitica, citopenie, epatite, nefrite<br>→ **Reazioni gravi** | Aztreonam o antibiotico non beta-lattamico |
| Orticaria, angioedema, anafilassi, asma, ipotensione<br>→ **Reazioni con caratteristiche IgE-mediate** | Cefalosporina di 3^, 4^ o 5^ generazione o carbapenemi **con test dose**<br><br>Aztreonam o antibiotico non beta-lattamico |

Sistema Socio Sanitario
Regione Lombardia
ASST Fatebenefratelli Sacco

Adapted from Pea, Viale et al. Critical Care, 2009

# A never-ending puzzle

**Disease severity**

- Clinical presentation, hemodynamic instability, multiorgan failure, patient setting
- Sepsis/septic shock warrants wider antimicrobial spectrum

**Risk factors and local epidemiology**

- Severe manifestation: comorbidities, compromised immunity
- MDR pathogens: indwelling devices, previous colonisation/infection, previous antibiotic therapy, long-term care facilities
- Local microbiological surveillance and updated reporting > cumulative AST

**Site of infection**

- Medical history, signs and symptoms, first level findings
- Guides further radiological and microbiological exams, and source control!
- Inform antimicrobial choice (tissue penetration)

**Patient physiopathology and PK/PD > TDM!**

- Kidney failure, need for hemofiltration
- Obesity
- Burns, hypoproteinemia
- Drug abuse (methadone!), drug-drug interactions

# Guidelines informed by local epidemiology

**8.15. Sepsi e shock settico di origine sconosciuta o ancora non determinata**

| Quadro clinico | Prima scelta | Alternative | Note |
|---|---|---|---|
| Paziente proveniente dalla comunità o ospedalizzato < 48h<br><br>Nessun fattore di rischio per ESBL<br><br>Non note colonizzazioni da MDRO | **Piperacillina/Tazobactam**<br>9 g dose carico in 1-2h, quindi 18 g/24h ev infusione continua (in alternativa 4.5 g/6h in infusione di 3-4h)<br>+<br>**Daptomicina**<br>10 mg/kg/24h ev<br>o, se sospetta localizzazione polmonare<br>**Linezolid** TDM !Controlla interazioni!<br>600 mg/12h ev<br>*Possibile ottimizzare infusione:*<br>600 mg dose carico in 1h, a seguire 1200 mg/24h in infusione continua | Allergia<br>**Ceftazidima**<br>2 g dose carico in 1-2h, quindi 6 g/24h ev infusione continua (in alternativa 2 g/8h in infusione di 3-4h)<br>o<br>**Meropenem**<br>2 g dose carico in 1-2h, quindi 2 g/8h ev in infusione di 4h<br><br>Alternativa a Daptomicina/Linezolid<br>**Vancomicina** TDM<br>20-30 mg/kg dose di carico, a seguire 15-20 mg/Kg/12h ev, *oppure*<br>15-20 mg/Kg dose carico, poi 30-40 mg/Kg/24h ev in infusione continua | Se shock settico e potenziale origine intraddominale/urinaria dell'infezione, valutare aggiunta di un aminoglicoside<br>**+ Amikacina**<br>20 mg/Kg ev per la prima dose<br>Dose e intervalli successivi da stabilire in base a **TDM** (concentrazione di valle).<br>Se TDM non disponibile, proseguire ogni 24 ore fino a stabilità emodinamica (massimo per 3 giorni)<br><br>Preferire Daptomicina al posto di Vancomicina se:<br>• Assenza di localizzazione polmonare dell'infezione<br>• Sospetto di infezione correlata a catetere vascolare<br>• eGFR basale < 30 ml/min<br>• Allergia a Vancomicina |
| Paziente ospedalizzato da > 48h<br><br>Oppure<br><br>Fattori di rischio ESBL | **Meropenem**<br>2 g dose carico in 1-2h, quindi 2 g/8h ev in infusione di 4h<br>+<br>**Daptomicina**<br>10 mg/kg/24h ev<br>o, se sospetta localizzazione polmonare<br>**Linezolid** TDM !Controlla interazioni!<br>600 mg/12h ev<br>*Possibile ottimizzare infusione:*<br>600 mg dose carico in 1h, a seguire 1200 mg/24h in infusione continua | Alternativa a Daptomicina/Linezolid<br>**Vancomicina** TDM<br>20-30 mg/kg dose di carico, a seguire 15-20 mg/Kg/12h ev, *oppure*<br>15-20 mg/Kg dose carico, poi 30-40 mg/Kg/24h ev in infusione continua<br><br>Se paziente con Allergia a beta-lattamici, esegui una corretta anamnesi (Meropenem è spesso un'opzione sicura) | |

# BSI in Italian ICUs

Sistema Socio Sanitario Regione Lombardia
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ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI · IRCCS

GiViTI
Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva

## BLOODICU bloodstream infections and their outcomes in ICU patients: insights from the PROSAFE study

02. Bacterial infection & disease
02b. Severe sepsis, bacteraemia & endocarditis (incl epidemiology, diagnosis, host biomarkers, treatment, and outcome prediction)

C. Genovese[1], M. Offer[2], G. Tricella[3], G. Scaglione[1], E. Palomba[1], G. Montrucchio[4], F. Agostini[5], F. Dore[3], G. Monti[6], B. Viaggi[7], A. Gori[1], S. Finazzi[3], M. Colaneri[2].
*[1]Department of Infectious Diseases, ASST Fatebenefratelli Sacco University Hospital - Milan (Italy), [2]Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan - Milan (Italy), [3]Laboratory of Clinical Data Science, Department of Public Health, Mario Negri Institute for Pharmacological Research IRCCS - Ranica (Italy), [4]Department of Surgical Sciences, University of Turin - Turin (Italy), [5]Anesthesia and Intensive Care 3, University Hospital City of Science and Health, CTO Hospital - Turin (Italy), [6]Dipartimento di Anestesia e Rianimazione, ASST Grande Ospedale Metropolitano Niguarda - Milan (Italy), [7]Department of Anaesthesiology, Neuro-Intensive Care Unit, Careggi University Hospital - Florence (Italy)*

ESCMID Global
Congress of the European Society of Clinical Microbiology and Infectious Diseases

- 10-years retrospective analysis
- 211’491 included patients
- 192 Italian ICUs

Genovese et al, ESCMID Global 2025

# BSI in Italian ICUs

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DIBIC – Dipartimento di Scienze Biomediche e Cliniche – Scientia et Salus

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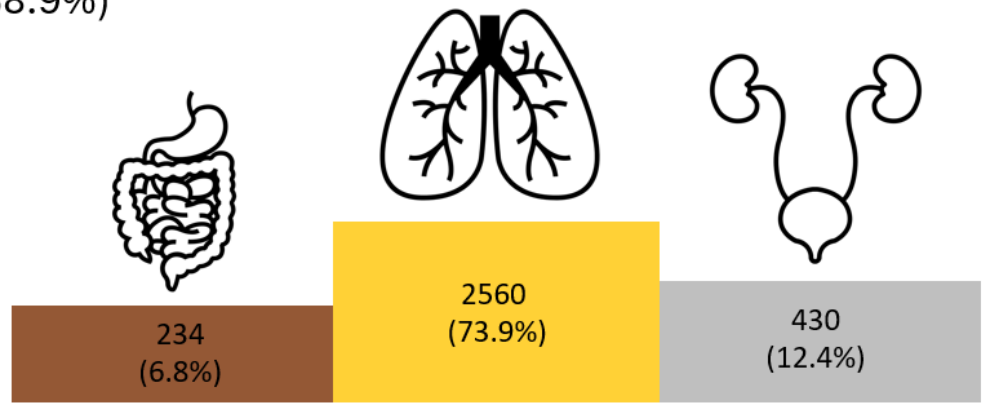
- 8909 BSI episodes from 2014 to 2023
- Prevalence: 4.2% in at risk patients
- 10 days (4.0 - 17.0) from ICU admission to BSI onset


- 3463 S-BSIs (38.9%)




**LOS ICU**

**Total**
5.0 (3.0, 11.0)

**BSI**
25.0 (16.0, 38.0)

**NO-BSI**
5.0 (3.0, 10.0)

P<0.001

**In-ICU mortality**

**Total**
17.4%

**BSI**
28.9%

**NO-BSI**
16.9%

P<0.001

Genovese et al, ESCMID Global 2025

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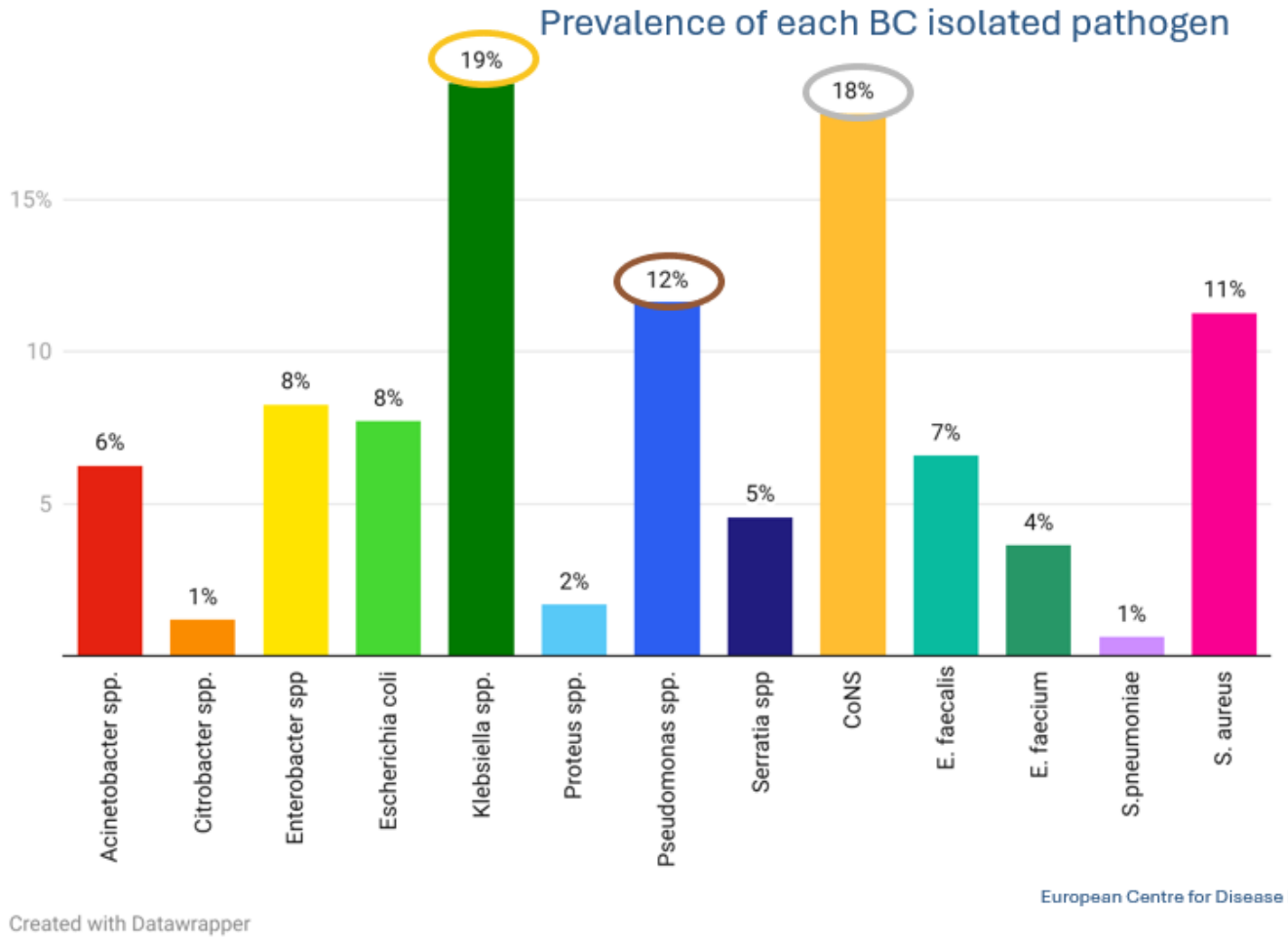
- 9956 isolated species:




vs:

- ECDC: CoNS 24.9% > *Enterococcus* spp. 19.1% > *Klebsiella* spp. 11.3%
- EUROBACT-2: *Klebsiella* spp. 27.9% > *Acinetobacter* spp. 20% > *E. coli* 15% GPB: *Enterococcus* spp. 35% > CoNS 30% > *S. aureus* 27%

*Intensive Care Med (2023) 49:178–190* https://doi.org/10.1007/s00134-022-06944-2
European Centre for Disease Prevention and Control. Healthcare-associated infections acquired in intensive care units. In: ECDC. Annual Epidemiological Report for 2021. Stockholm: ECDC; 2024.

Genovese et al, ESCMID Global 2025

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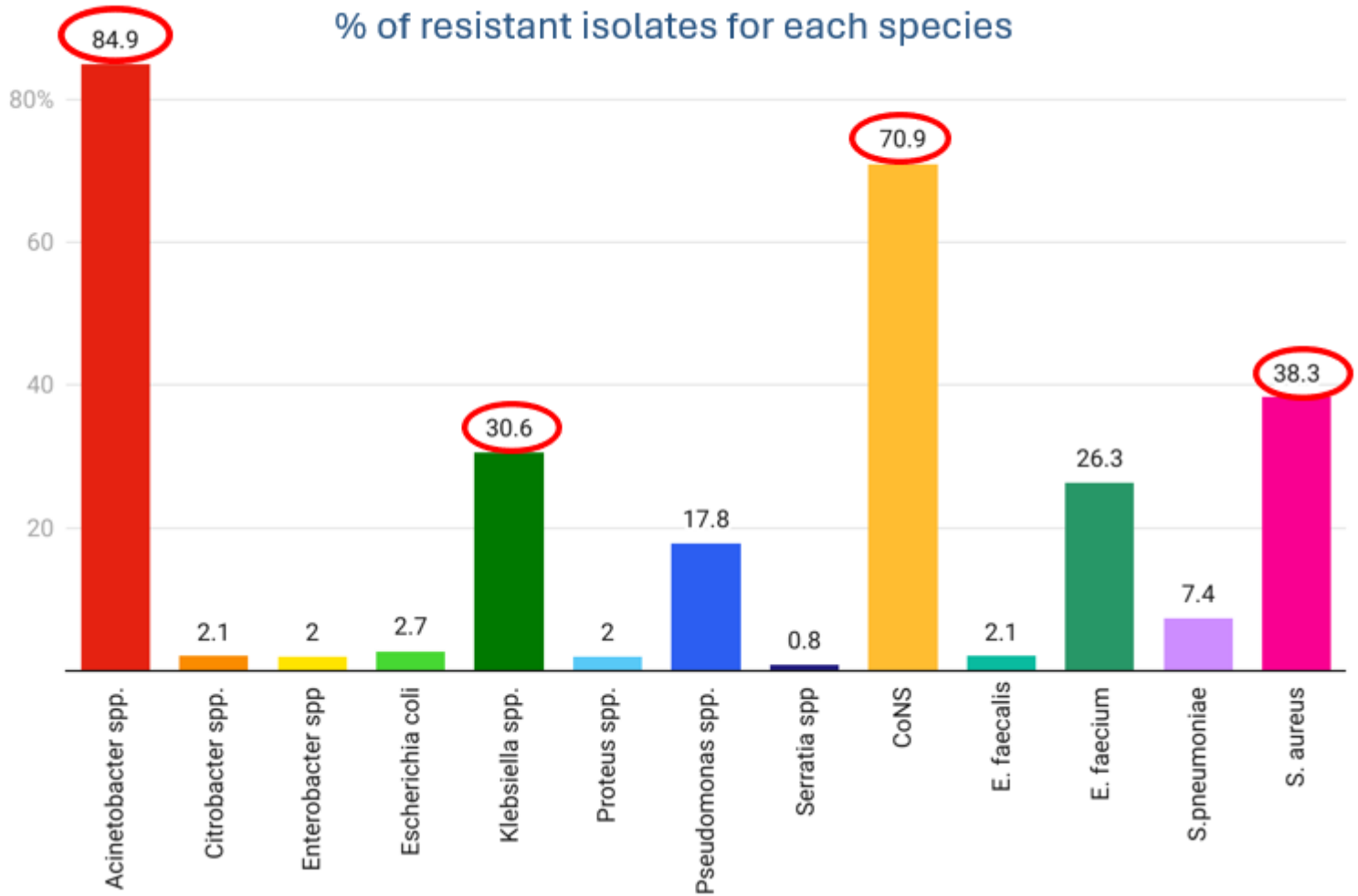
- 8561 tested species:




Genovese et al, ESCMID Global 2025

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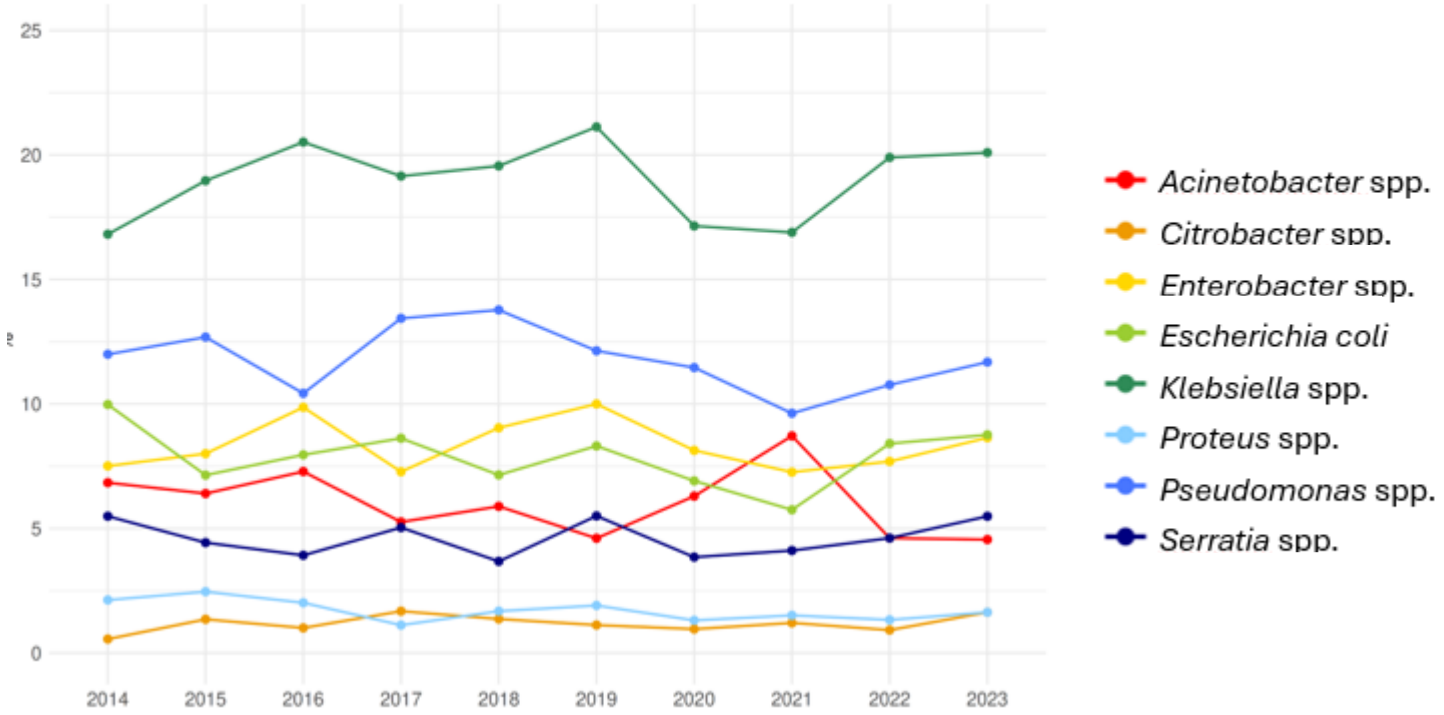
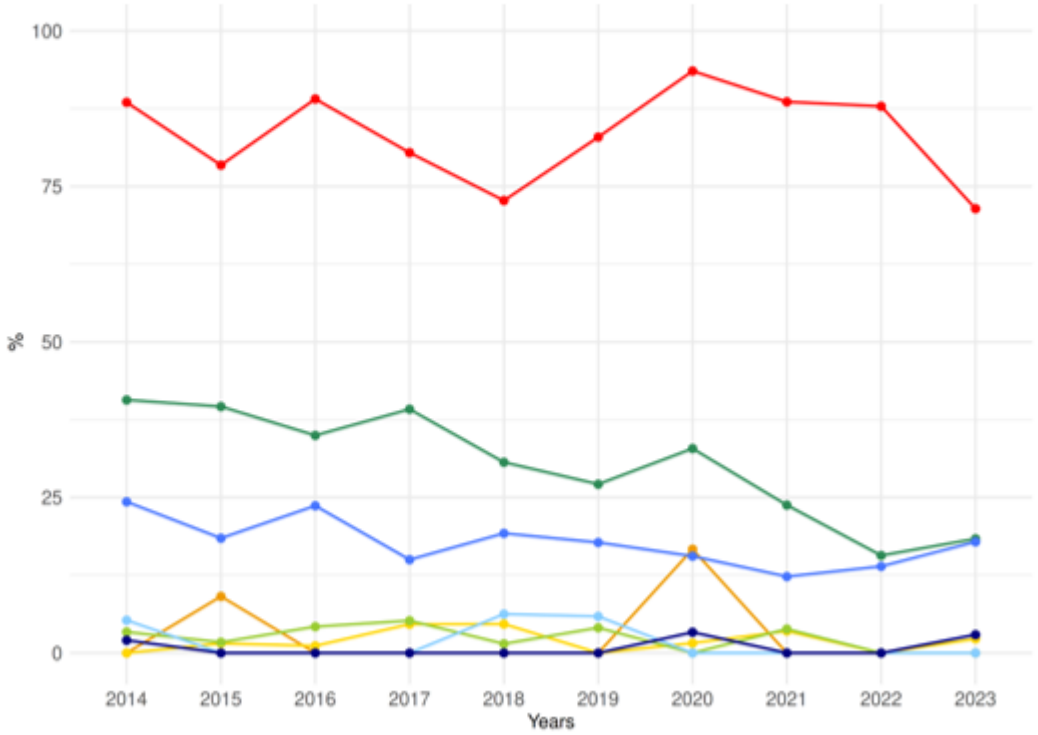
## GNB BSI from 2014 to 2023




## CR-GNB BSI from 2014 to 2023




Genovese et al, ESCMID Global 2025

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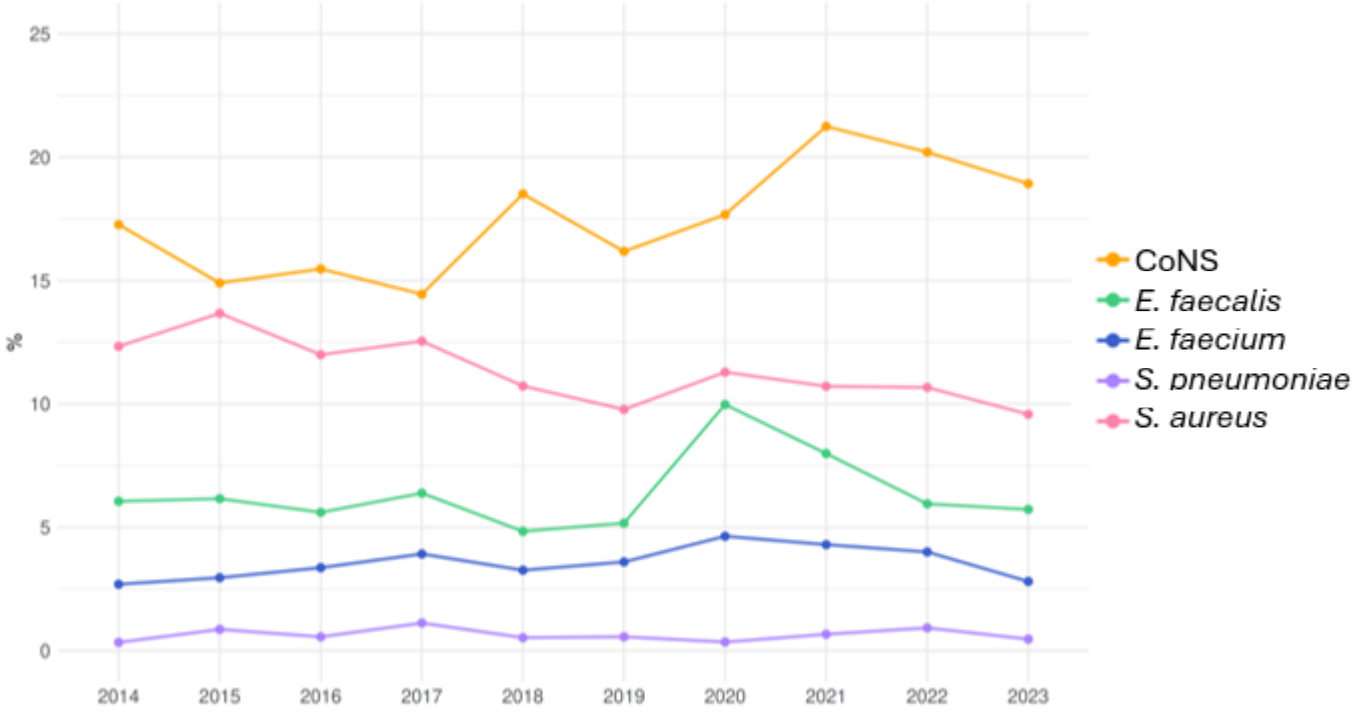
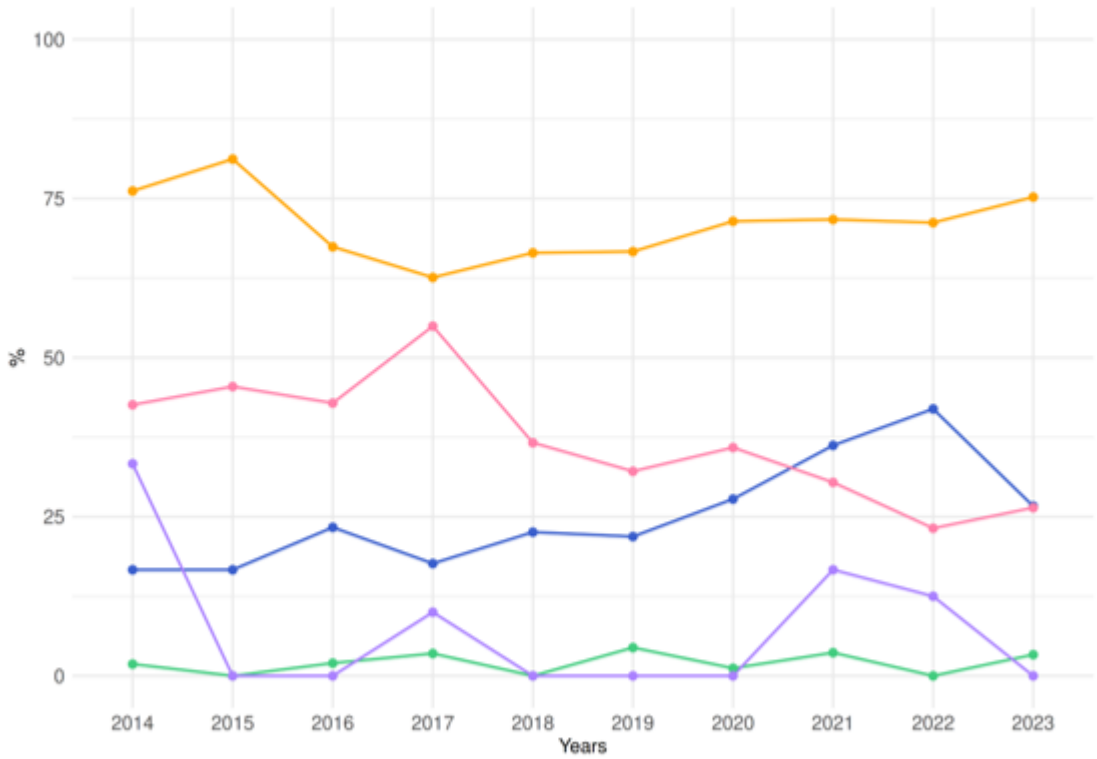
*Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva*

## GPB BSI from 2014 to 2023




## GPB-DR BSI from 2014 to 2023




CoNS, coagulase negative staphylococci; *E. faecalis*, *Enterococcus faecalis*; *E. faecium*, *Enterococcus faecium*; *S. aureus*, *Staphylococcus aureus*; *S. pneumonaie*, *Streptococcus pneumoniae*; DR for S. aureus and CoNS is defined by methicillin resistance, DR for Enterococci is defined by vancomycin resistance, DR for *S. pneumoniae* is defined by penicillin resistance.

Genovese et al, ESCMID Global 2025

# Final considerations

Effective management of BSI requires close and timely collaboration between infectious disease specialists and clinical microbiologists

Implementation of the most recent technologies on positive cultures—such as rapid incubation systems and syndromic panels—is mandatory (starting with critical patients?)

Clinical metagenomics from whole blood holds great promise, especially in selected cases, but is currently limited by high costs, along with analytics and interpretative challenges

The adoption of rapid diagnostic technologies must be integrated within antimicrobial stewardship programs to improve clinical outcomes (and costs!)

Empiric antibiotic therapy should be individualized based on patient-specific characteristics (e.g., disease severity, risk factors, PK/PD considerations) and guided by thorough knowledge of antimicrobial agents and local epidemiology

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