
Names of God

# Same-day antimicrobial susceptibility test using acoustic enhanced flow cytometry

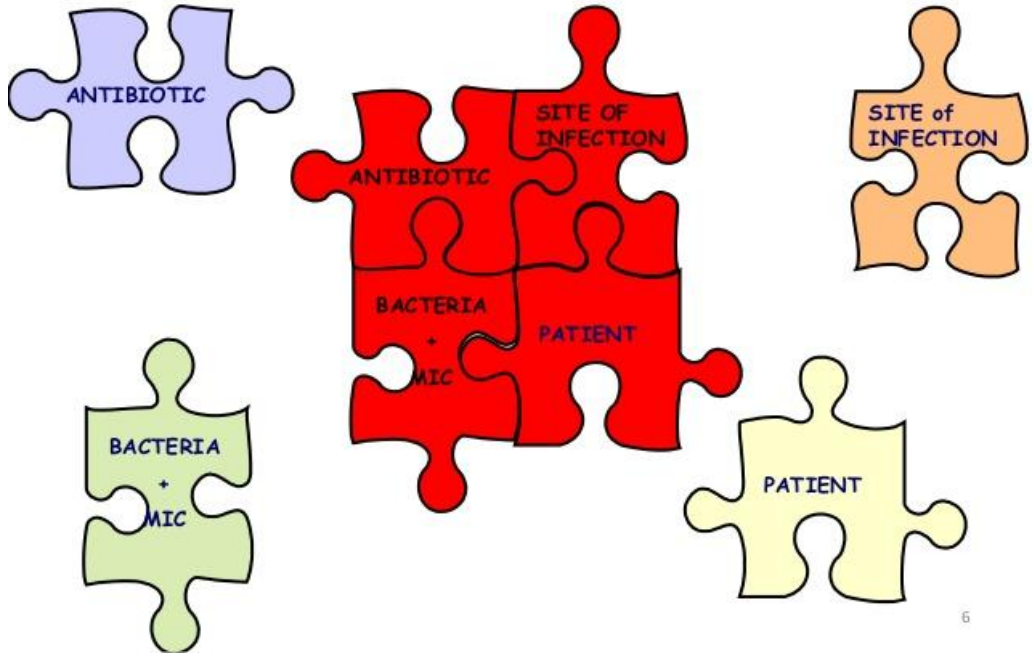


Mohammad Sadegh Damavandi,
Ph.D. student of medical bacteriology,
Department of Microbiology, School of Medicine,
Isfahan University of Medical Sciences, Isfahan,
Iran

# Antimicrobial susceptibility test (ASTs):

A. To optimized antimicrobial therapy
B. Antimicrobial resistance surveillance
C. New antimicrobial agent discovery

A




B

GLASS
Global AMR Surveillance System

C

# Antimicrobial susceptibility test

- Most often expressed in a binary form as **sensitive** or **resistant**
- In some instances, antimicrobial susceptibility needs to be expressed as a **quantitative** measurement known as the **MIC**
- The current international AST **reference** method is the **broth microdilution** (BMD) version of the MIC
- Currently using classical culture-dependent microbiology methods that provide a susceptibility profile within **48 h**, or **longer**

# Why we need faster, more accurate laboratory detection of ASTs?

- Antimicrobial resistance (AMR) is a global problem
- AMR is expected to be responsible for 10 million deaths annually by **2050**
- an estimated **30–50%** of all antimicrobial prescriptions are unnecessary (USA!!!)
- **Excessive** and otherwise **inappropriate** prescription of antibiotics promote resistance
- Serious **life-threatening** infections

In 2015, the White House released the **National Action Plan** for **Combating Antibiotic-Resistant Bacteria**, **targeting a 50% reduction** of inappropriate antibiotic prescribing in the outpatient setting by **2020**

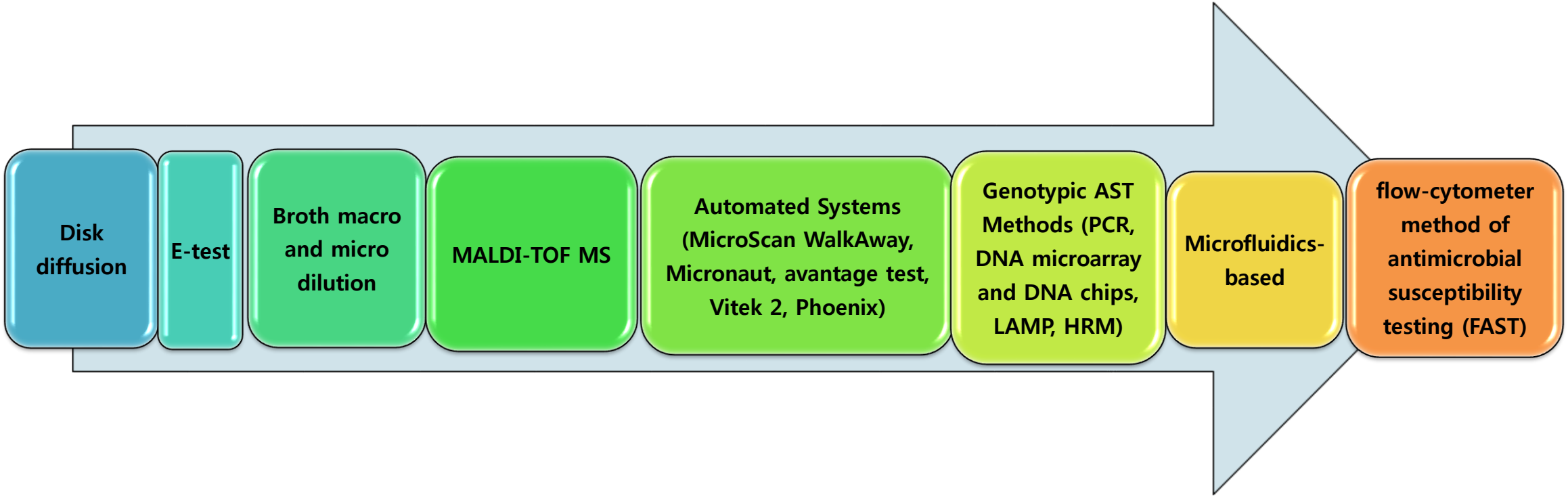
to address the problem of resistance could result in:

**10m deaths by 2050**

**Costing £66 trillion**

# Old and new antimicrobial susceptibility test methods


**Slow** the Emergence of Resistant Bacteria and **Prevent** the Spread of Resistant Infections



# Flow-cytometer method of antimicrobial susceptibility testing (FAST)

## Methods

### Software

Data machines were designed, assembled and run using open access data-mining software (Orange v3.20, University of Ljubljana, Slovenia) [9]; under a Creative Commons license. Orange was run under Windows 10 (Microsoft, CA, USA). Statistical analysis was conducted in Prism v8 (GraphPad, San Diego, CA, USA).

### Bacterial strains

*Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* –2841 (clinical), *Klebsiella pneumoniae* ATCC 700603, *K. pneumoniae* ATCC 700603, *K. pneumoniae* ATCC BAA-1705, *K. pneumoniae* ATCC BAA-1706, *K. pneumoniae* ATCC 13883, *Proteus mirabilis* –9545 (clinical), *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 33592, *S. aureus* –6885 (clinical), *Enterococcus faecalis* ATCC 29212. Clinical isolates: *E. coli* 1A and *S. aureus* 9B. Curated by Western Australian

# Methods con…

## Antimicrobial susceptibility series

**We used commercial, custom, pre-dispensed 96-well microtitre plates containing dilution series of antimicrobial agents for standardized broth microdilution MIC**

### Enterobacterales

SEMPA1, containing amikacin (0.5–32 μg $ml^{-1}$), aztreonam (1–64 μg $ml^{-1}$), ciprofloxacin (0.12–8 μg $ml^{-1}$), colistin (0.25–32 μg $ml^{-1}$), cefepime (1–32 μg $ml^{-1}$), gentamicin (0.25–16 μg $ml^{-1}$), imipenem (0.25–32 μg $ml^{-1}$), levofloxacin (0.12–8 μg $ml^{-1}$), meropenem (0.12–32 μg $ml^{-1}$), piperacillin-tazobactam (1/4-64/4 μg $ml^{-1}$), trimethoprim-sulphamethoxazole (0.12/2.38-16/304 μg $ml^{-1}$), ceftazidime (0.5–32 μg $ml^{-1}$) and tobramycin (0.25–16 μg $ml^{-1}$).

### Gram positive cocci

SEMSE3, containing amikacin (2–64 μg $ml^{-1}$), azithromycin (0.5–8 μg $ml^{-1}$), ciprofloxacin (0.12–8 μg $ml^{-1}$), clarithromycin (0.25–8 μg $ml^{-1}$), clindamycin (0.12–4 μg $ml^{-1}$), cefoxitin (1–16 μg $ml^{-1}$), gentamicin (0.12–4 μg $ml^{-1}$), levofloxacin (0.12–8 μg $ml^{-1}$), linezolid (0.5–16 μg $ml^{-1}$), moxifloxacin (0.06–4 μg $ml^{-1}$), norfloxacin (1–16 μg $ml^{-1}$), ofloxacin (0.25–4 μg $ml^{-1}$), penicillin (0.03–0.5 μg $ml^{-1}$), teicoplanin (0.25–16 μg $ml^{-1}$), tobramycin (0.12–4 μg $ml^{-1}$) and vancomycin (0.5–16 μg $ml^{-1}$).

# Methods con…

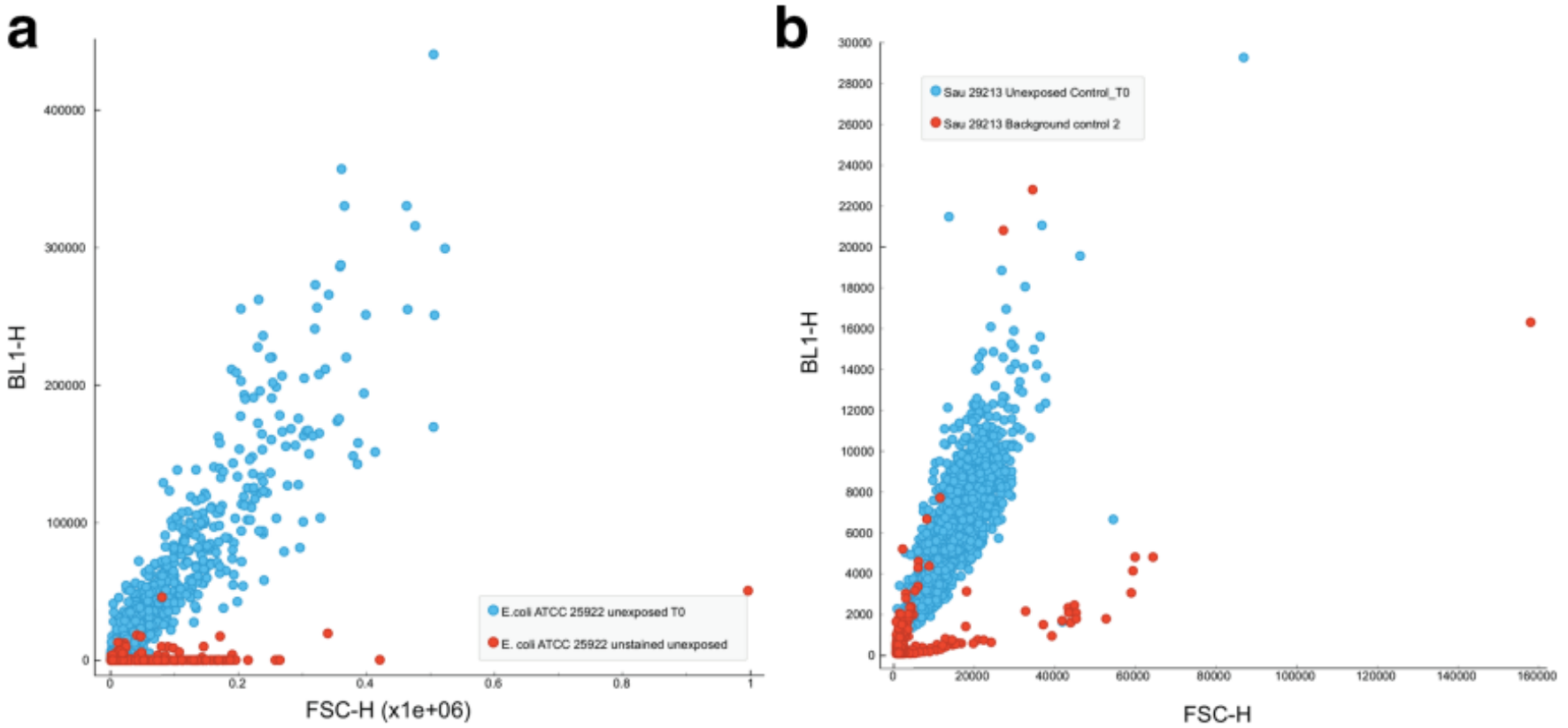
## Bacterial analysis:

**An acoustic flow cytometer (Attune NxT, ThermoFisher Scientific, Eugene, OR, USA) was coupled to a 96-well auto sampler to generate well-by well analysis of SYTO9 nucleic acid intercalating dye (ThermoFisher Scientific, Eugene, OR, USA) -stained bacterial cells after co-incubation with a series of increasing concentrations of the antimicrobial agents.**

A. **The 96-well plate was sealed and incubated without shaking for 1 h (Enterobacterales), or 3 h (Gram-positives)**
B. **20 µl of 5 mM SYTO9 → with 400 r.p.m. shaking, for 8 min**
C. **The 96-well plate was then inserted into the flow cytometer auto-sampler for data acquisition by the flow cytometer.**

# Data generation for AST

# Step one
## data machine 1: antimicrobial unexposed population determiner

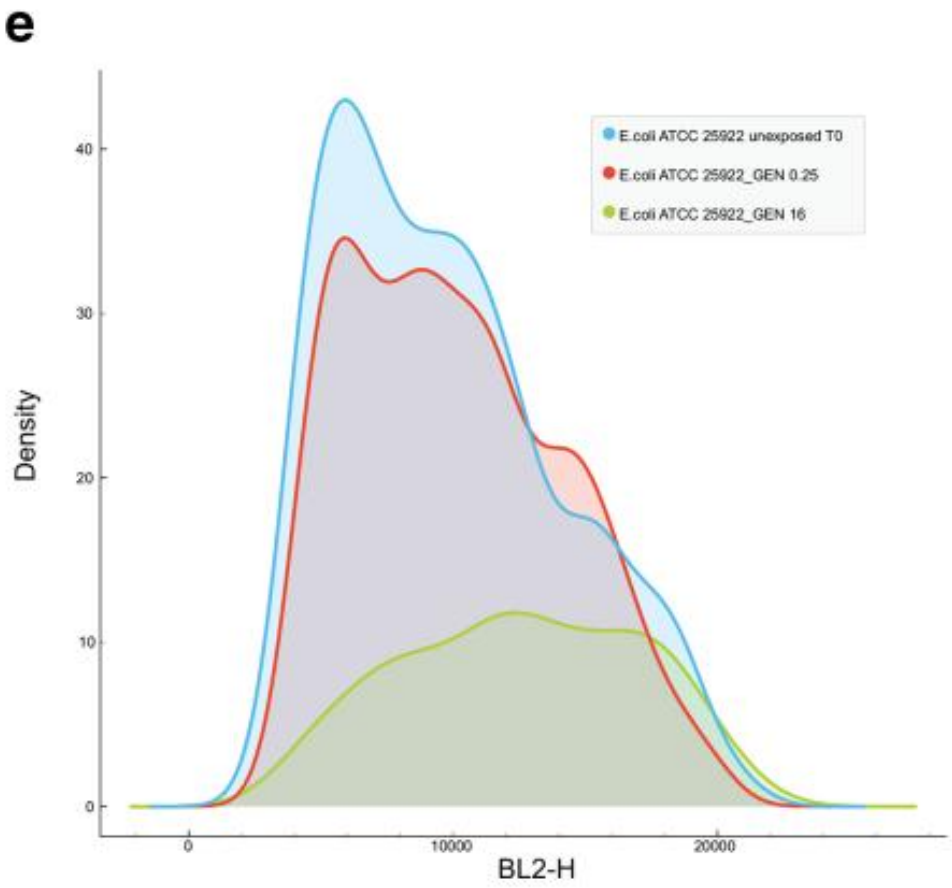



Antimicrobial-unexposed population. Antimicrobial unexposed bacteria (blue) and background particulate noise (red). antimicrobial agent-unexposed population (AUP)

# Step one

## Data machine 2: ordinal antimicrobial susceptibility classifier

We concatenated the unexposed bacterial suspension data with the lowest and uppermost antimicrobial-exposed bacterial suspension files.

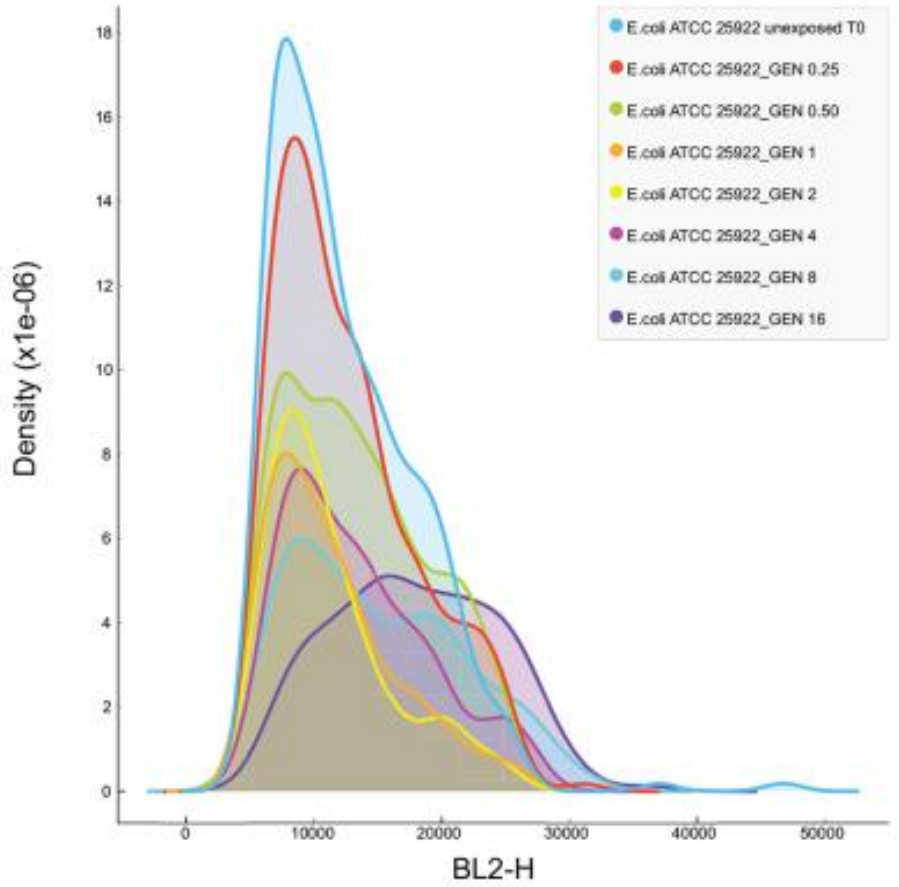



Density histogram of antimicrobial-unexposed population (AUP, blue), and lowest (red) and highest (green) Gentamicin-concentration-exposed *E. coli* ATCC 25922.

# Step one

## Data machine 3: antimicrobial susceptibility classifier for predicted inhibitory concentration

A PIC was determined from the antimicrobial–bacterial combination series when a >50 % reduction in frequency **density from the AUP density curve** was observed. The PIC was then interpreted against the **2019 EUCAST** susceptibility test standards.



Step two

Expanded challenge panel

**AST was performed in parallel by FAST and Sensititre broth microdilution (BMD) methods against three different classes of antimicrobial agent.**

**Nine Gram-negative, and five Gram-positive bacterial species**

**Step three**
**Clinical application**

**The final version of these data machines was then used to process FAST data from new blood culture isolates**

# Results

Using a 50 % fall in peak event density on the most highly ranked channels, the approximate categoric agreement between machine-learning analysis of all SEMPA1 and SEMSE3 MICs was 91.07 % when compared with proprietary flow-cytometry software (FlowJo) and 89.29 % when compared with BMD. (**BMD results fell within the same interpretive category)**

The approximate essential agreement between machine learning and proprietary software was 96.43 % and with BMD was 100%. **(3 results were within ±2 dilutions of the BMD reference value).**

**Table 1.** Data-machine development and calibration series, antimicrobial susceptibility test results

| Species, strain | Antimicrobial agent | BMD[a] | FAST | | BP[d] | S-R[e] |
|---|---|---|---|---|---|---|
| | | | ps[b] | sml[c] | | |
| *E. coli* | Amikacin | 2 | 1 | 2 | 8 | S |
| ATCC 25922 | Aztreonam | 1 | 1 | 1 | 1 | S |
| | Ciprofloxacin | ≤0.12 | ≤0.12 | ≤0.12 | 0.25 | S |
| | Colistin | 1 | ≤0.25 | ≤0.25 | 2 | S |
| | Cefepime | ≤1 | ≤1 | ≤1 | 1 | S |

# Clinical application results

**Table 3.** Clinical isolates, single-pass antimicrobial susceptibility test results

| Species, isolate | Antimicrobial agent | BMD[a] | FAST | | BP[d] | S-R[e] | corrn[f] |
|---|---|---|---|---|---|---|---|
| | | | ps[b] | sml[c] | | | |
| *E. coli* | Piperacillin/tazobactam | 4 | 2 | >64 | 8 | R | 4/S |
| 1A | Gentamicin | 0.5 | 0.5 | 0.5 | 2 | S | |
| | Meropenem | ≤0.12 | ≤0.12 | ≤0.12 | 2 | S | |
| *S. aureus* | Penicillin | >0.5 | >0.5 | >0.5 | 0.12 | R | |
| 5B | Cefoxitin | >16 | 16 | 16 | 4 | R | |
| | Vancomycin | 1 | 0.5 | 1 | 2 | S | |

*a*, BMD, broth microdilution.
*b*, ps, proprietary software.
*c*, sml, supervised machine learning.
*d*, BP, EUCAST susceptible breakpoint (μg $ml^{-1}$).
*e*, S-R, sensitive/resistant categorization.

# Conclusions

**Though the rapid generation of AST data by FAST is valuable in time-critical bacterial infections such as septicemia and bacterial pneumonia, it needs to be complemented by the speed and consistency offered by an automated analytical pipeline.**

Currently may not be available in many clinical microbiology laboratories

In conclusion, supervised machine learning **enabled** us to determine AST classifications **without** the high-end analytic skills of an expert flow-cytometer user or dedicated flow cytometry analytic software.

The combination of machine learning with the FAST method generated same-day AST results and has the potential to aid early **antimicrobial treatment decisions**, Surveillance and detection of resistance.

# Supported by

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Categories

Accelerating Science Behind the Bench General FAST: Flow Cytometry-Assisted Antimicrobial Susceptibility Testing

## FAST: Flow Cytometry-Assisted Antimicrobial Susceptibility Testing

By Behind The Bench Staff
02.25.2019

Dr. Tim Inglis shares with us his findings on FAST methods of antibiotic resistance detection and most recently, on flow cytometry-assisted antimicrobial susceptibility testing using the Attune NxT Flow Cytometer.

**Tim Inglis, Ph.D.**

# Thank You So Much!