

**Welcome**

**to**

**the CDS Workshop**

Melbourne 2014

Jane Hanrahan, Chris McIver & Syd Bell

# Jeanette Pham PhD



# The CDS Reference Lab has moved to Kogarah



# Present Members of Our Team



Jane Hanrahan

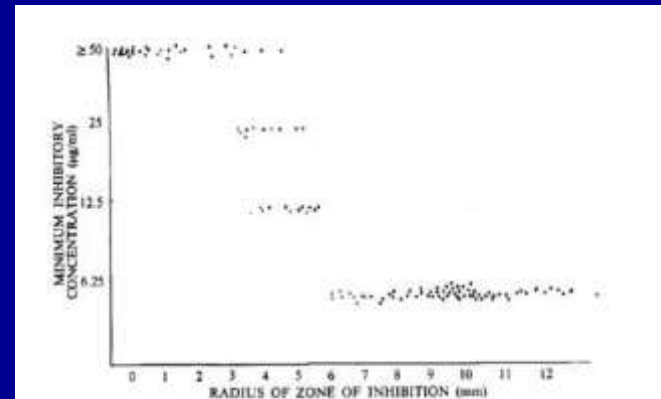
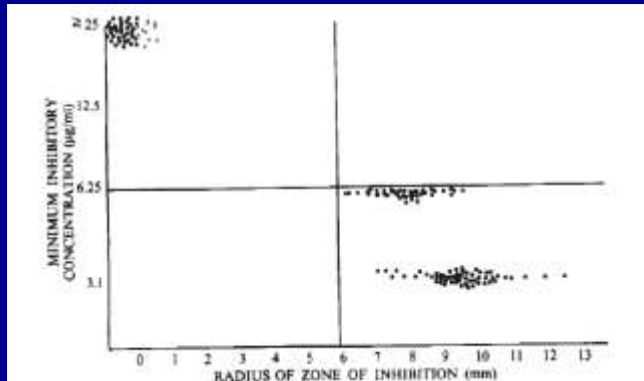
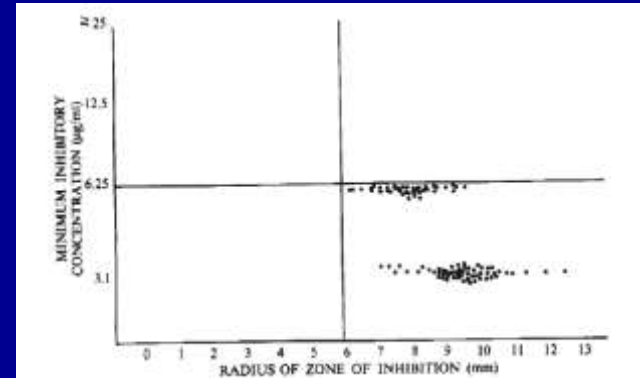
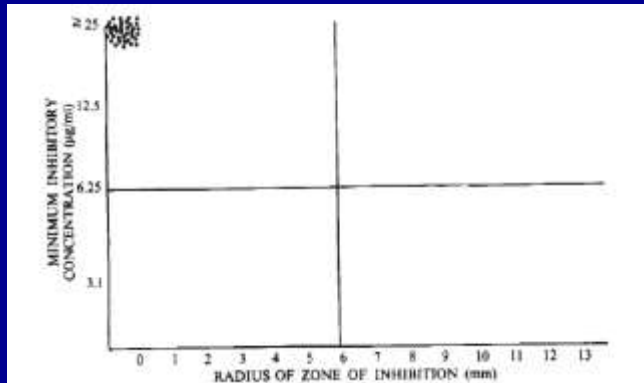


Chris McIver



Peter Newton

# Four Categories of Distribution of Susceptibility



# Accepted Error of MIC Determination

Irrespective of whether by broth or by agar

Error = plus or minus one dilution

If MIC is reported at say 8 mg per litre

It means it lies

somewhere between 4 and 16 mg per litre

# What is a Calibrated Disc Test ?

A calibrated antibiotic disc test is where the inhibitory zone size is calibrated to Minimum Inhibitory Concentration of each of the antibiotics as determined by a quantitative technique.

# Putting the ISO20776 Furphy to rest

- The WHO accepts agar dilution as a reference method for calibration of antibiotic disc testing.
- The FDA specifies agar dilution as a reference standard for assessing all methods of antibiotic susceptibility testing.
- BSAC accepts agar dilution as a method for determining reference MICs.
- The two biggest users of disc tests calibrated by agar dilution have been ISO accredited for > 20 years.



# A “ True” Susceptible MIC

An MIC where a clinical correlation has been established that demonstrates that an infection caused by that particular organism with an MIC of that value or less responds favourably to treatment with the antibiotic tested.

# Other types of Susceptible MIC

- Those where the species consistently has isolates with a low MIC or has a bimodal distribution of high and low MICs and the antibiotic has been effective with other species with similar low MICs.
- Those where the susceptibilities are distributed in a continuous distribution and arbitrary breakpoints are established.

# Harmonisation of Susceptible Breakpoints

## ENTEROBACTERIACEAE

No. of Antibiotics Listed in each method:

CDS 42    Eucast 25    Both 24

Agreement 21    Disagreement 3

( Aztreo (CLSI)

Norflox

Ertapenem (CDS)

# Harmonisation of Susceptible Breakpoints

## Staph. aureus

No. of Antibiotics Listed in each method:

CDS 29    Eucast 24    Both 24

Agreement 20    Disagreement 4

( Benpen (CLSI)    Rifamp (CLSI)    Tetra (CLSI)    Teic (CLSI) )

# Harmonisation of Susceptible Breakpoints

## Pseudomonas

No. of Antibiotics Listed in each method:

CDS 24    Eucast 15    Both 14

Agreement 11    Disagreement 3

(Aztreo (CLSI)

Cefepime

Doripenem (CLSI)

# NDM-1

## A case study of the first NDM-1 producing isolate in Australia

Jane Hanrahan  
CDS Laboratory  
St. George Hospital Kogarah

# NDM-1 – What is it?

- *New Delhi metallo- $\beta$ -lactamase* (NDM-1)
- The newest subgroup to the metallo- $\beta$ -lactamase family
- Ambler class B, Bush Group 3
- Cases of NDM-1 infections are caused by gram negative bacteria from the *Enterobacteriaceae* family, *Acinetobacter spp.* and *Pseudomonas spp.*
- Epidemiological studies indicate NDM-1 originated in India
- Believed Indian subcontinent and Pakistan are a major reservoir for NDM-1 producing bacteria

# NDM-1 Global Dissemination

- First reported case in Sweden 2009
  - Patient transferred from India
  - *Klebsiella pneumoniae* (strain 05-506) from urine
  - *Escherichia coli* isolated from rectal culture
- Reports now extend to UK, Europe, USA, Africa, Middle East, Asia, New Zealand and Australia
- Incidence of infection is increasing
- 6 allelic variations NDM-1 discovered



# NDM-1 Molecular Studies

- *bla*<sub>NDM-1</sub> gene identified on several plasmids
- Multiple antibiotic resistant genes can exist within the same region
- Potential for multiple drug resistant phenotypes

# NDM-1 Molecular Studies

- *bla*<sub>NDM-1</sub> gene located in a highly mobile genetic element
- Ability to transfer into various bacterial genera is unpredictable
- Transfer can be achieved quite easily and quickly

# Case Study – Patient History

- 67 year-old man presented to St. George Hospital, Kogarah ED – feeling unwell
- Clinical history
  - transferred from Bangladesh ICU
  - treated for 12 days with meningoencephalitis
  - showed signs of further deterioration
- Admitted into St. George ICU
- CSU sample obtained

# Case Study – Isolate from CSU

## MICROSCOPY

White Cell Count >100x 10<sup>6</sup>/L

Red Cell Count >100x 10<sup>6</sup>/L

No Epithelial Cells

Organism present: rods

- Isolation of a multidrug resistant *E.coli* from CSU (*E. coli* 271)
- Resistant to all  $\beta$ -lactam antibiotics – including carbapenems
- Resistant to all aminoglycosides, fluoroquinolones, nitrofurantoin and sulfonamides
- Susceptible only to tetracycline, tigecycline, fosfomicin and colistin.

# Case Study - Molecular Investigation

- Multiplex PCR for common MBL genotypes
  - **NEGATIVE** result
- ESBL PCR
  - **CTX-M-15** (common ESBL gene)
  - **TEM-1** (penicillinase gene)
- Multiplex PCR screening for 16S rRNA methylase-encoding genes
  - Two methylase genes – *ArmA* and *RmtB*  
(confers resistance to aminoglycosides)

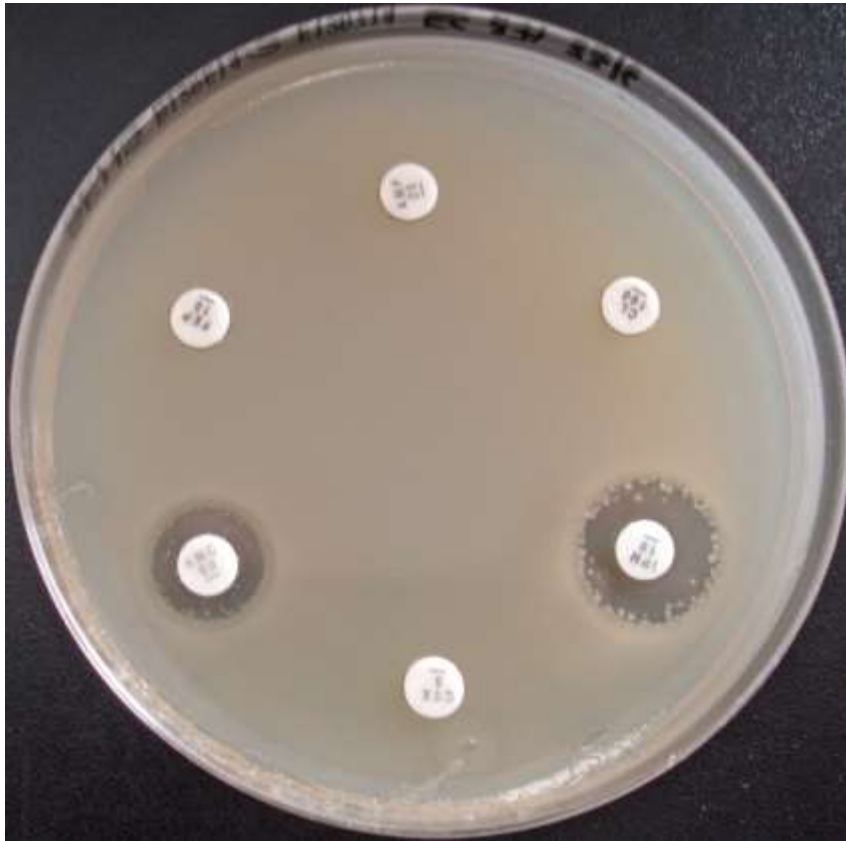
# Case Study - Molecular Investigation

- Further molecular studies and sequencing identified

***bla*<sub>NDM-1</sub> gene**

# **Phenotypic expression of NDM-1 using the CDS method**

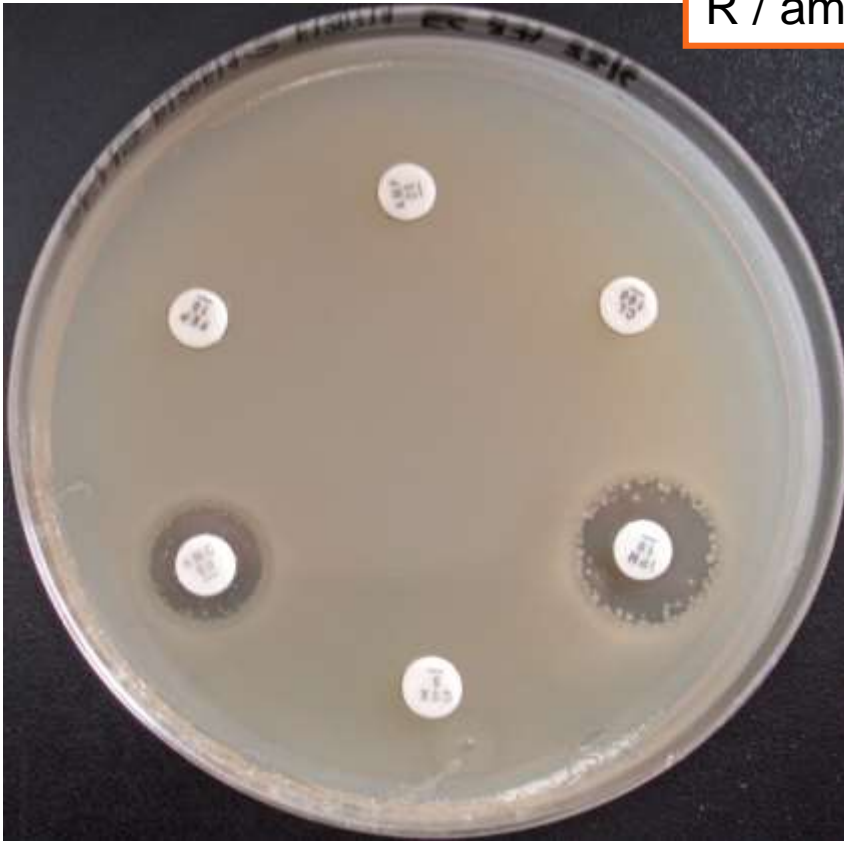
# *E. coli* 271 isolate



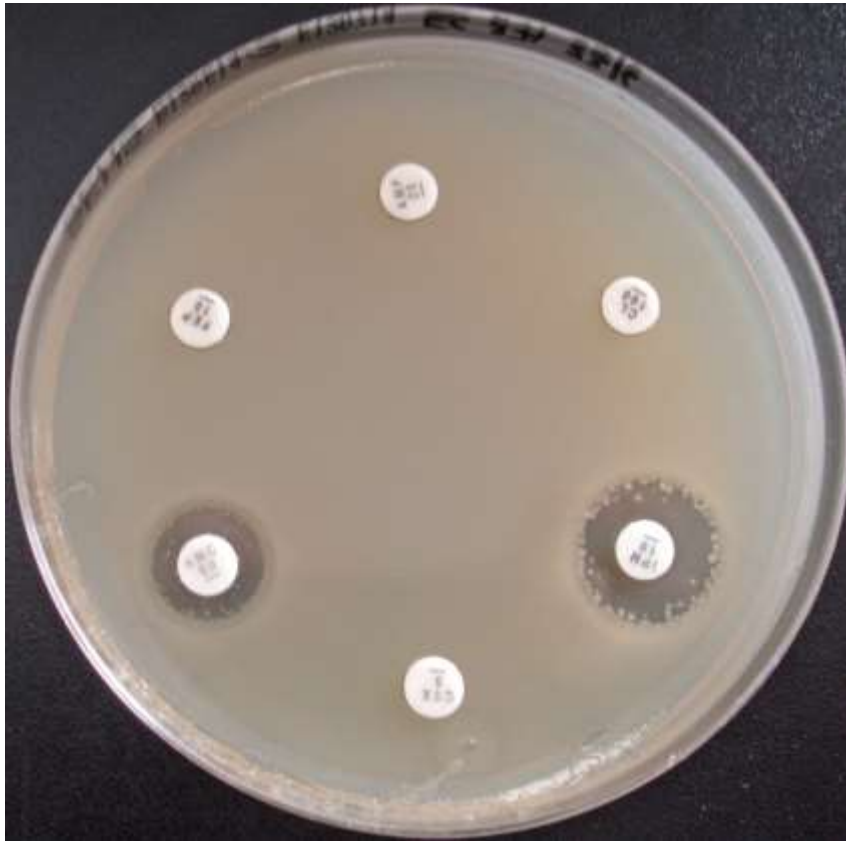


# *E. coli* 271 isolate

R / aminoglycosides

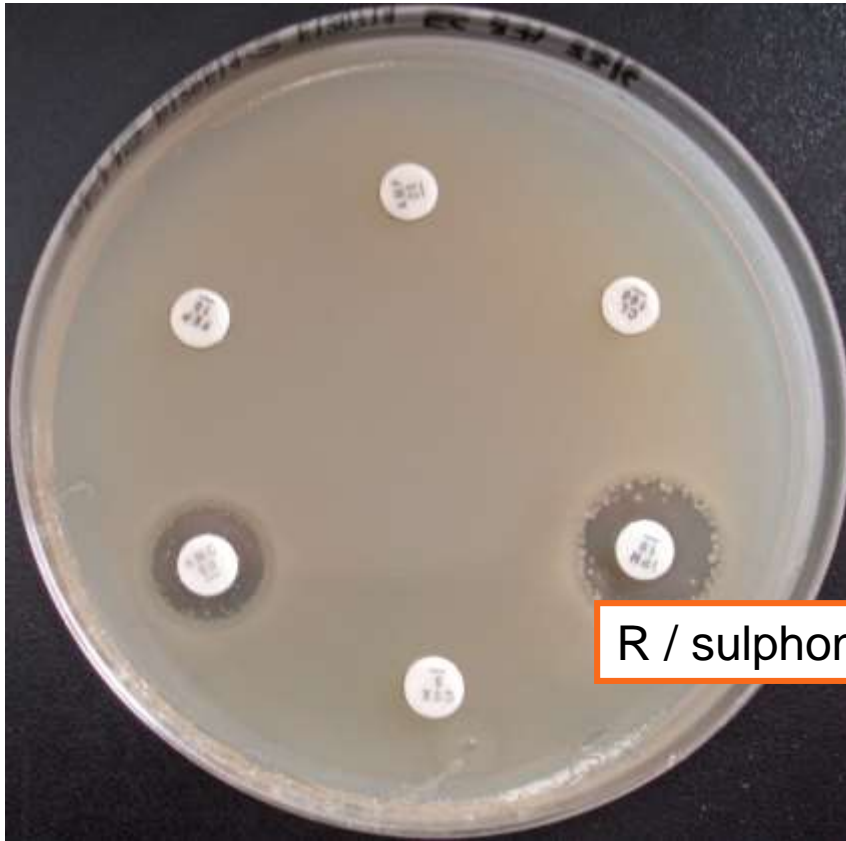


# *E. coli* 271 isolate



R / fluoroquinolones

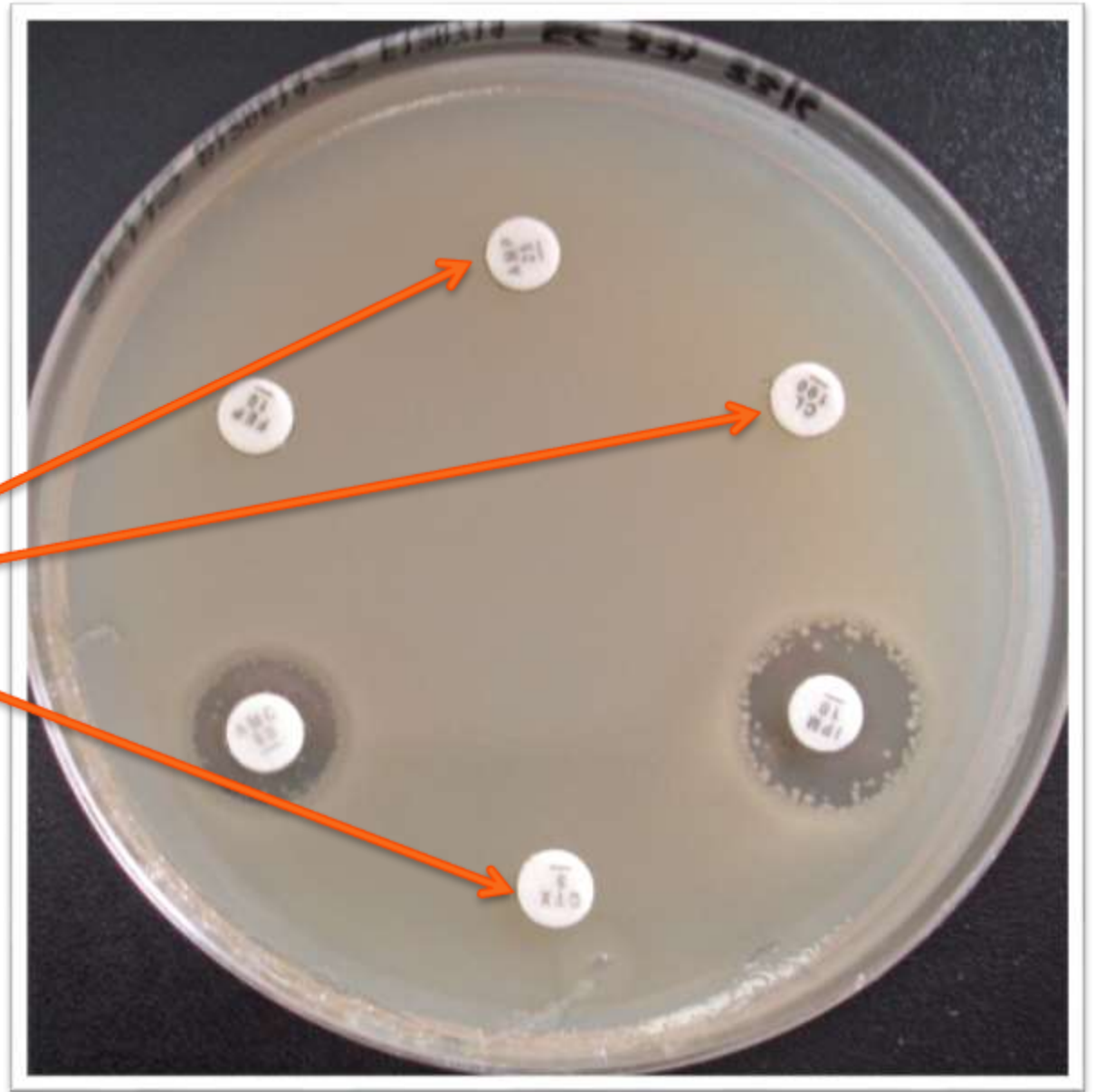
# *E. coli* 271 isolate



R / sulphonamide

*E. coli* 271 isolate

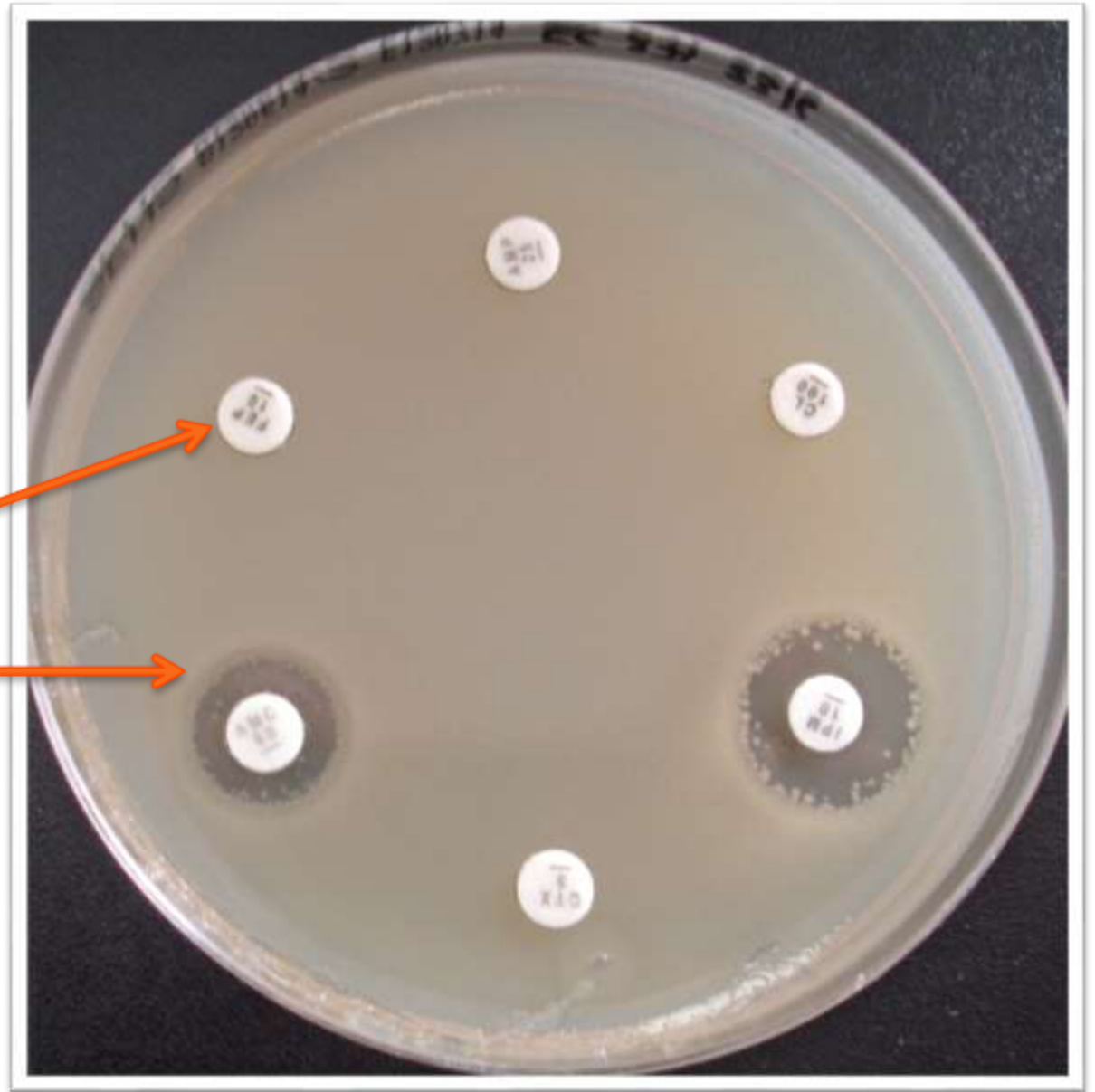
• R /  $\beta$ -lactam  
antibiotics



*E. coli* 271 isolate

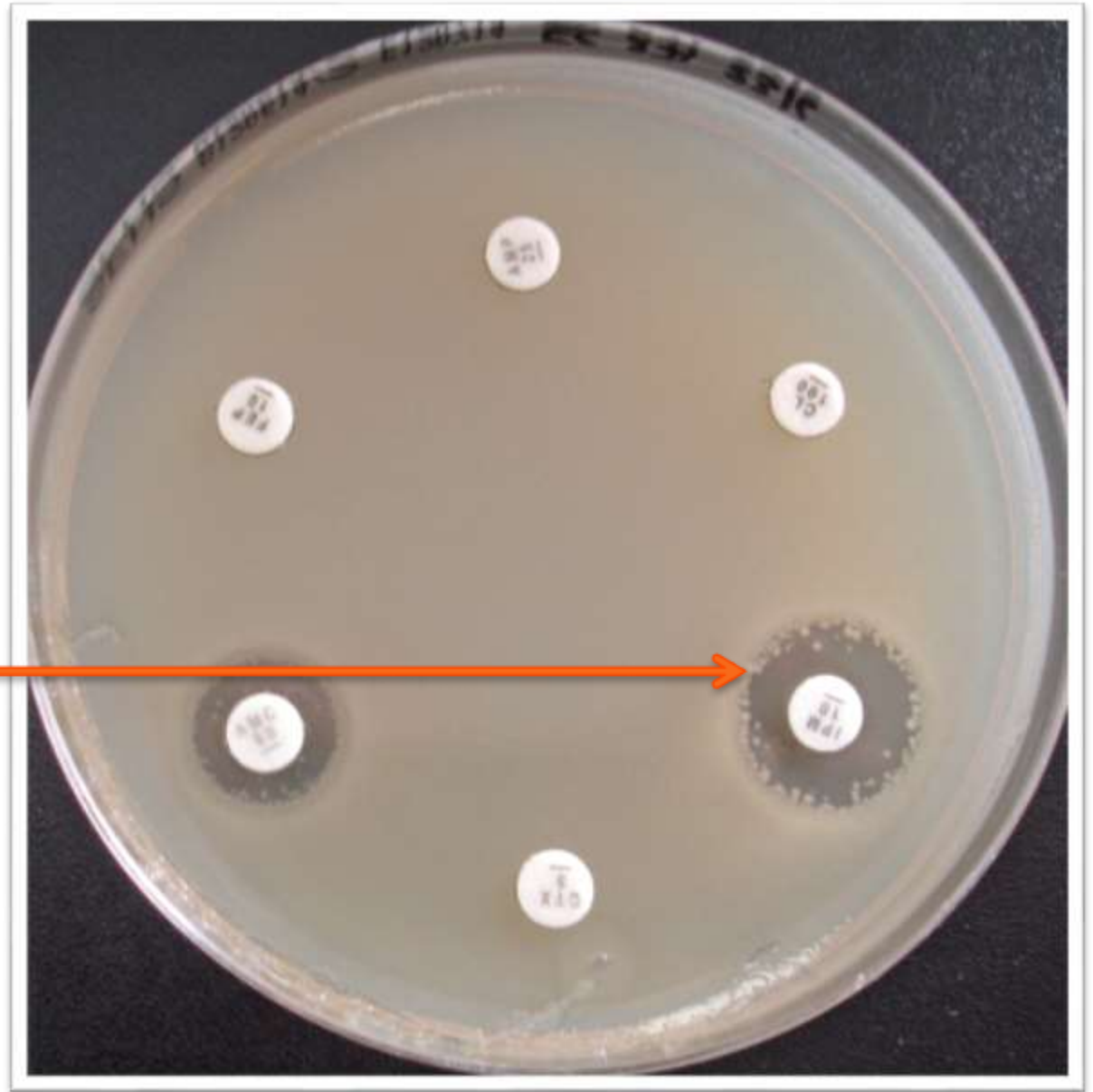
• **R / cefepime** (FEP 10)

• **R / augmentin** (AMC60)



*E. coli* 271 isolate

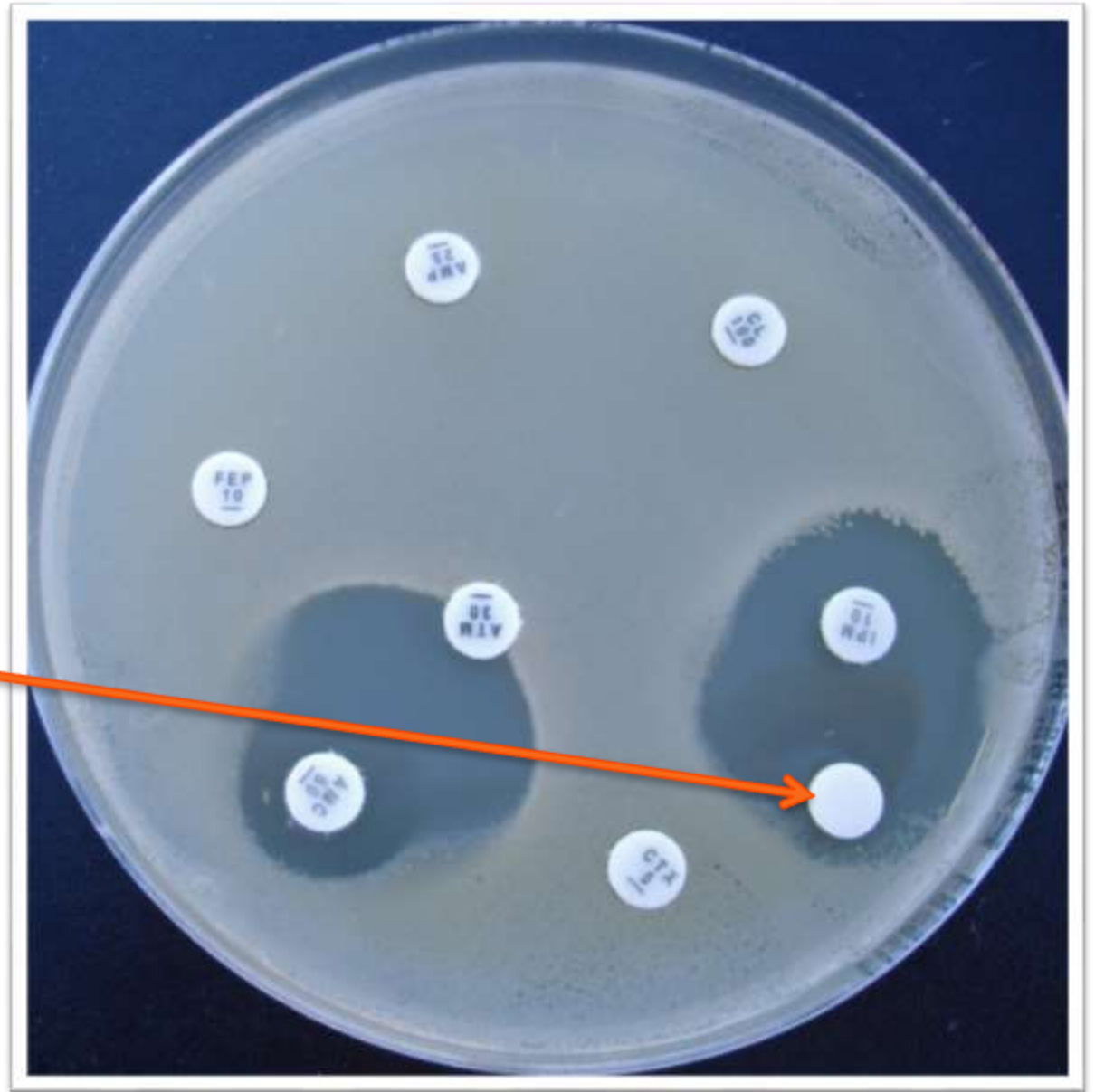
- **Reduced zone around imipenem (IPM 10)**
- **Resistant colonies around zone edge**



*E. coli* 271 isolate

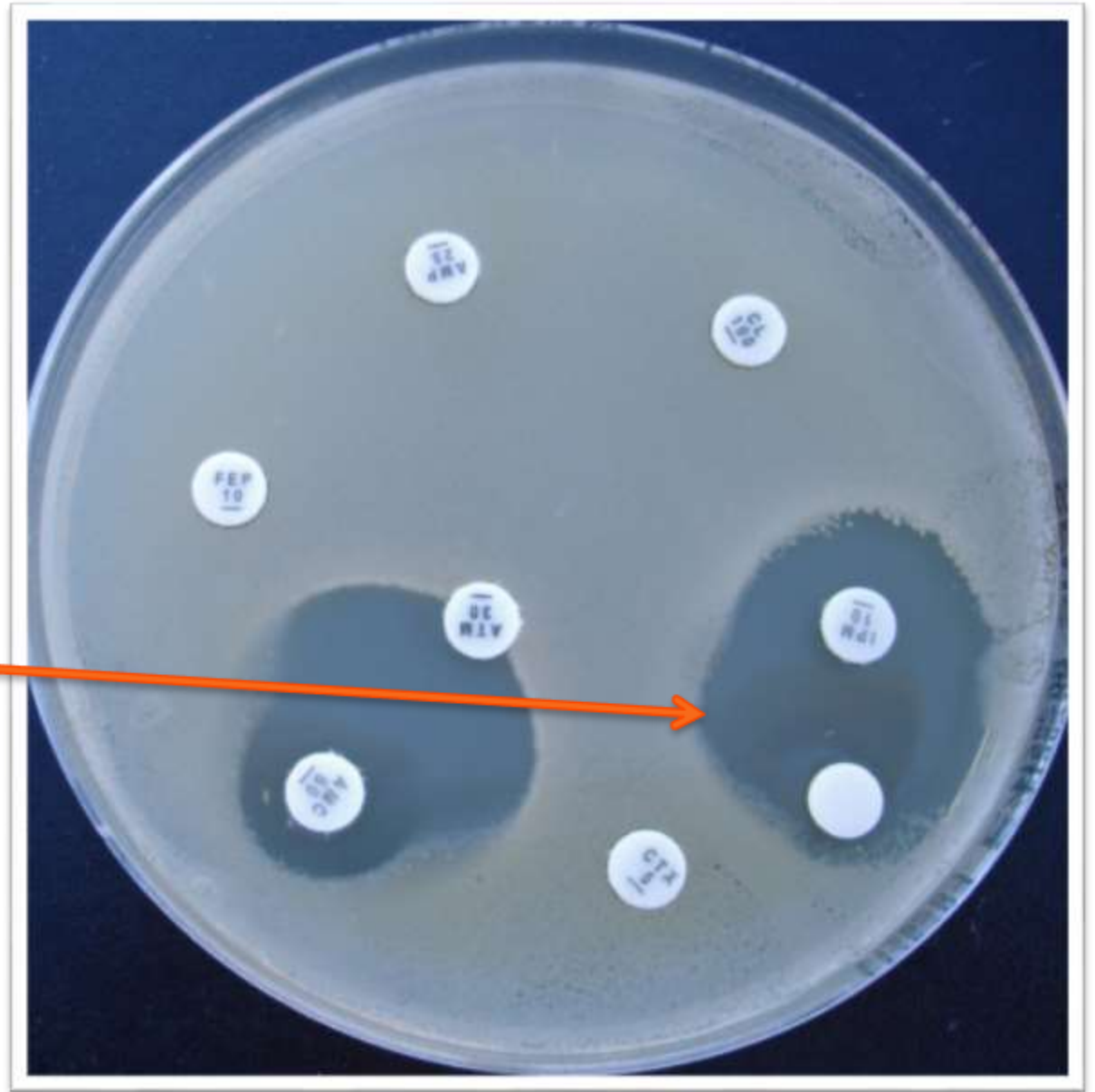
• **Addition of EDTA disc\* against a carbapenem disc**

• \* (25µl of 0.05M EDTA per disc)



*E. coli* 271 isolate

- Chelation of  $Zn^{2+}$  by EDTA disc
- Opens zone around imipenem towards EDTA



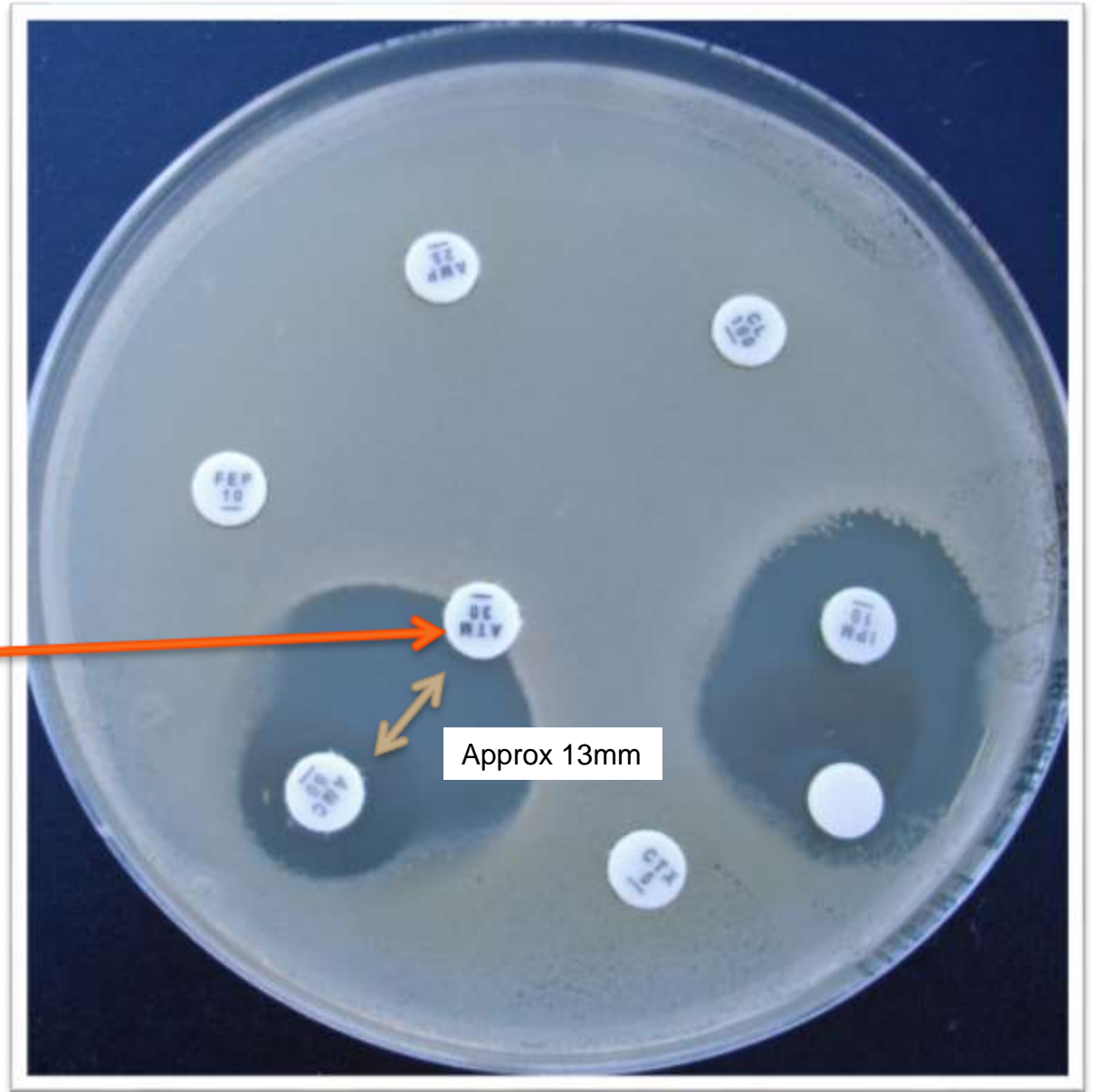


# Conclusion of phenotypic tests

**metallo- $\beta$ -lactamase  
present**

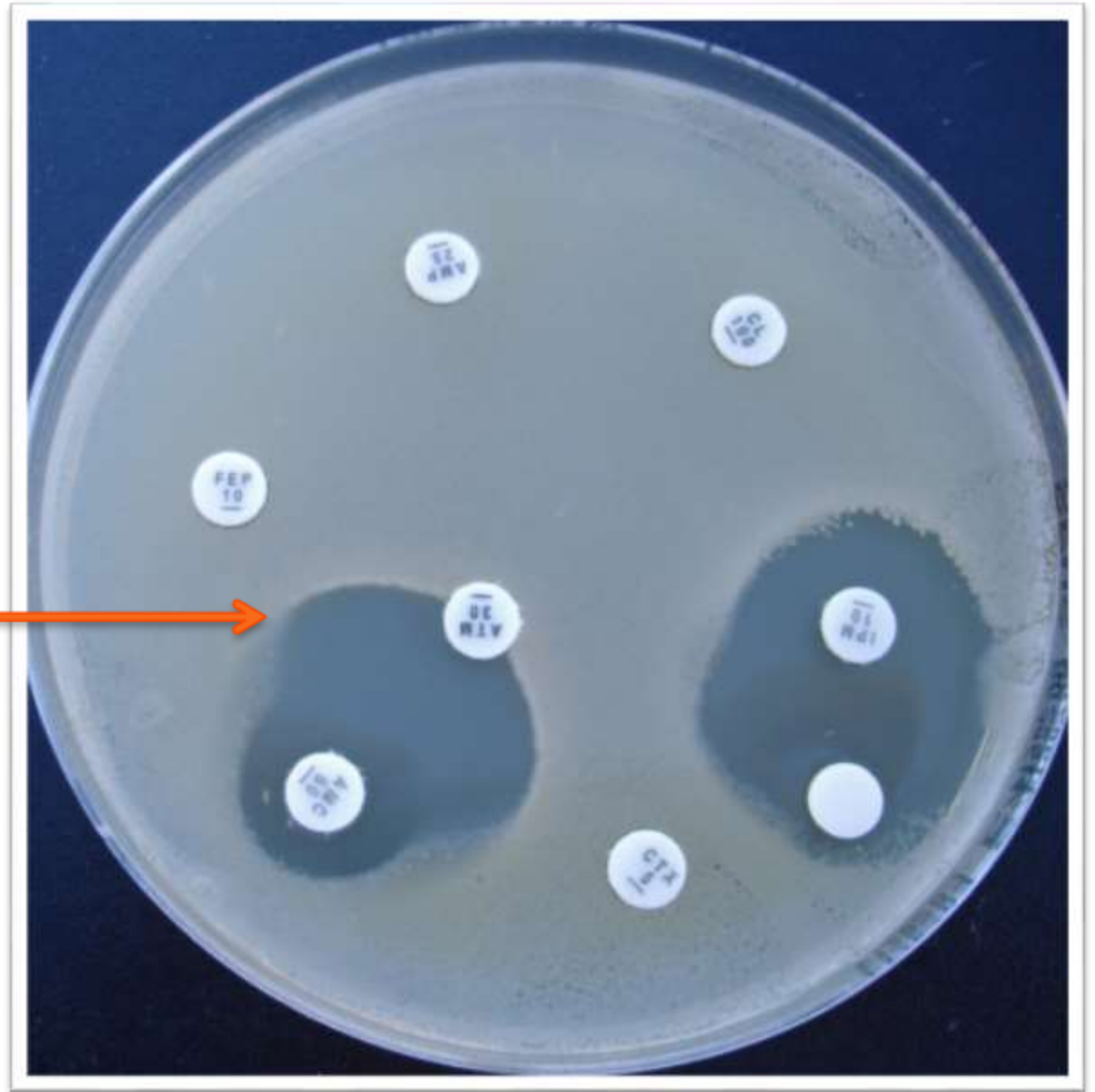
*E. coli* 271 isolate

- Addition of aztreonam (ATM 30) disc
- Placed 13mm away from augmentin (AMC 60) disc



*E. coli* 271 isolate

- **Synergy between augmentin (AMC 60) and aztreonam (ATM 30)**

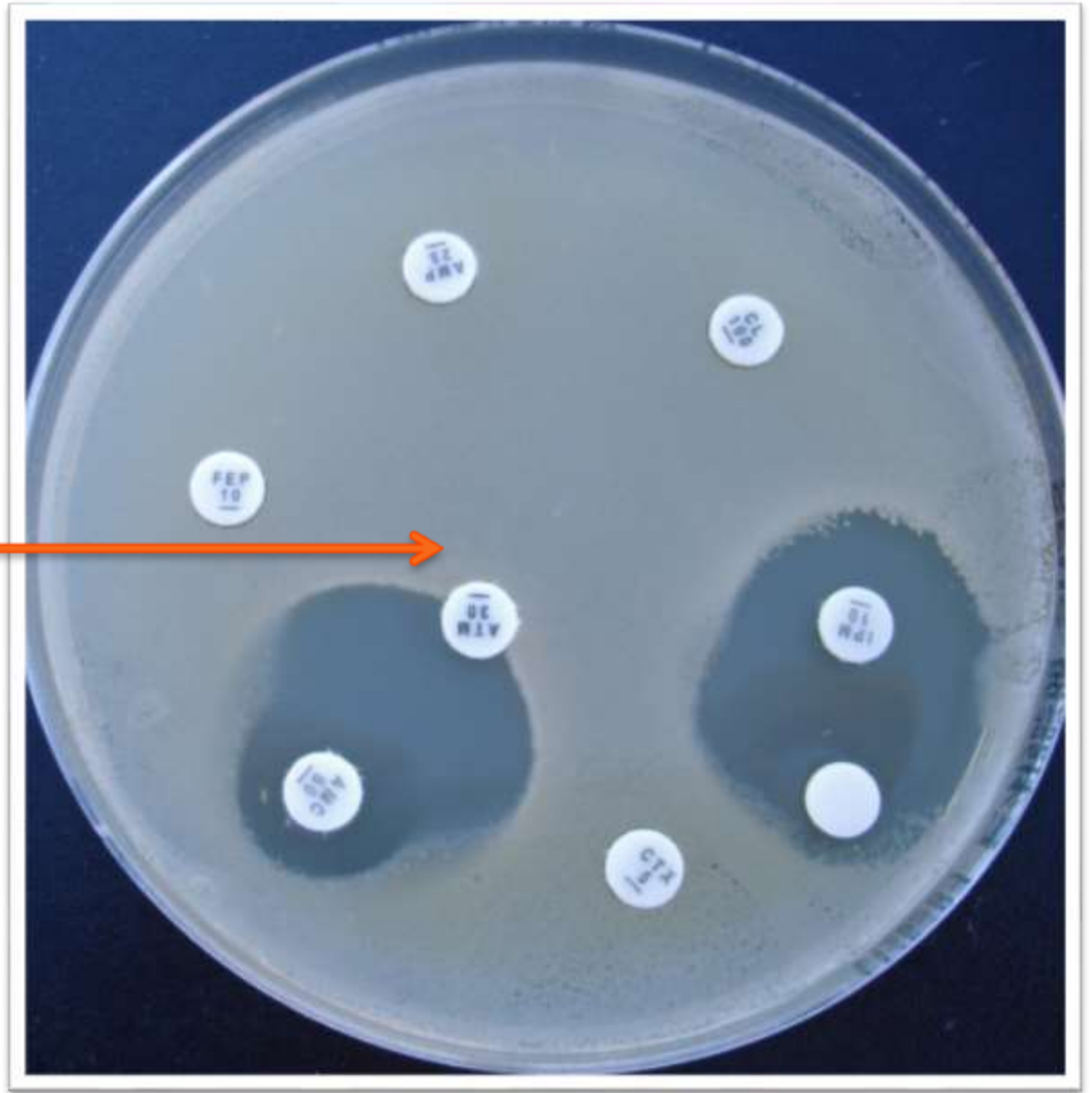


# Conclusion of phenotypic tests

**ESBL present**

*E. coli* 271 isolate

- **Resistant to aztreonam** (ATM 30)



*E. coli* 271 isolate

- **Resistant to aztreonam** (ATM 30)

MBLs hydrolyze all  $\beta$ -lactams, including carbapenems

**EXCEPT AZTREONAM** (monobactam)



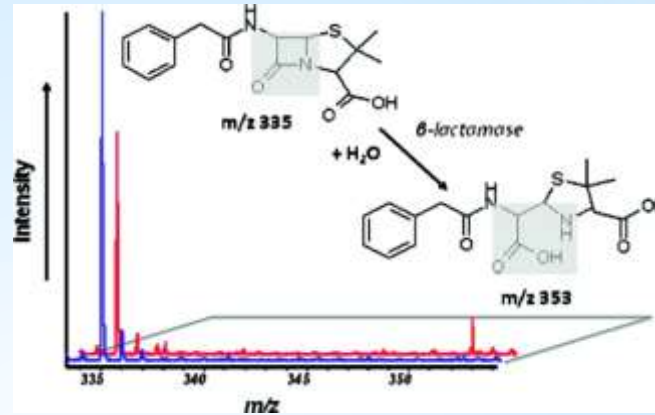
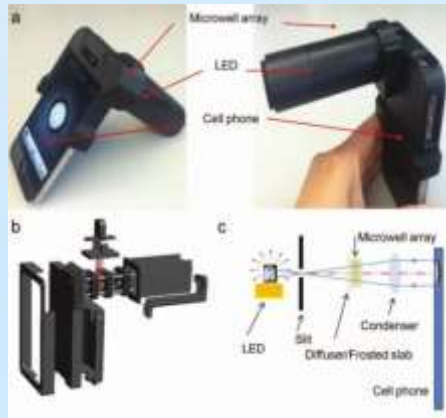
# Conclusions

- Concern over the rapid global dissemination of NDM-1
- Be aware of the emergence of NDM-1 cases in Australia
- NDM-1 is phenotypically identical to other MBLs
- Use same detection methods for MBL – EDTA disc
- Phenotype will show multi-drug resistance
- Other  $\beta$ -lactam resistance mechanisms may be present e.g. ESBL – addition of ATM disc helpful
- Definitive diagnosis of NDM-1 by molecular confirmation
- Importance of molecular typing of MBL isolates

# References

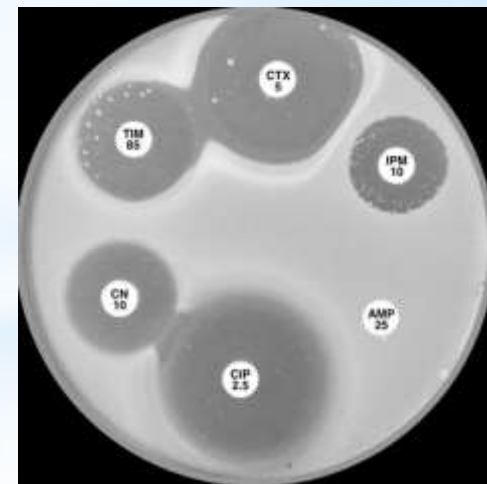
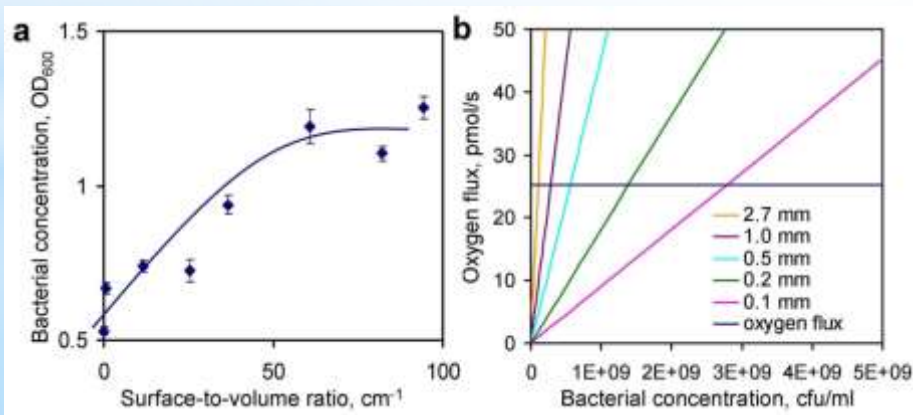
1. **Poirel, L., E. Lagrutta, P. Taylor, J. Pham, and P. Nordmann.** 2010. Emergence of Metallo- $\beta$ -Lactamase NDM-1-Producing Multidrug-Resistant *Escherichia coli* in Australia. *Antimicrob. Agents Chemother.* **54**:4914–4916.
2. **Yong, D., M. A. Toleman, C. G. Giske, H. S. Cho, K. Sundman, K. Lee, and T. R. Walsh.** 2009. Characterization of a new metallo- $\beta$ -lactamase gene,  $bla_{NDM-1}$ , and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* **53**:5046–5054.
3. **Kumarasamy, K. et al.** 10 August 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* **10**:597– 602. [Epub ahead of print.]
4. **J. Kamile Rasheed, et al.** 2013. New Delhi Metallo- $\beta$ -Lactamase–producing Enterobacteriaceae, United States. *Emerging Infectious Diseases.* **19**:870-878.
5. **R. A. Bonomo.** 2011. New Delhi Metallo-b-Lactamase and Multidrug Resistance: A Global SOS?. *Clinical Infectious Diseases.* **52**:485-487.
6. **Kaase, M., P. Nordmann, T. A. Wichelhaus, S.G. Gatermann, R. A. Bonnin, and L. Poirel.** 2011. NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. *J Antimicrob Chemother.* **66**: 1260–1262





# My journey with the CDS test and frontier antibiotic susceptibility testing

- A report of the ASA meeting, 2014



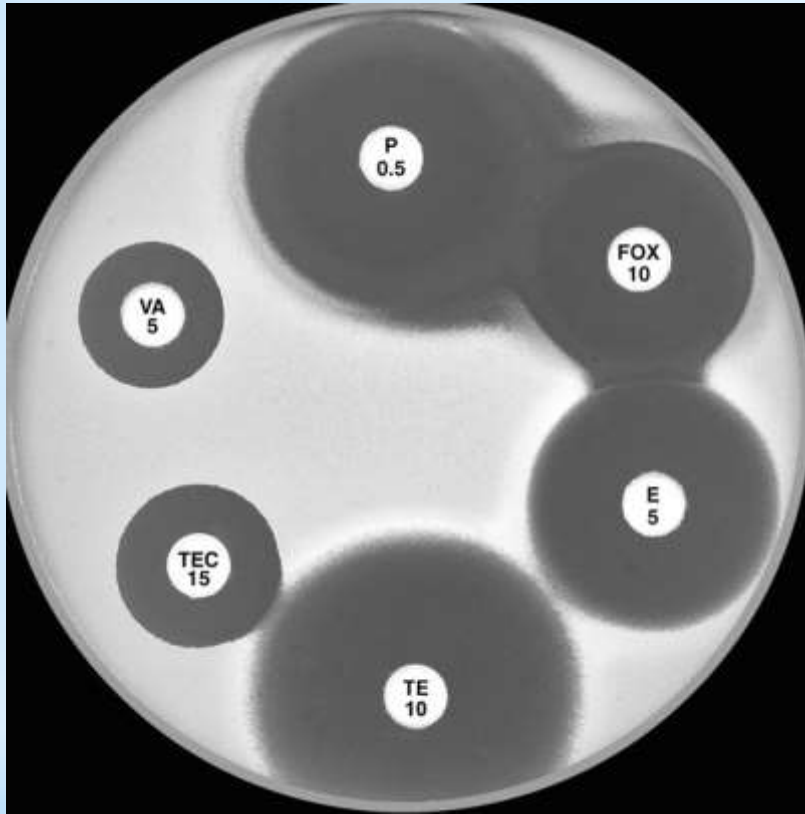
Christopher Mclver







# Calibrated dichotomous susceptibility (CDS) test



**Over 40 years in use**

**Standardized and reproducible**

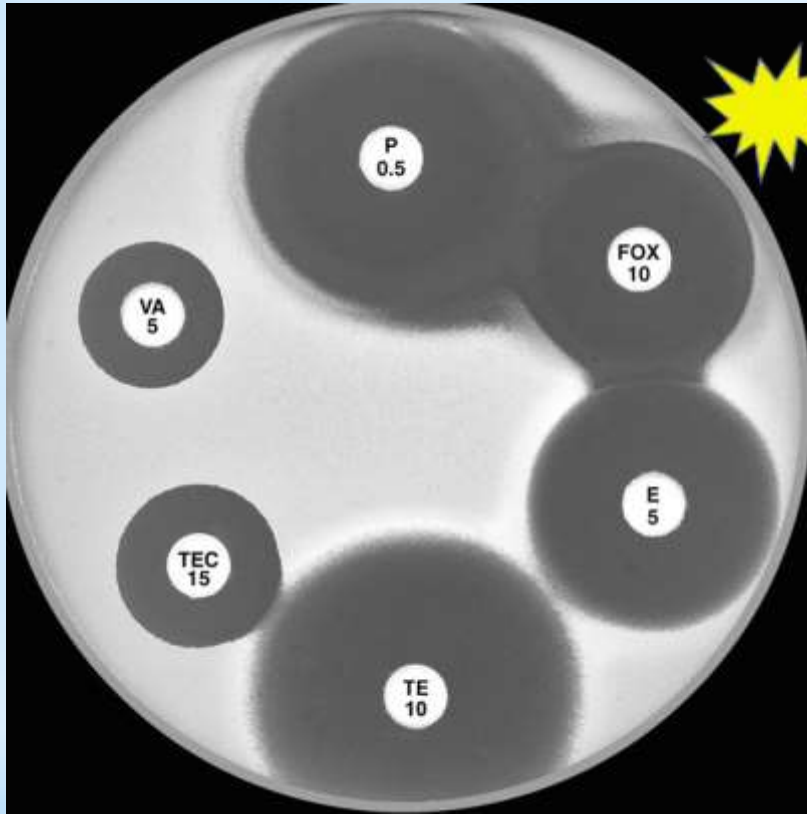
**Current and novel antibiotics**

**Methods to detect  
resistance mechanisms**

**Support application in clinical and veterinary laboratories**

- Website
- Publications
- Expert advice

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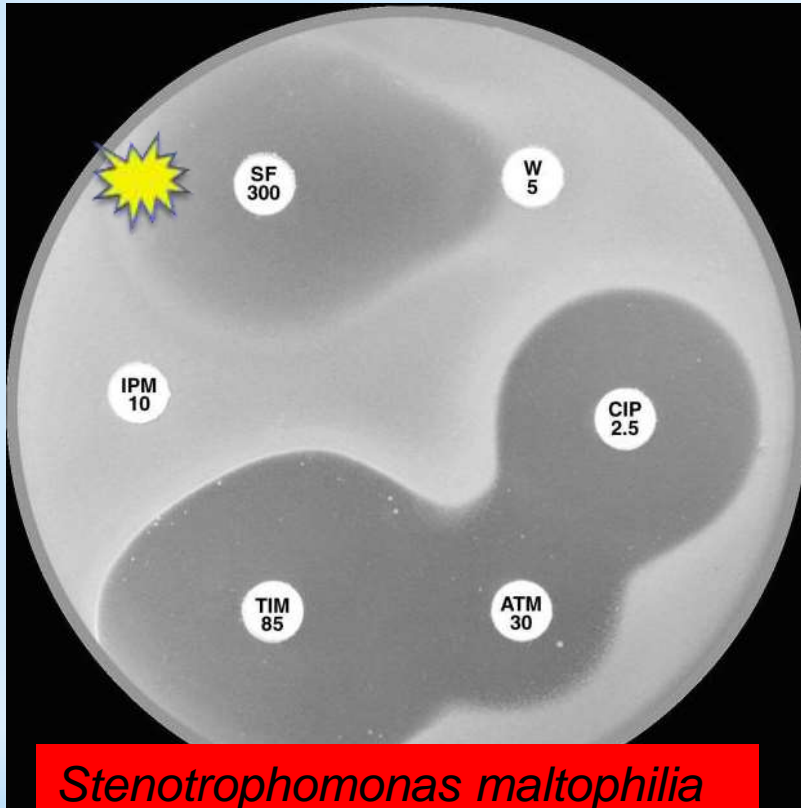
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*Stenotrophomonas maltophilia*

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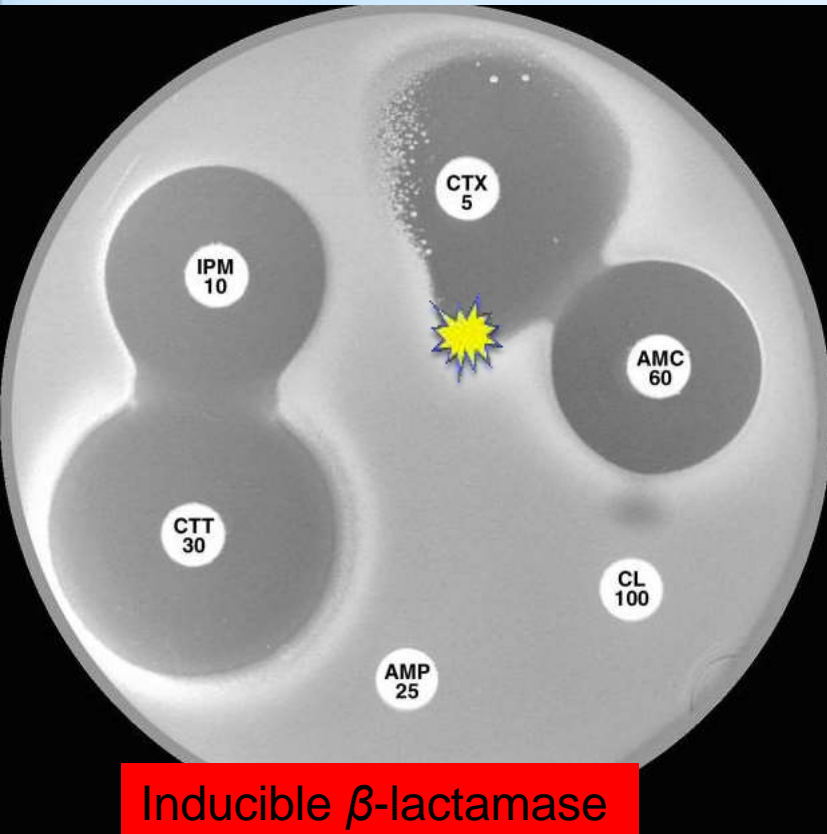
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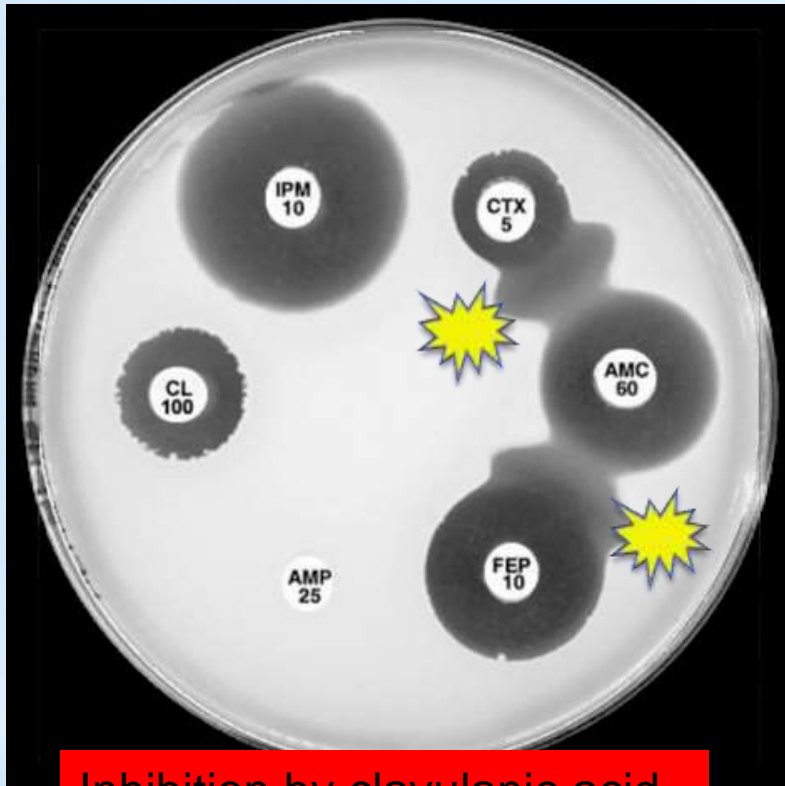
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Inhibition by clavulanic acid

Over 40 years in use

Standardized and reproducible

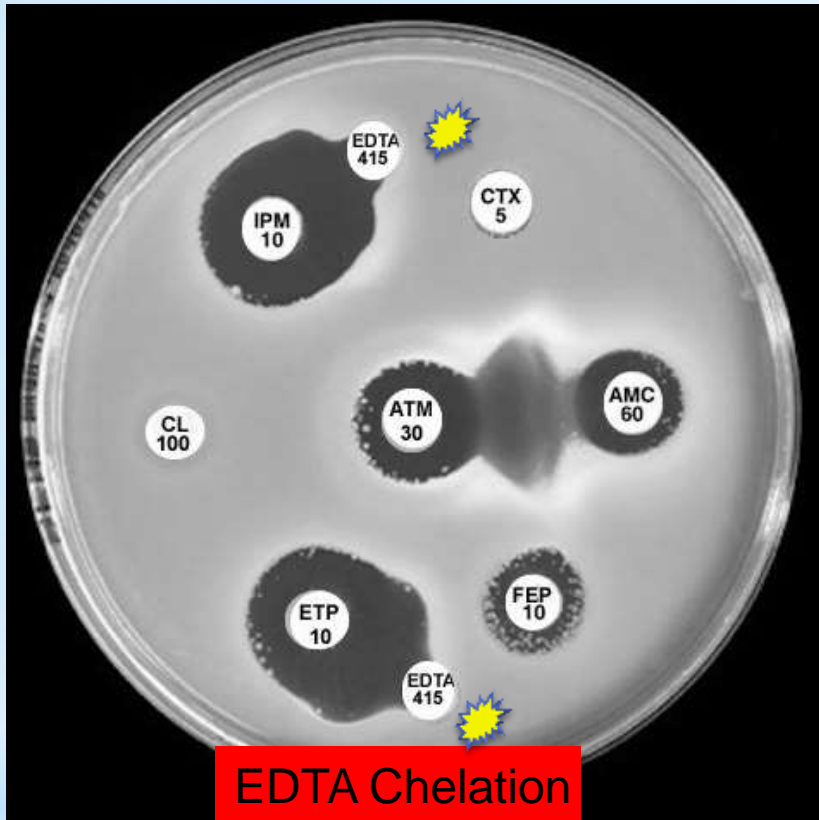
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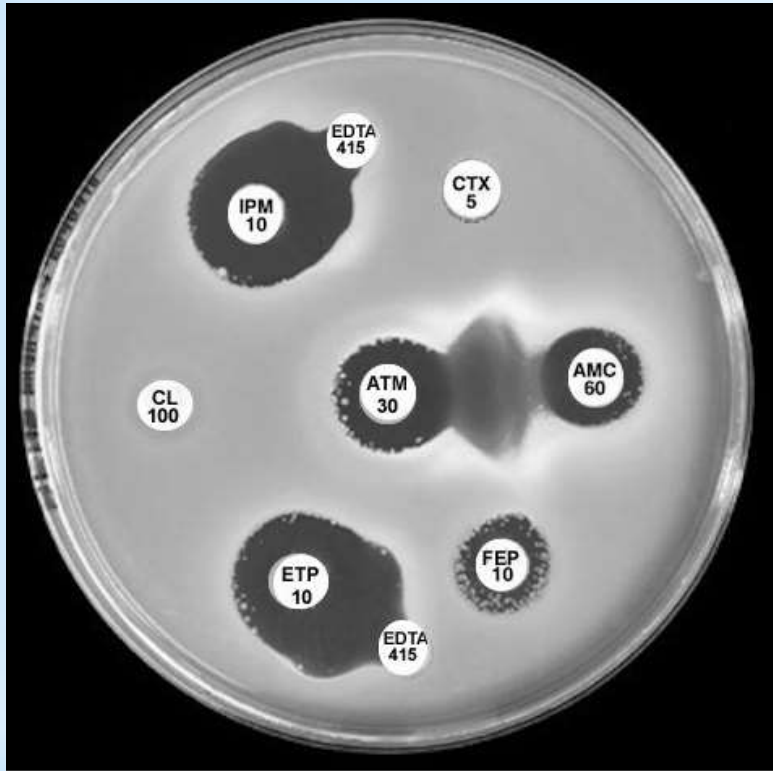
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Susceptibility testing cysteine auxotrophs of enterobacteria

The detection and classification of integrons in clinical isolates of the *Enterobacteriaceae*

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Molecular detection of resistance-determinants

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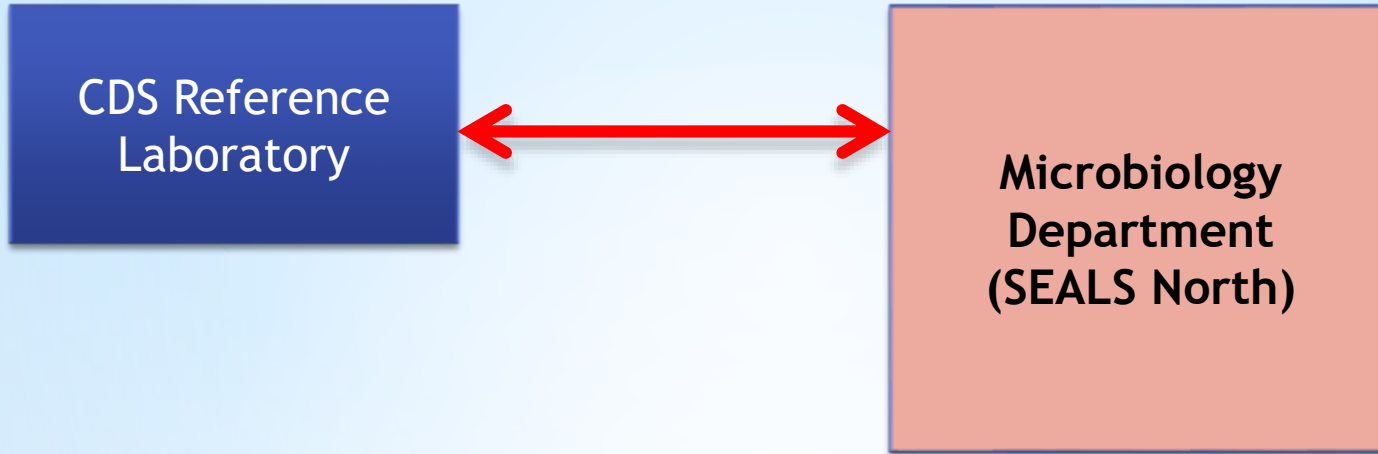
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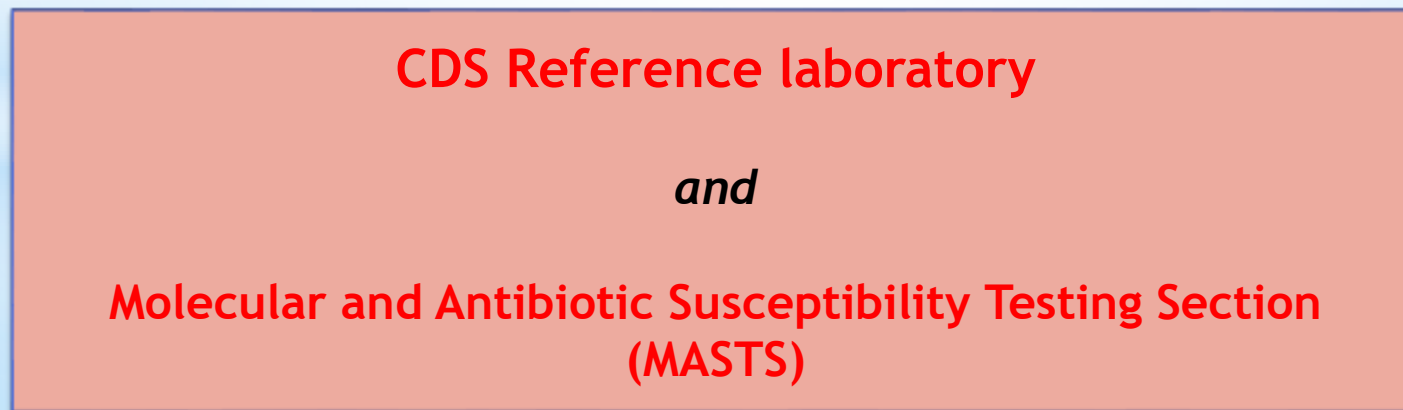
# Location: Prince of Wales Hospital, Randwick



## Location: Prince of Wales Hospital, Randwick



## Location: St George Hospital, Kogarah



# **IMPERATIVES FOR RAPID SUSCEPTIBILITY PROFILING**

**Judicious use antibiotics**

**Increasing costs of medical intervention**

**Increasing resistance to commonly used antimicrobial agents**

**Emerging resistance mechanisms**

**Stagnated development of antimicrobial agents**

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Cell phone based micro-photometric system

Magnetic bead biosensor

High surface-to-volume ratio microchannels

Microcalorimetry

MALDI-TOFF





# Microfluidics

High surface-to-volume ratio micro-channels

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High surface-to-volume ratio micro-channels

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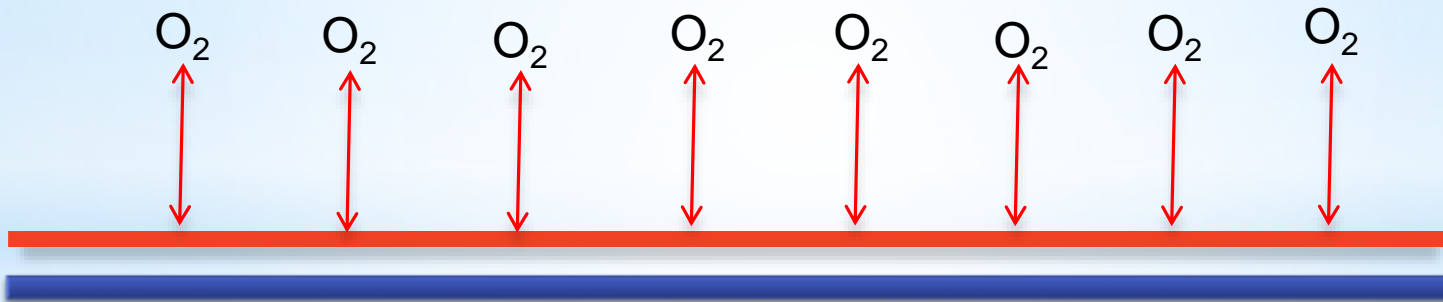
# Microfluidics

High surface-to-volume ratio micro-channels

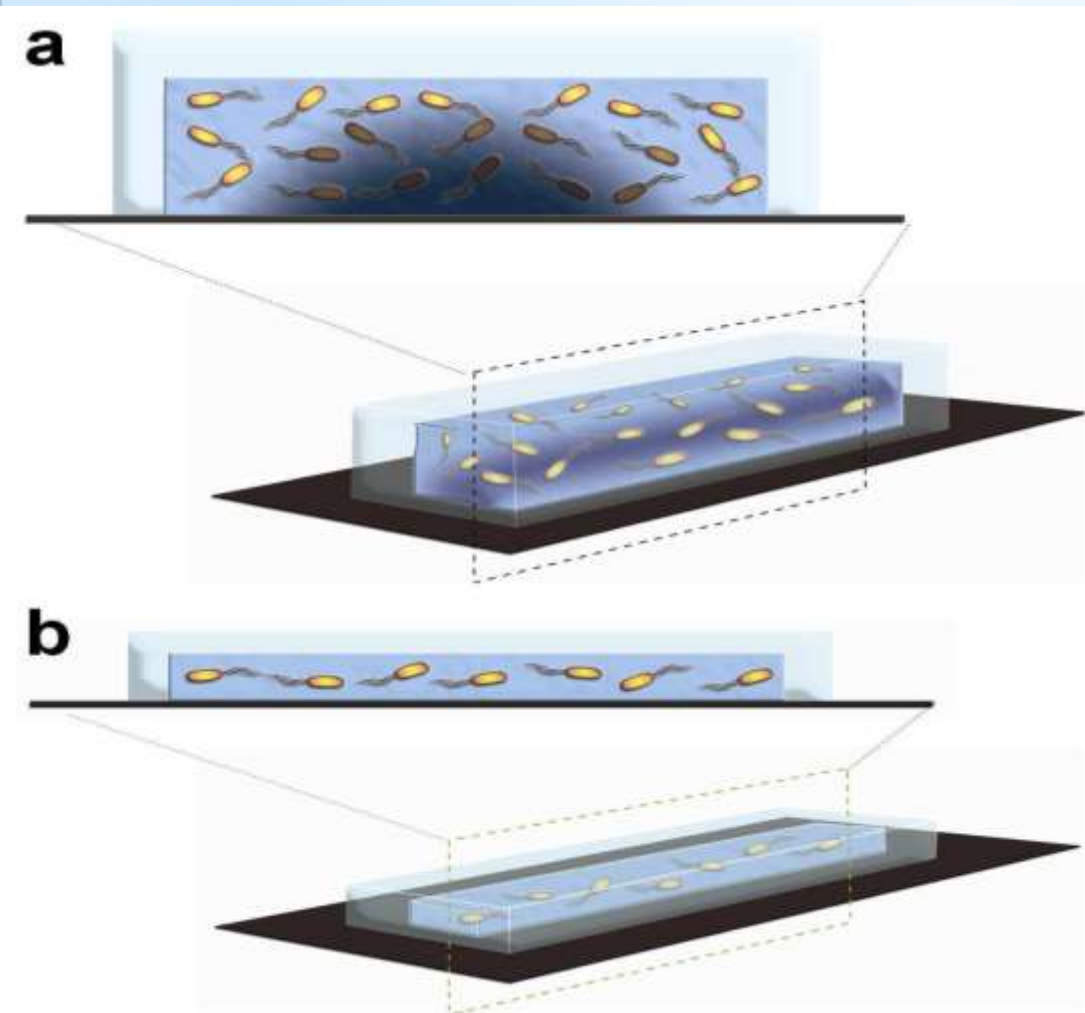


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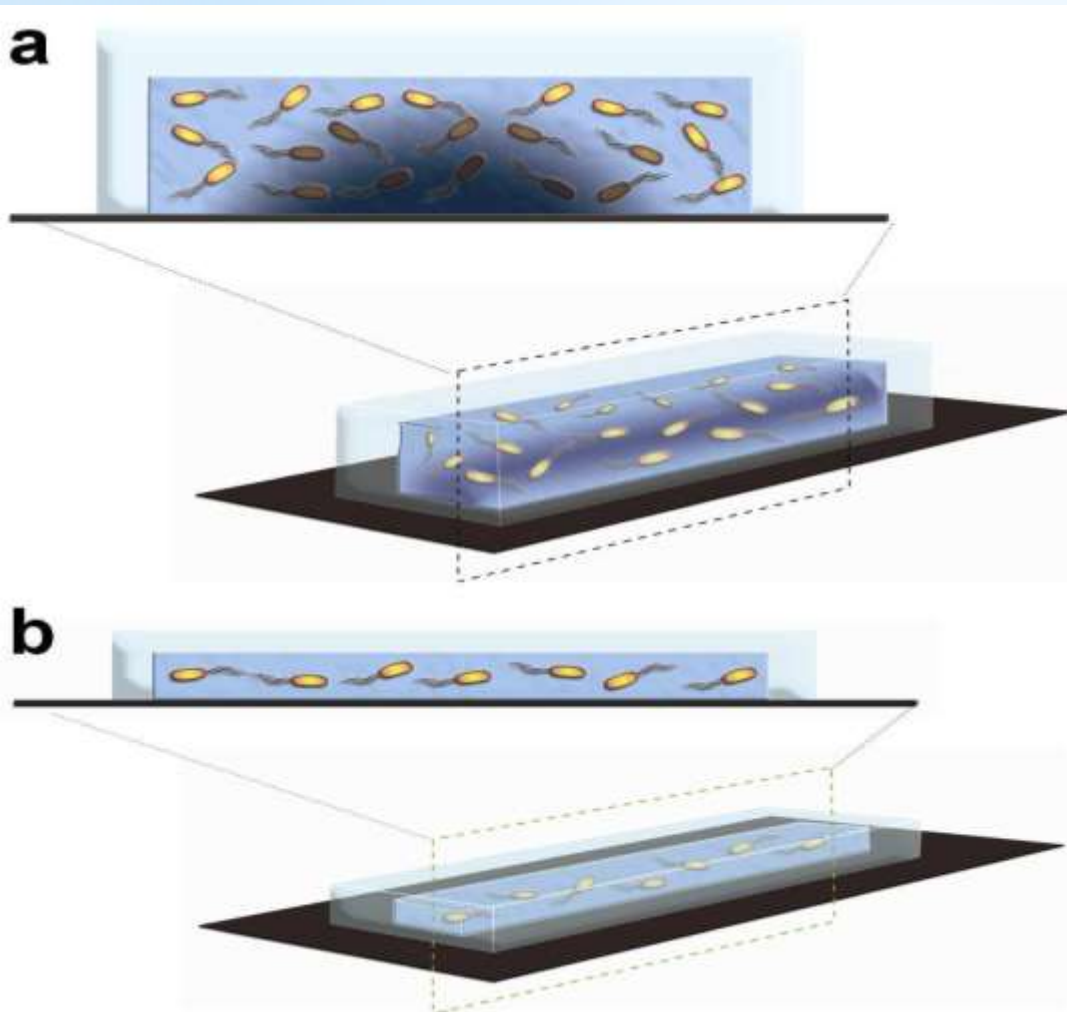
High surface-to-volume ratio micro-channels



Amount of oxygen required to sustain growth  $\alpha$  to the number of bacteria

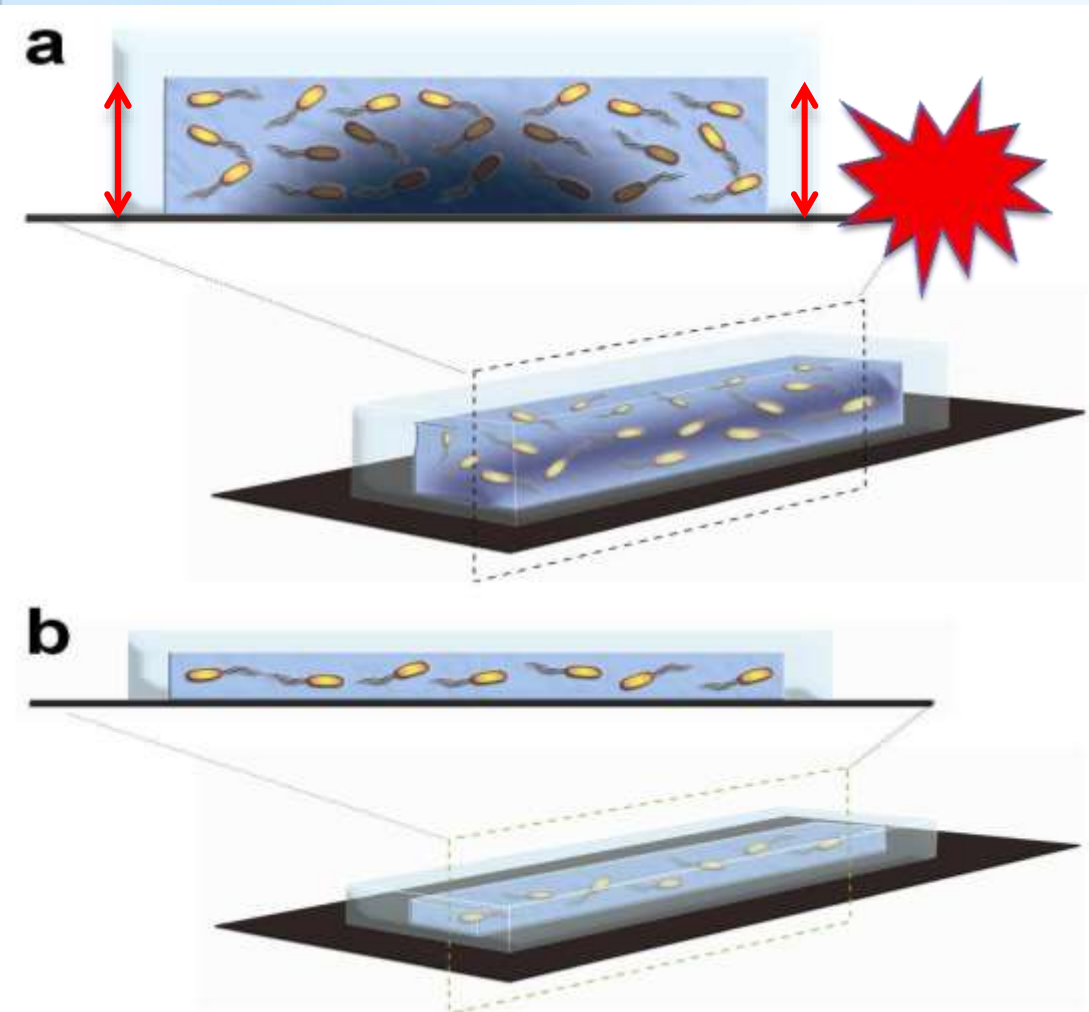


# Amount of oxygen delivered to bacteria $\propto$ surface area



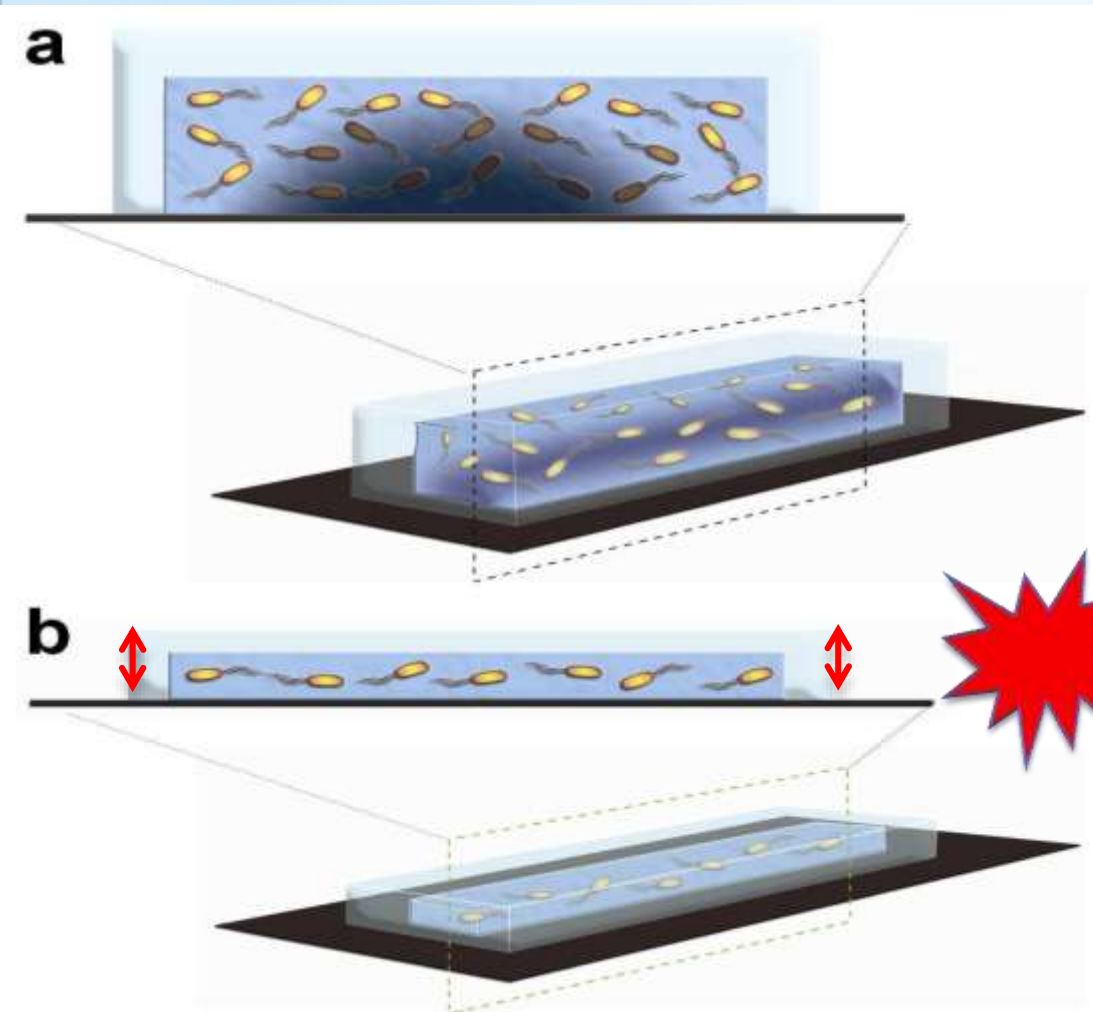
Amount of oxygen required to sustain growth  $\alpha$  to the number of bacteria

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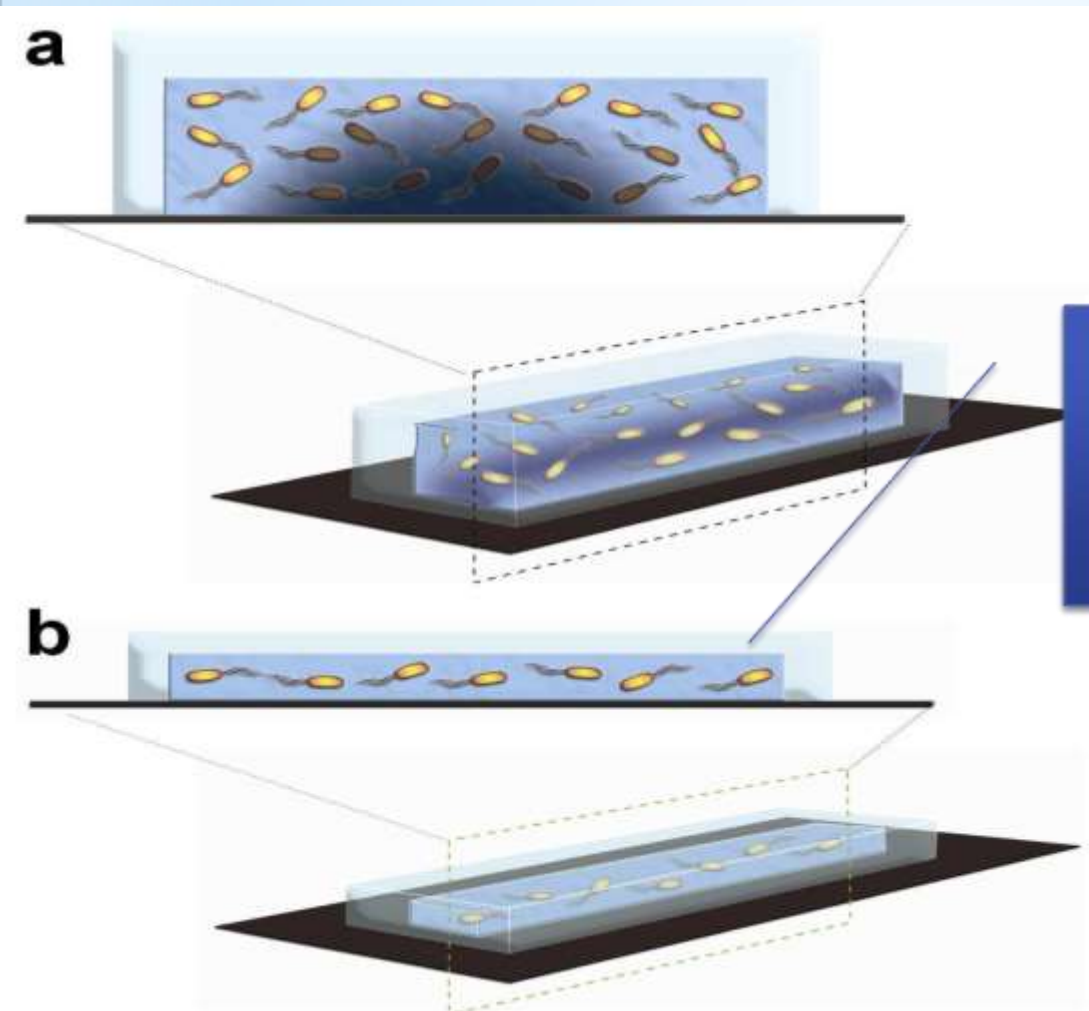
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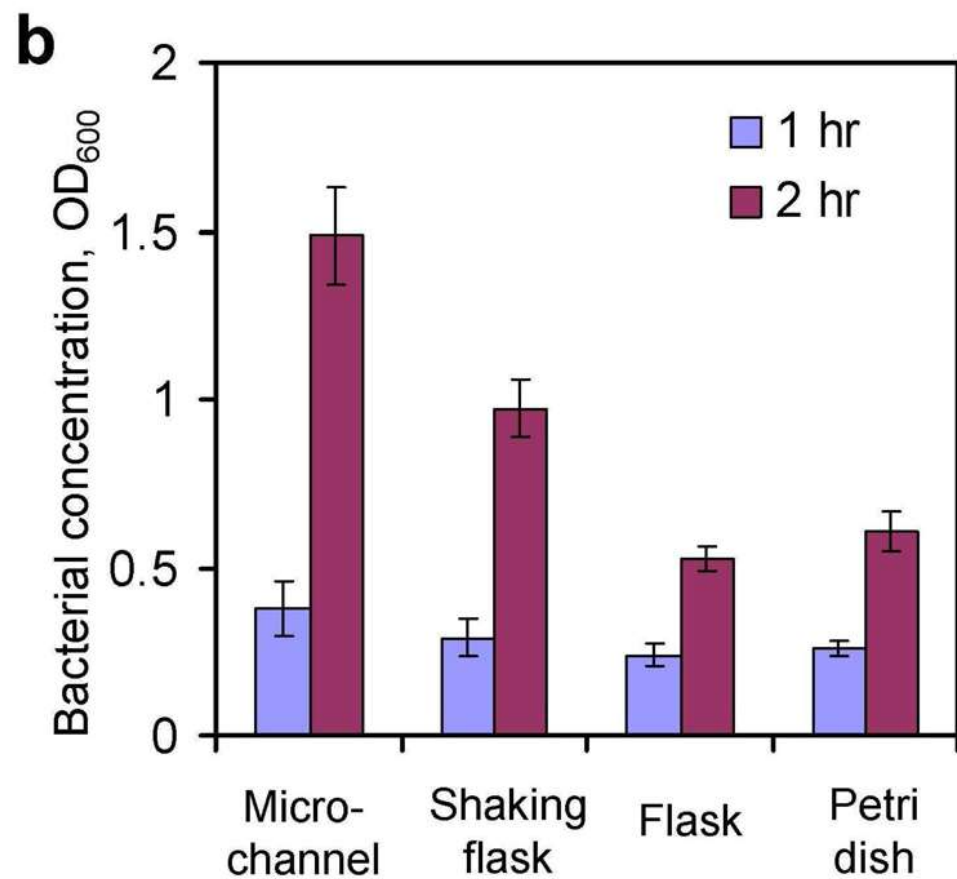
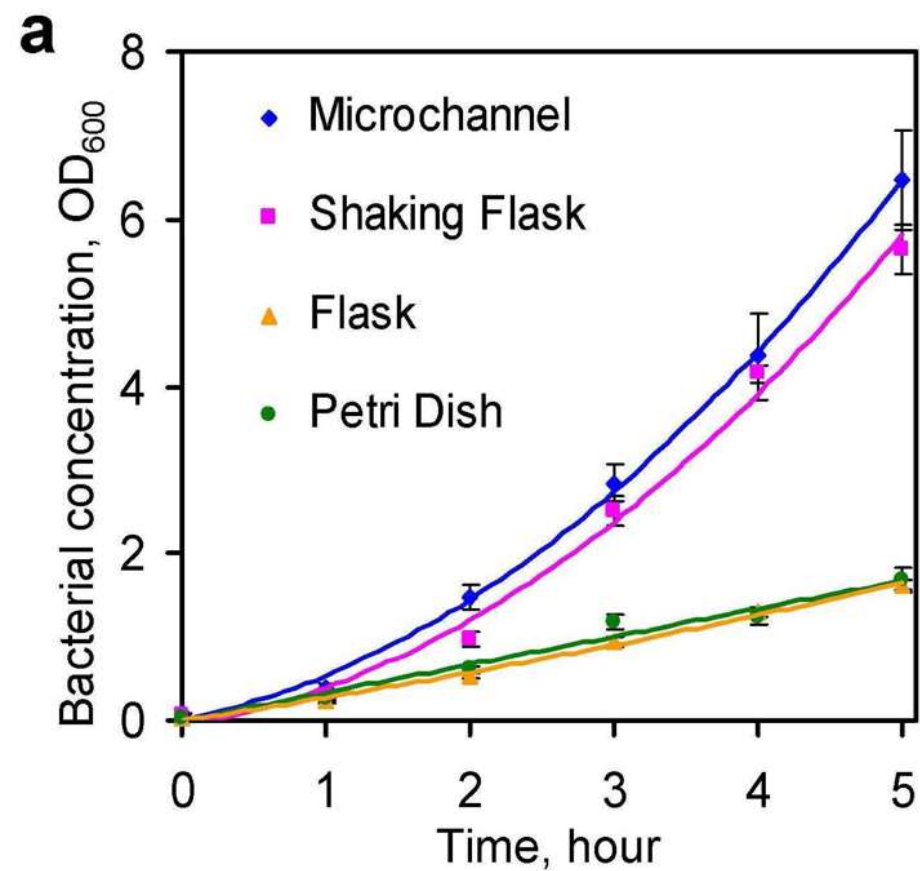
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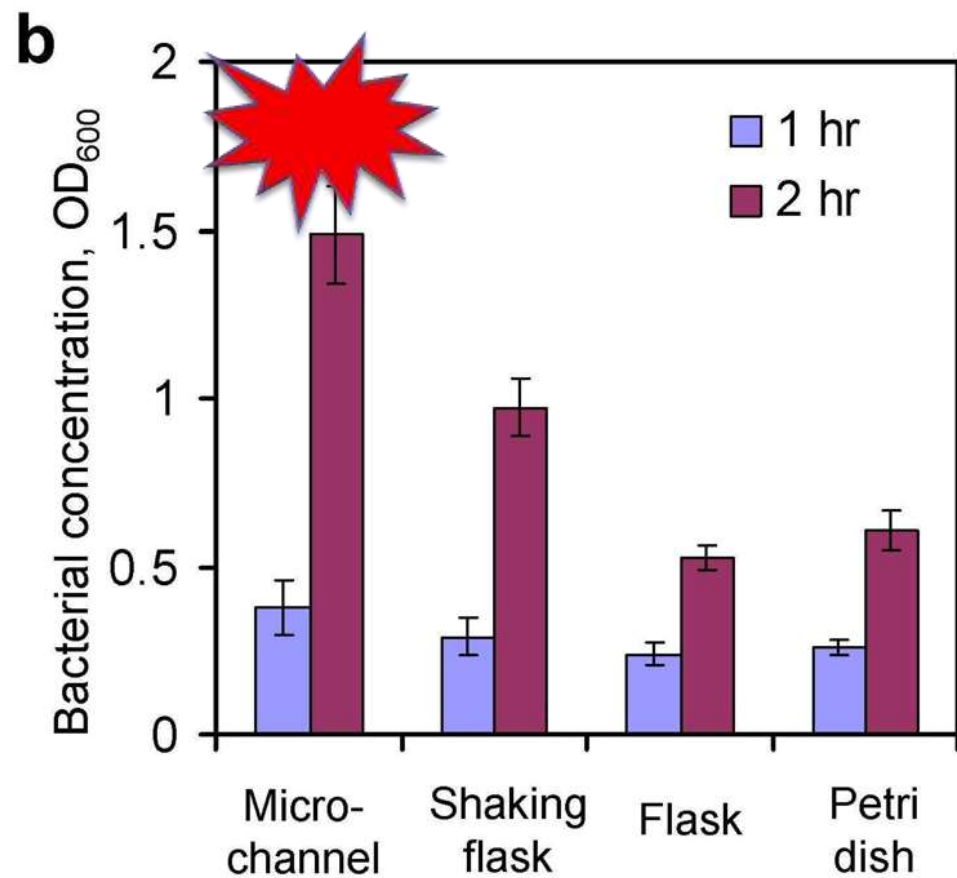
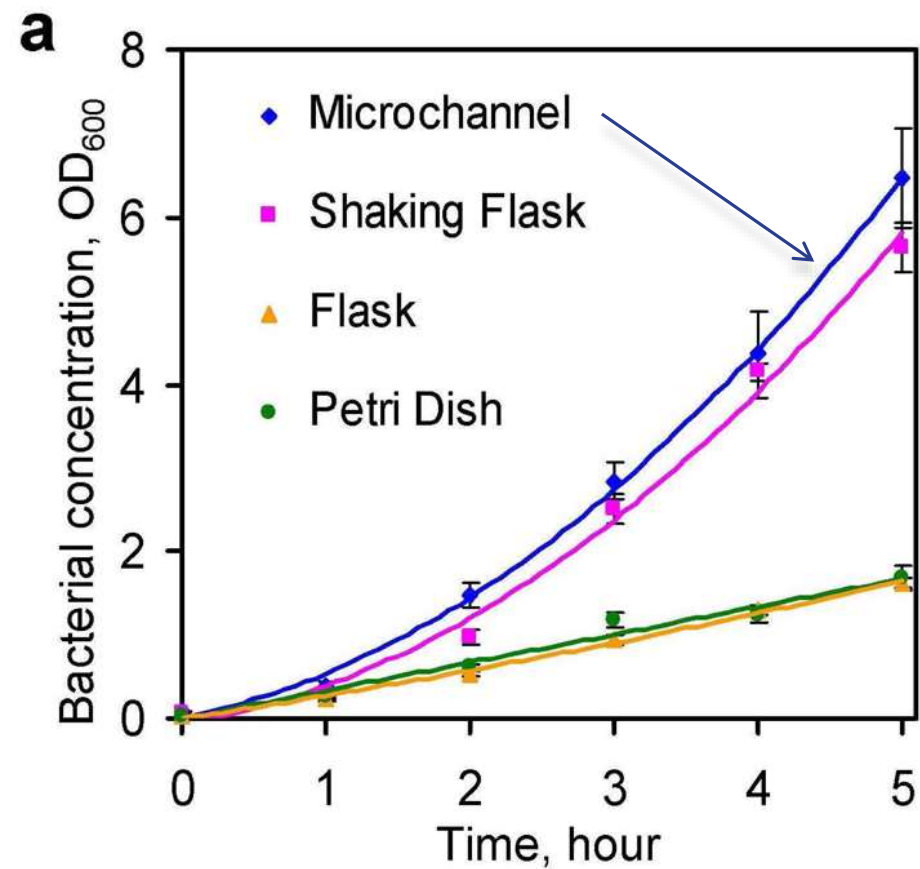
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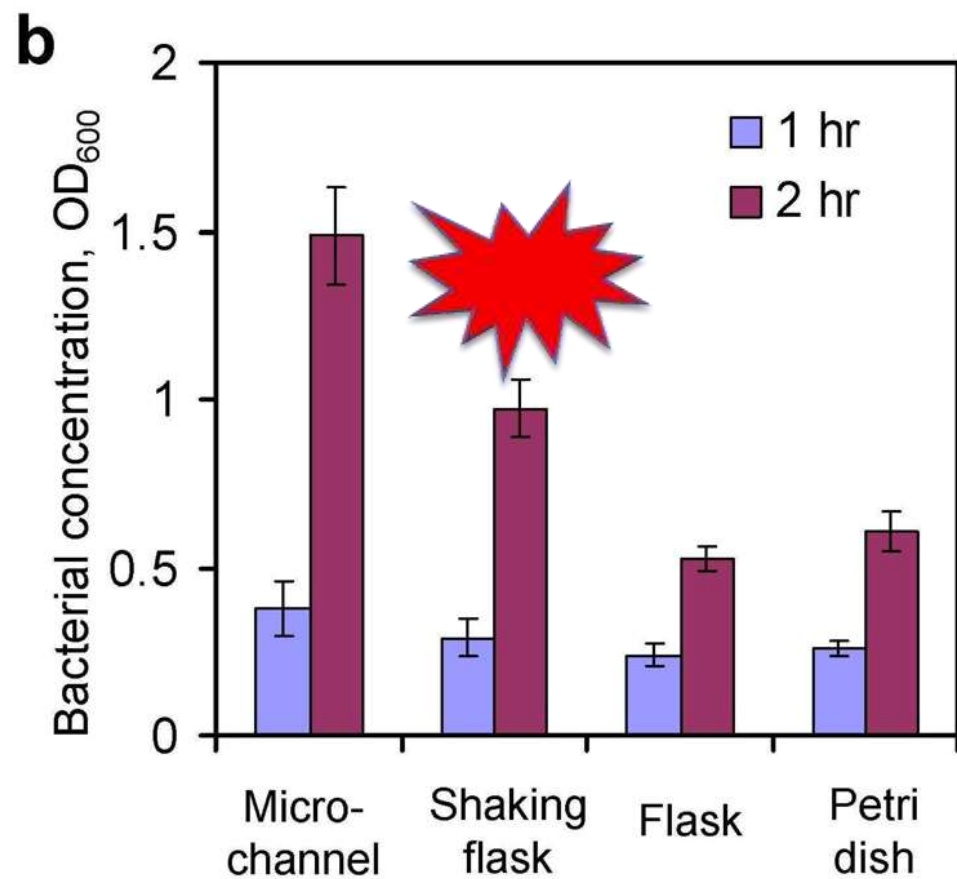
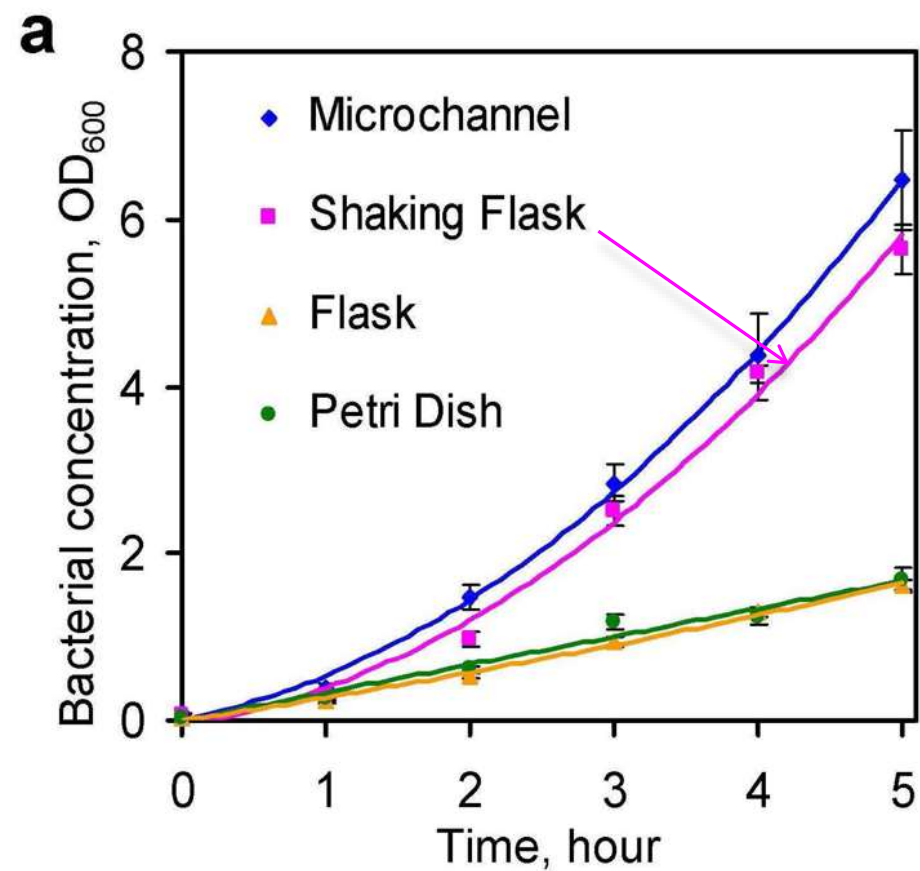


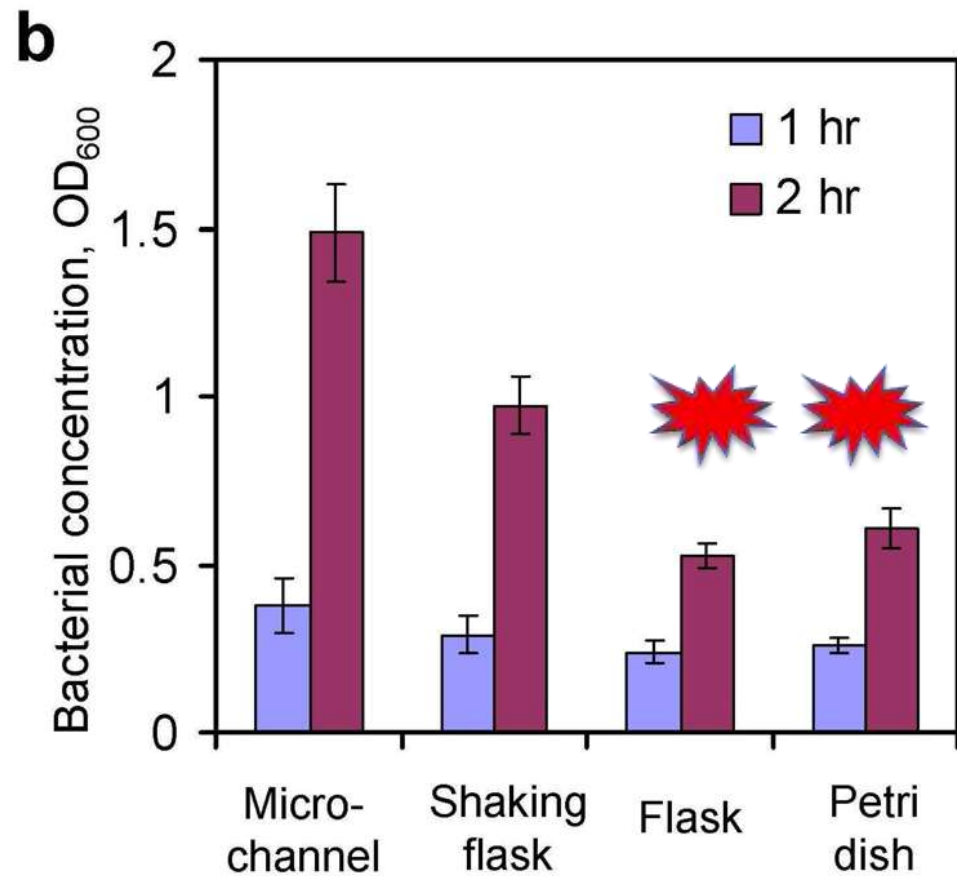
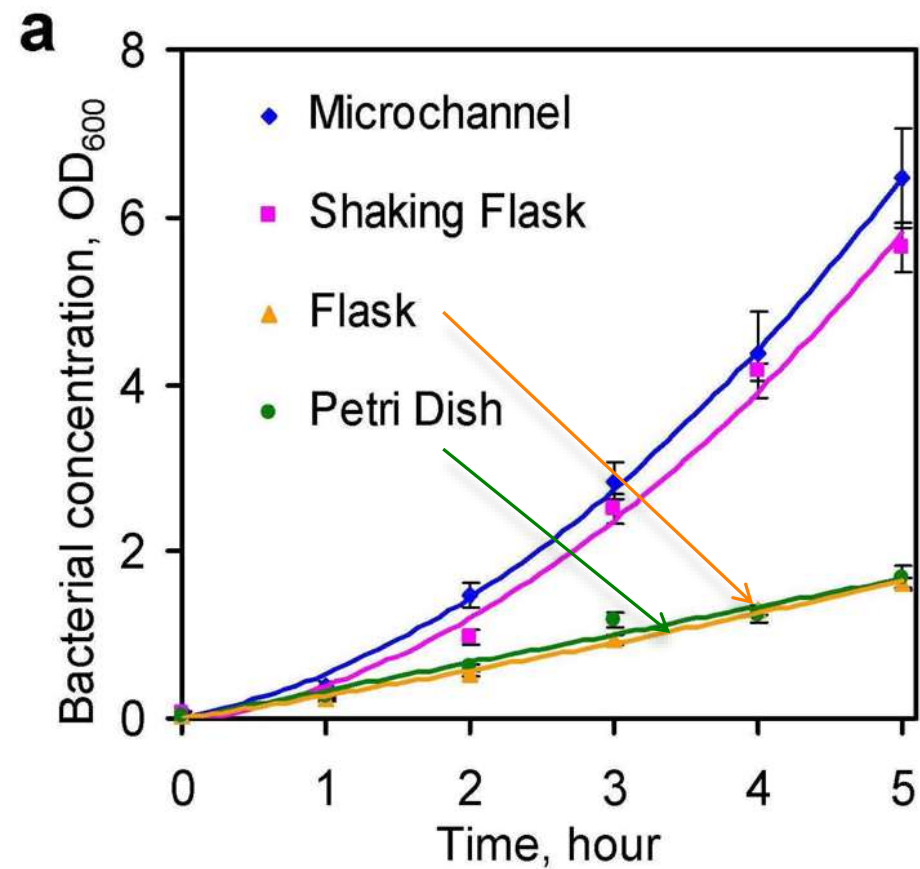
Relatively abundant oxygen is available for bacterial culture at the microscale

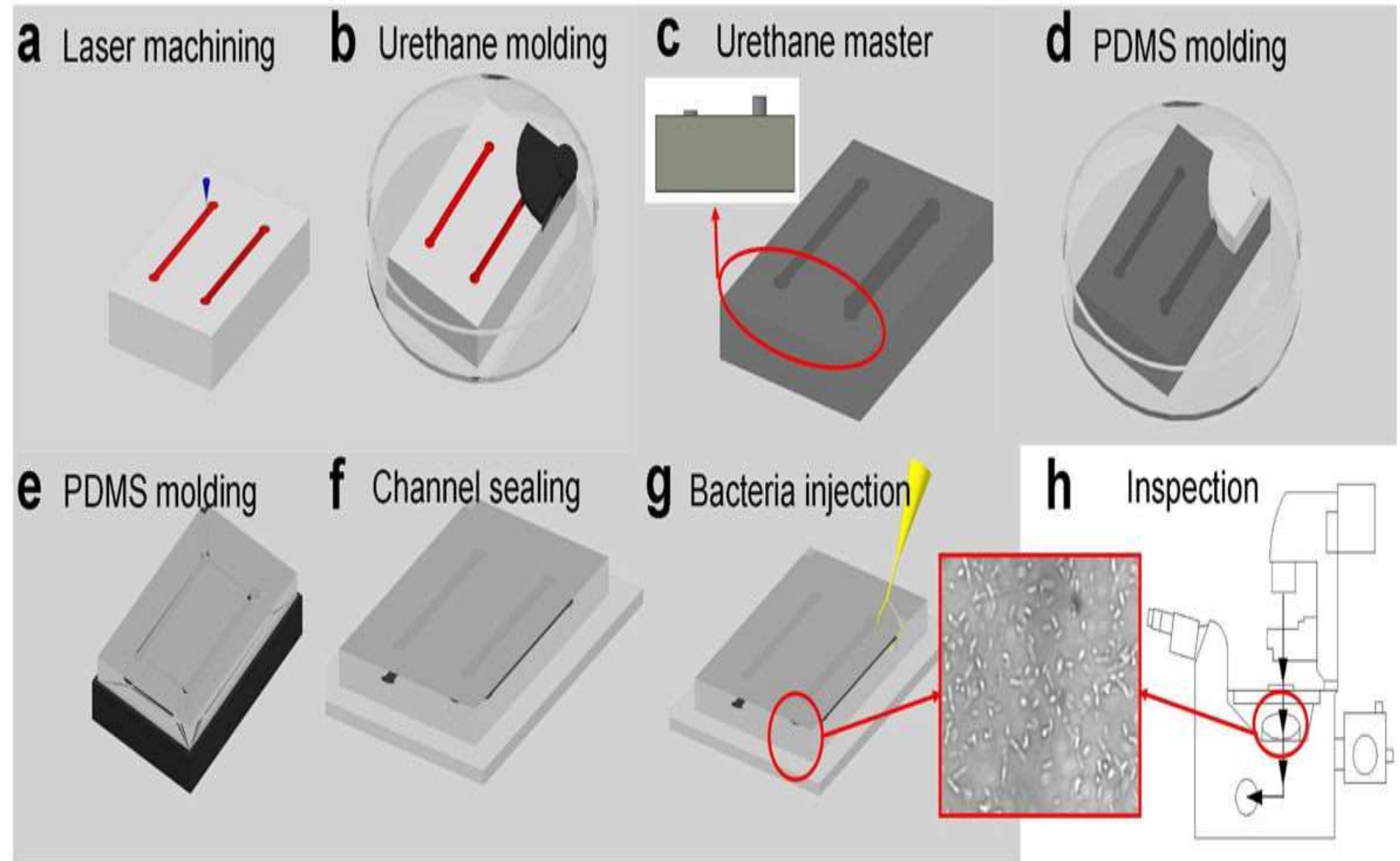












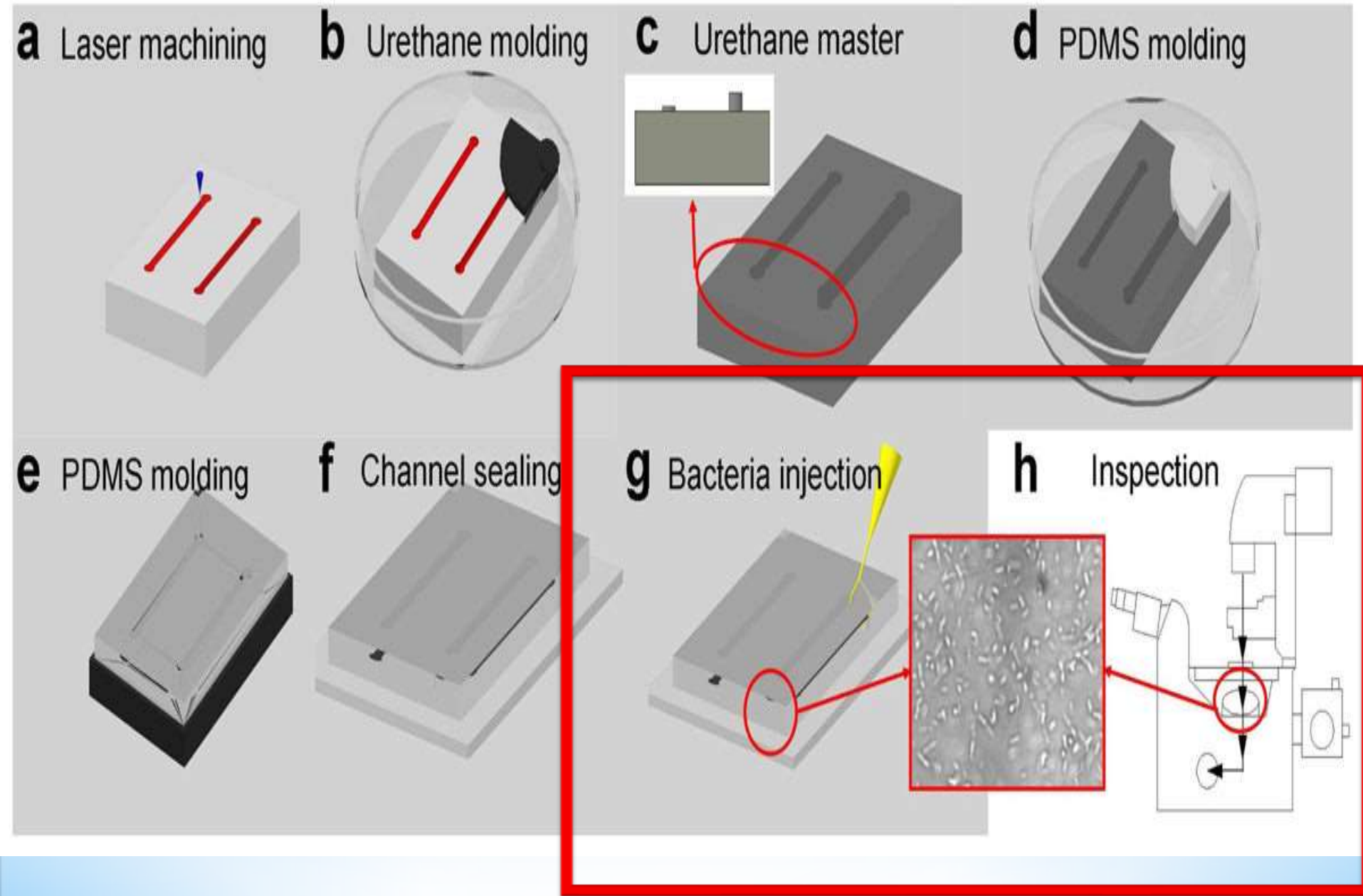


Figure 2. Polydimethylsiloxane microwell assay with precoated antibiotics for minimum inhibitory concentration determination and antimicrobial resistance profiling.

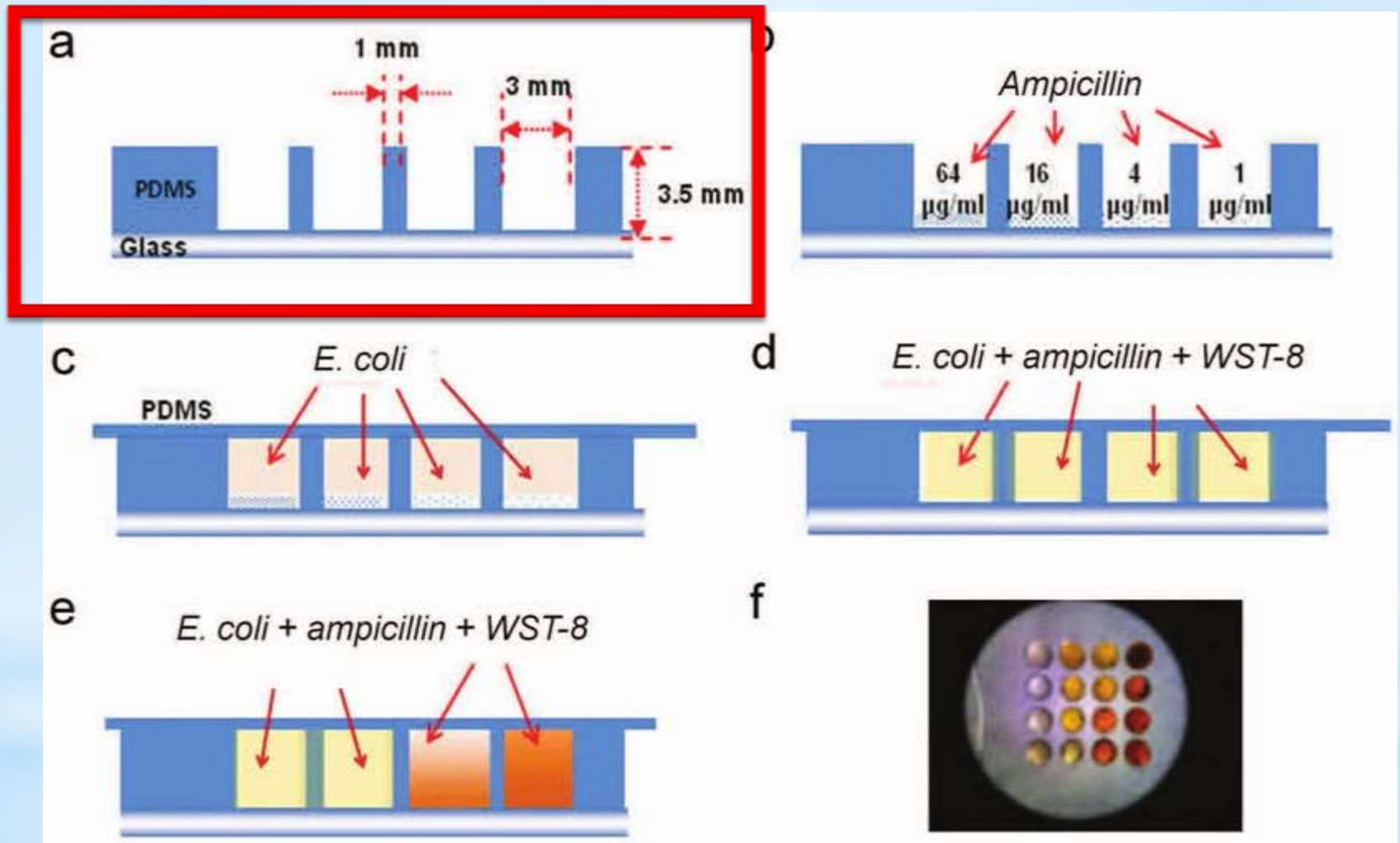




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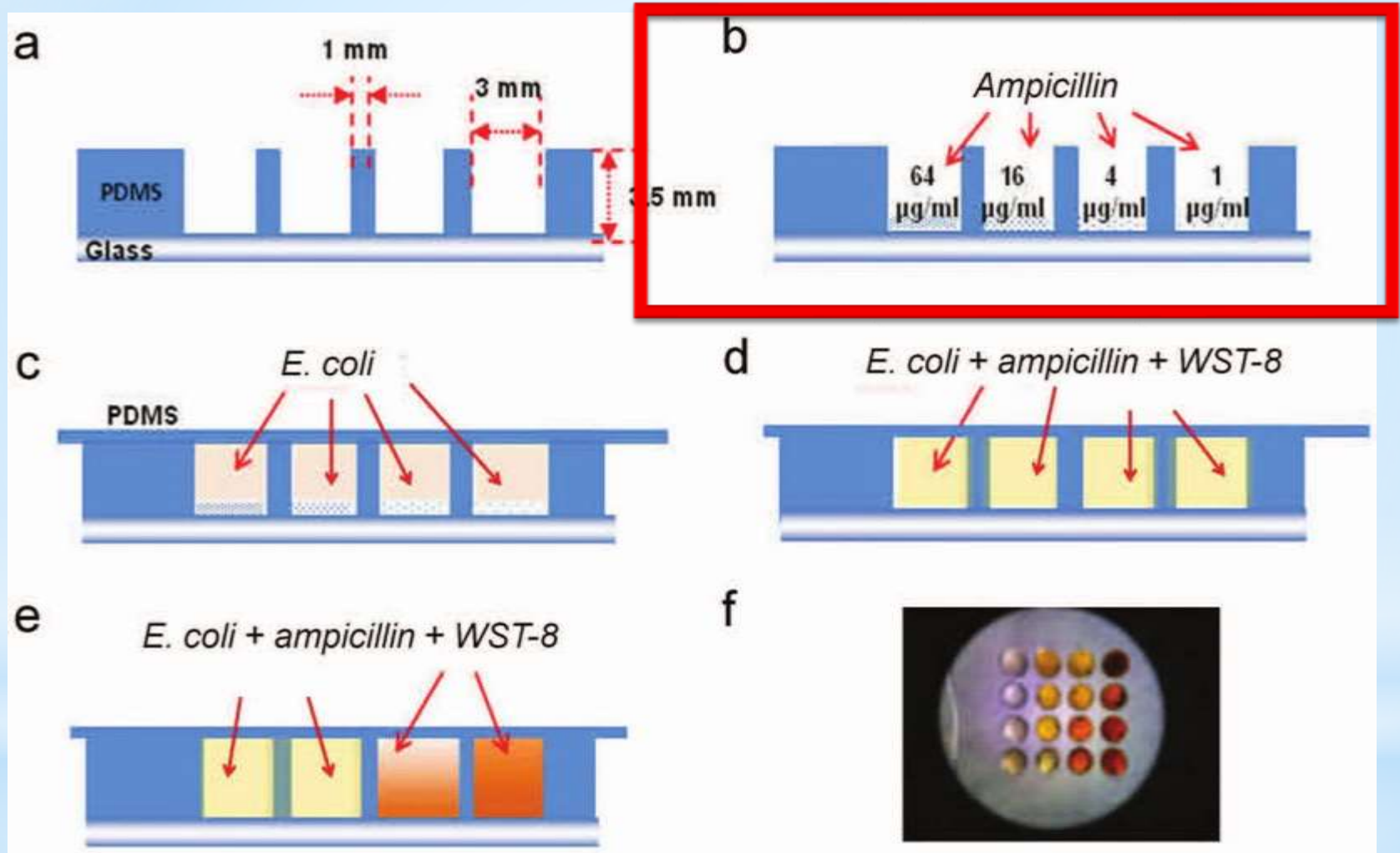
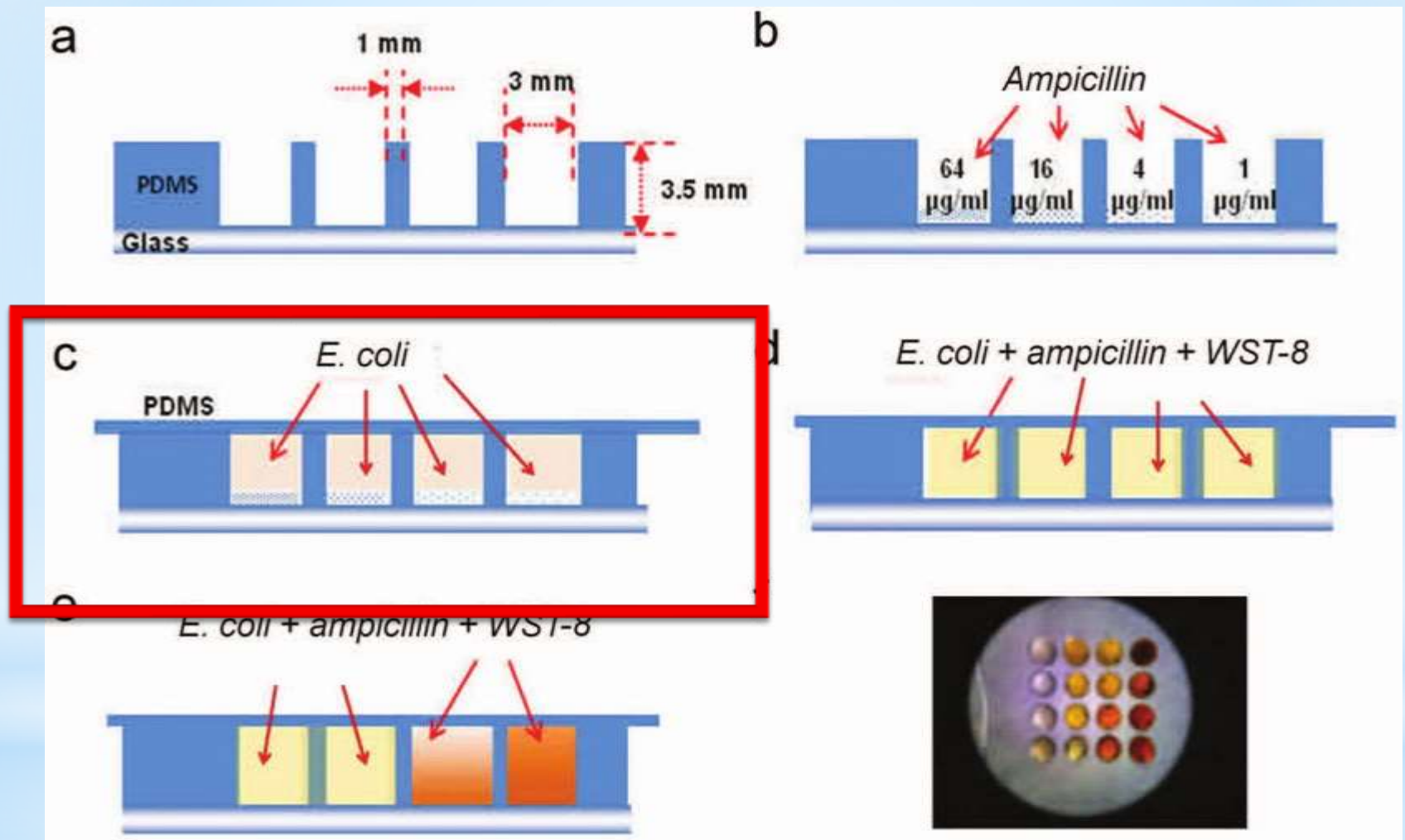


Figure 2. Polydimethylsiloxane microwell assay with precoated antibiotics for minimum inhibitory concentration determination and antimicrobial resistance profiling.



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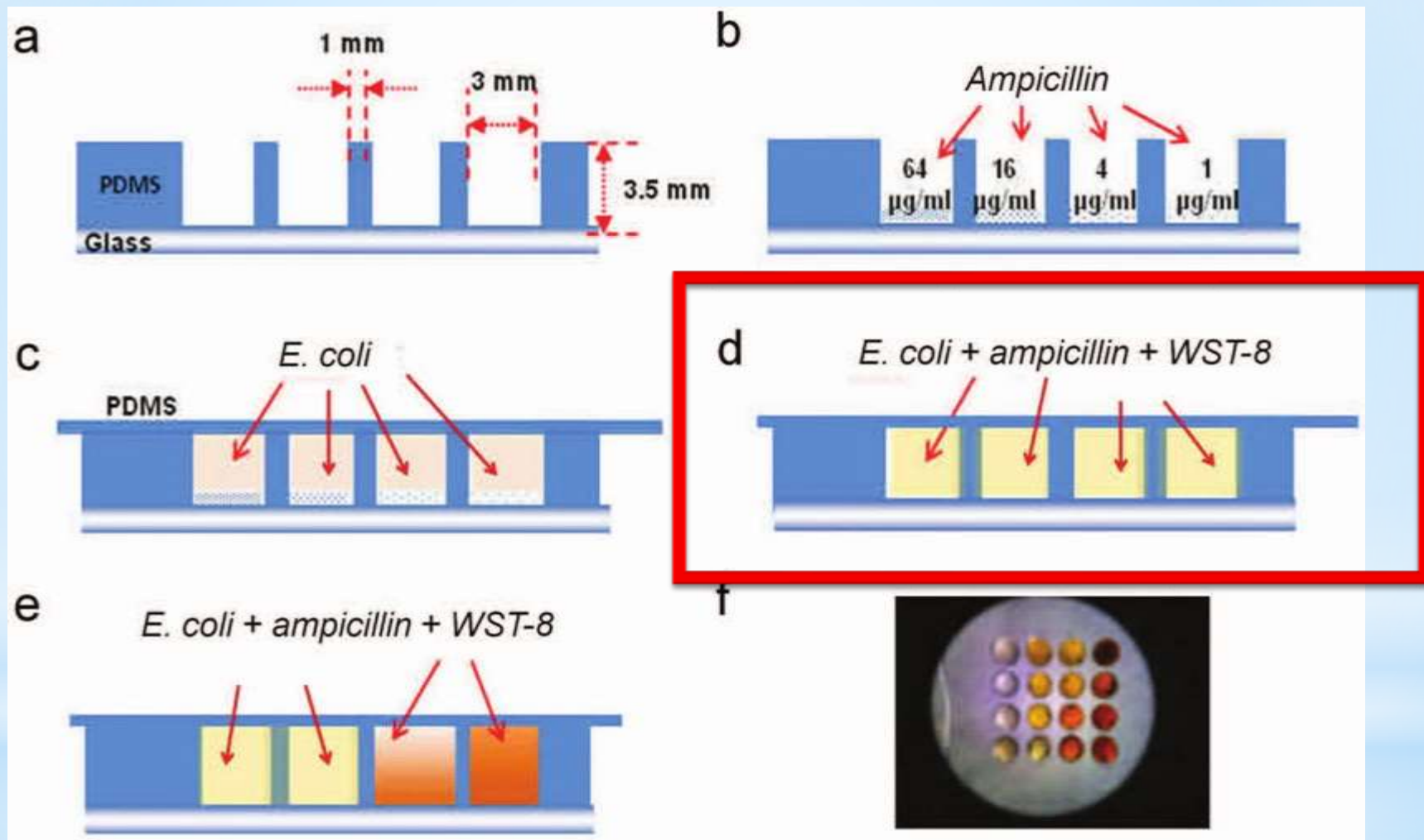
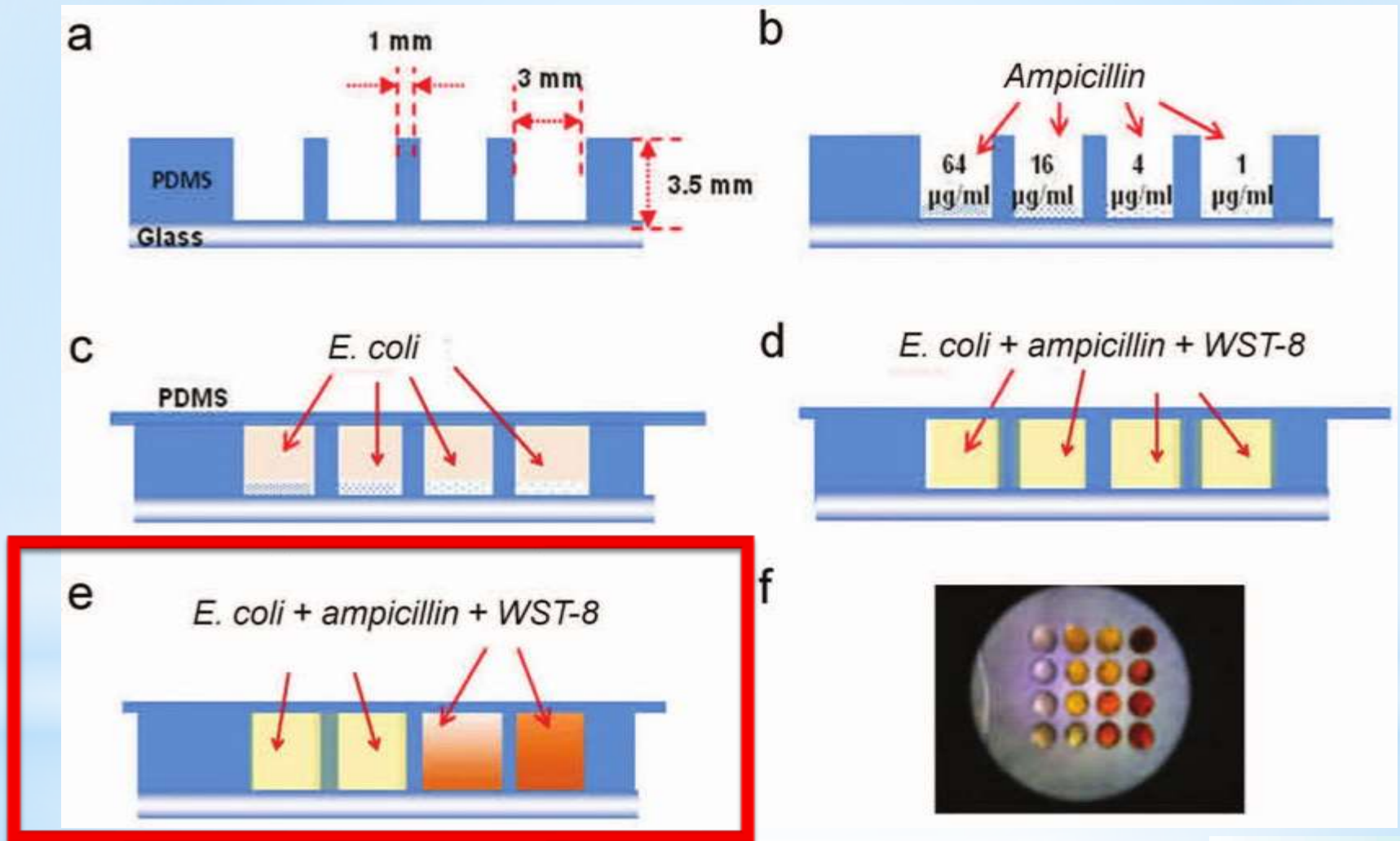
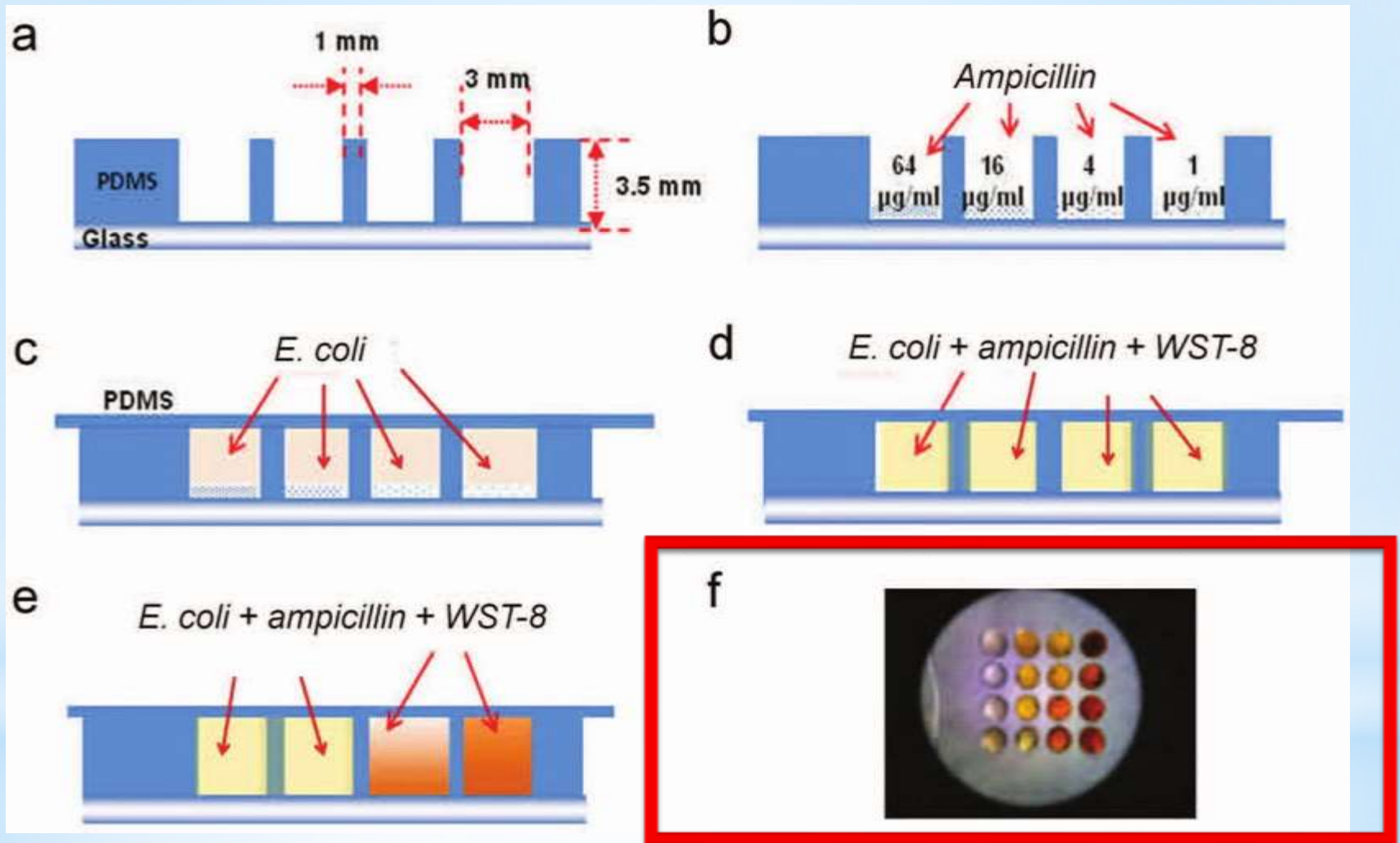


Figure 2. Polydimethylsiloxane microwell assay with precoated antibiotics for minimum inhibitory concentration determination and antimicrobial resistance profiling.

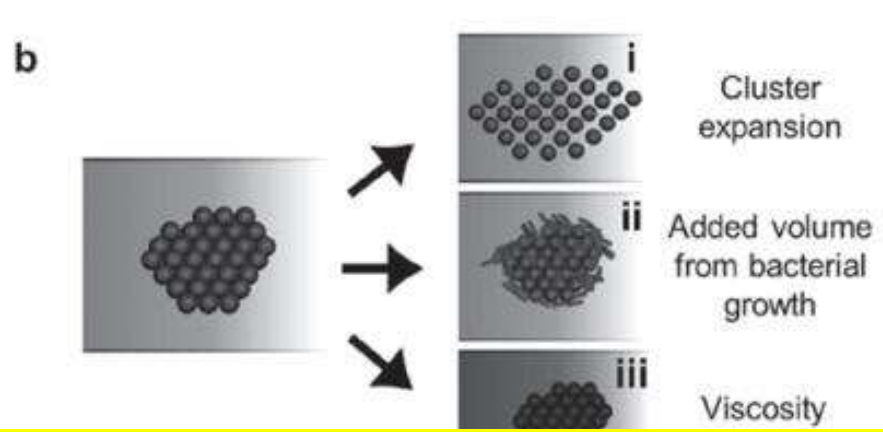
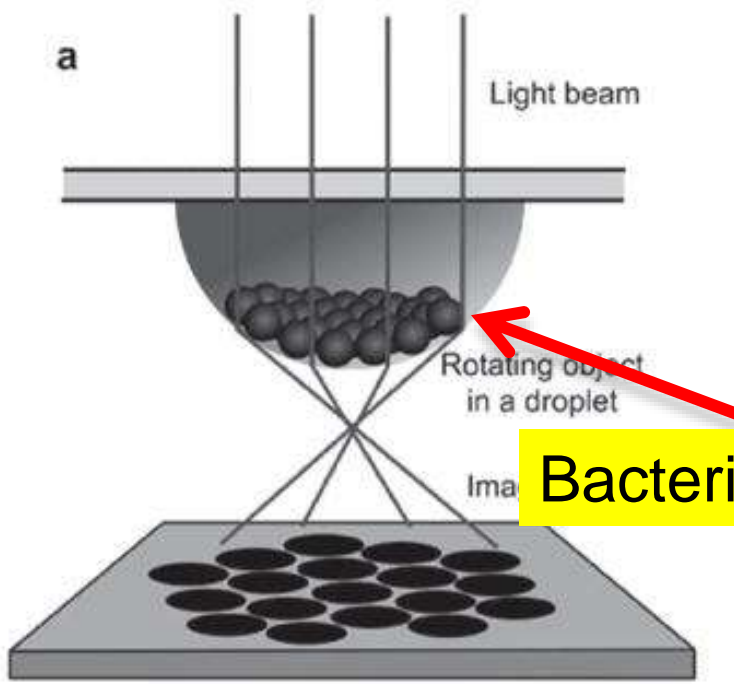


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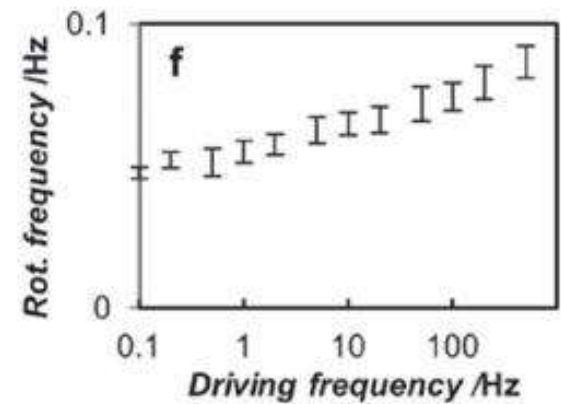
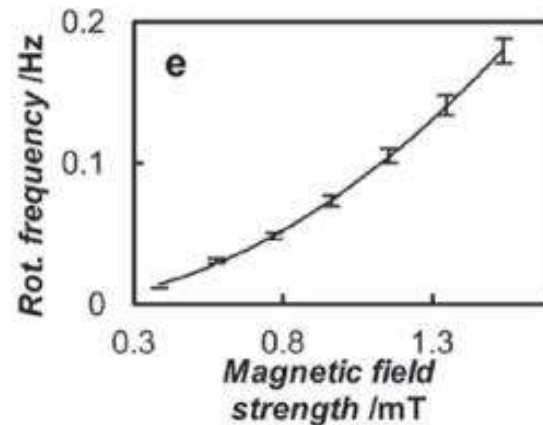
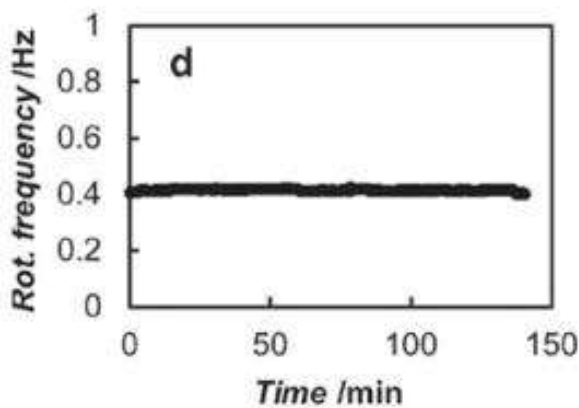
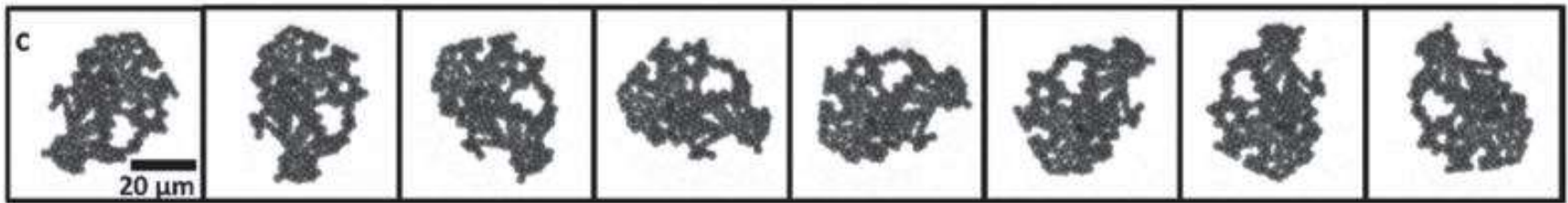
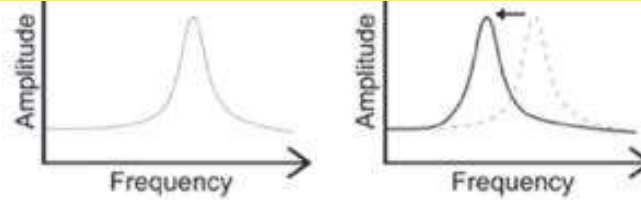
Figure 2. Polydimethylsiloxane microwell assay with precoated antibiotics for minimum inhibitory concentration determination and antimicrobial resistance profiling.

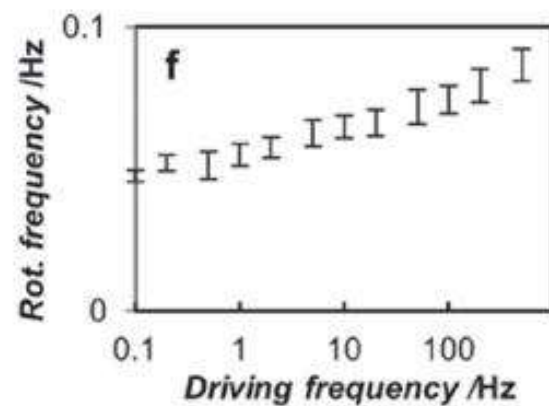
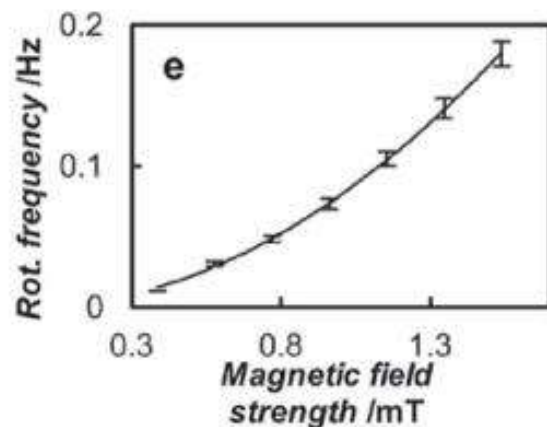
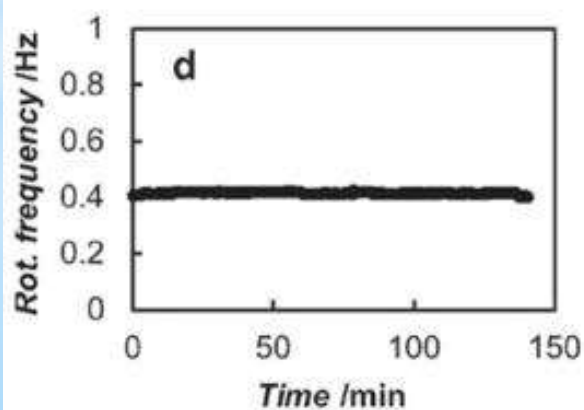
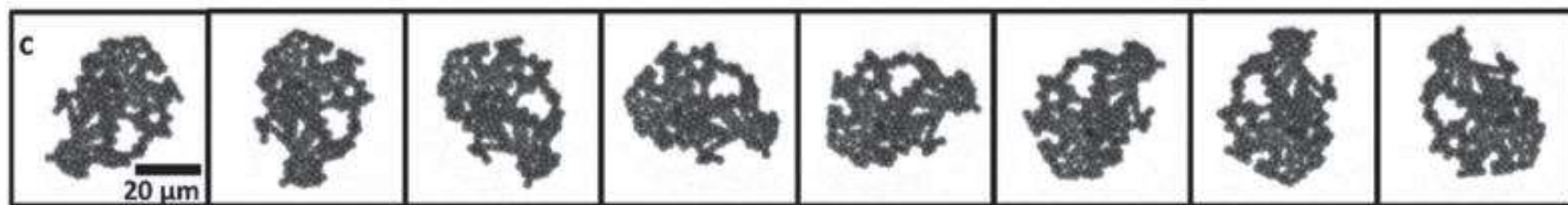
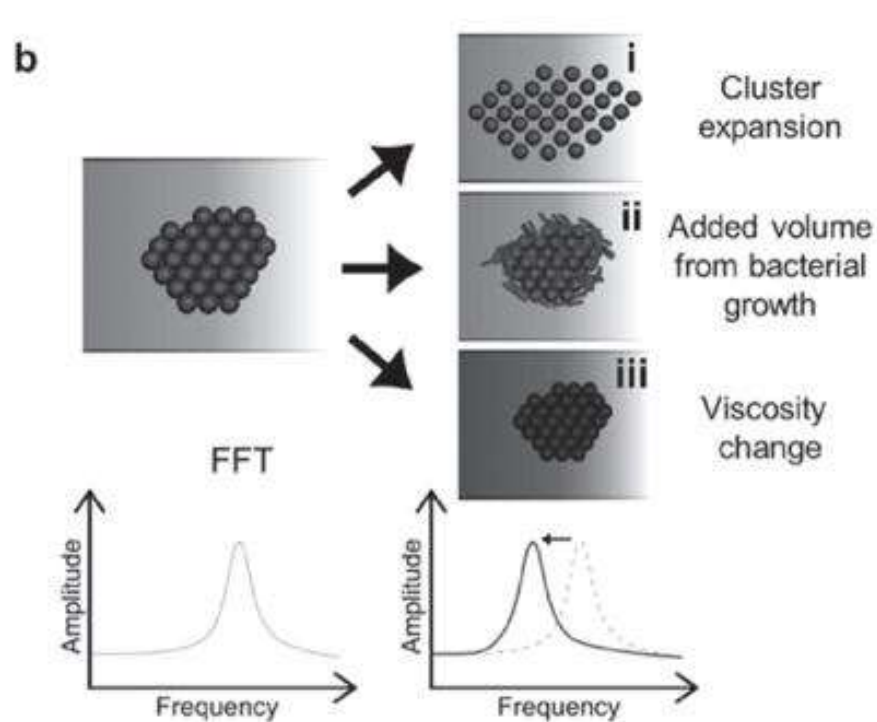
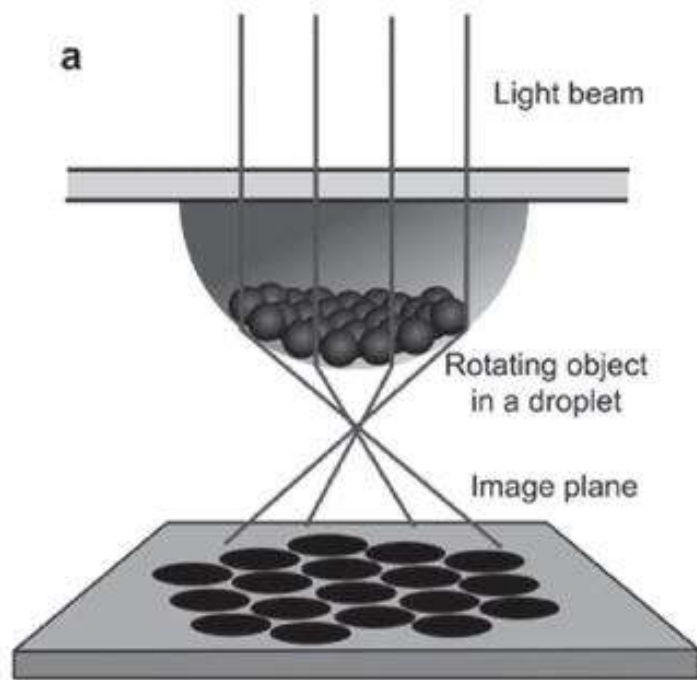


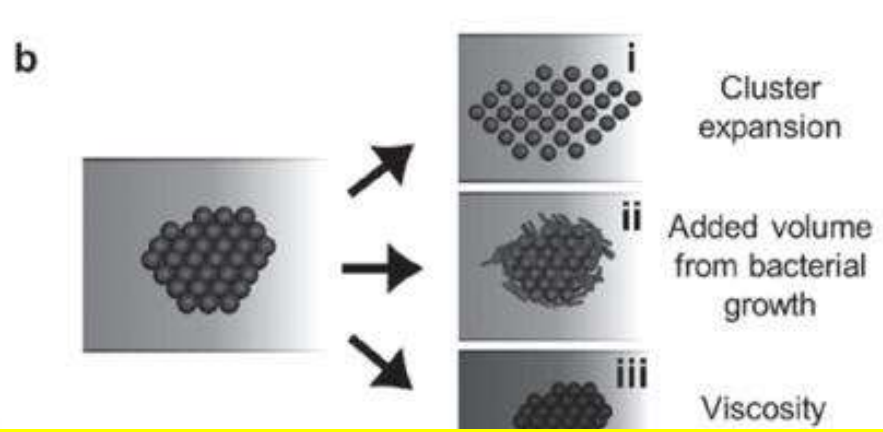
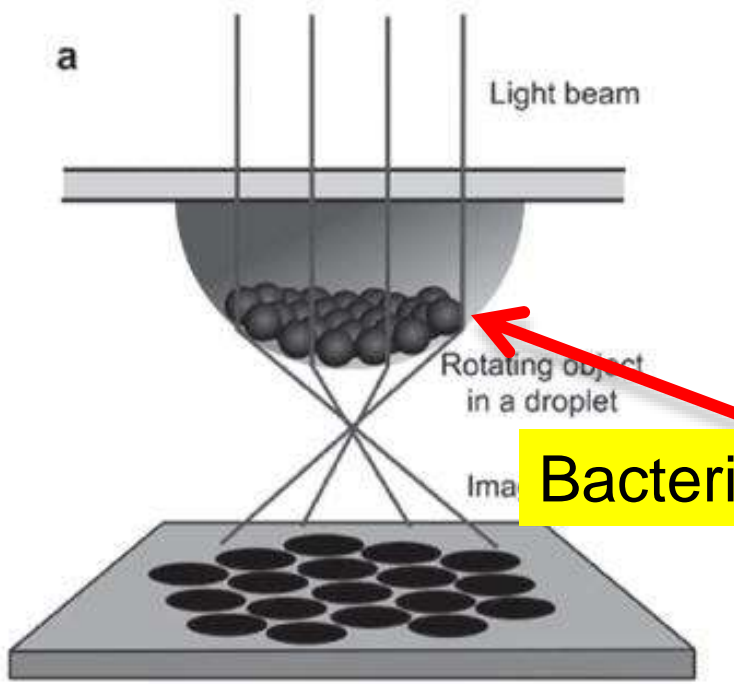
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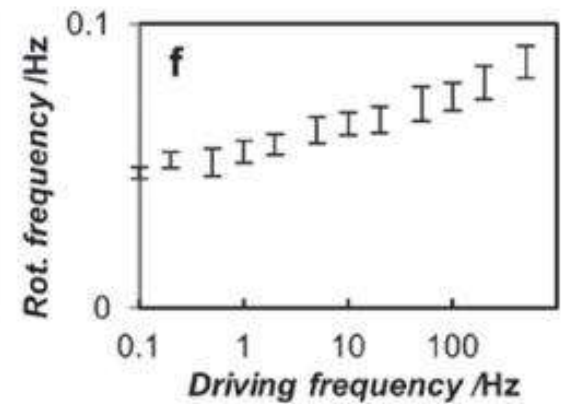
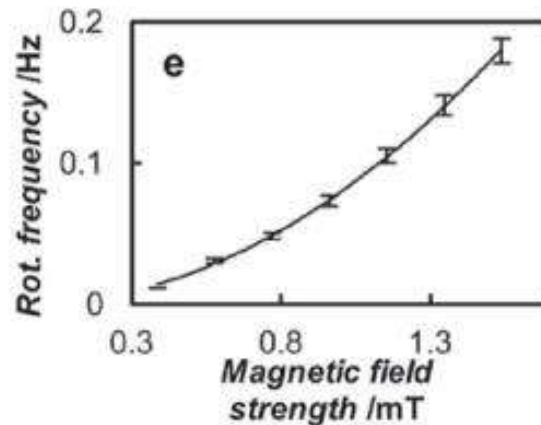
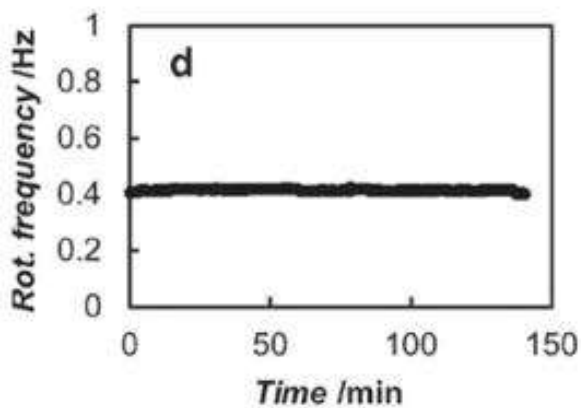
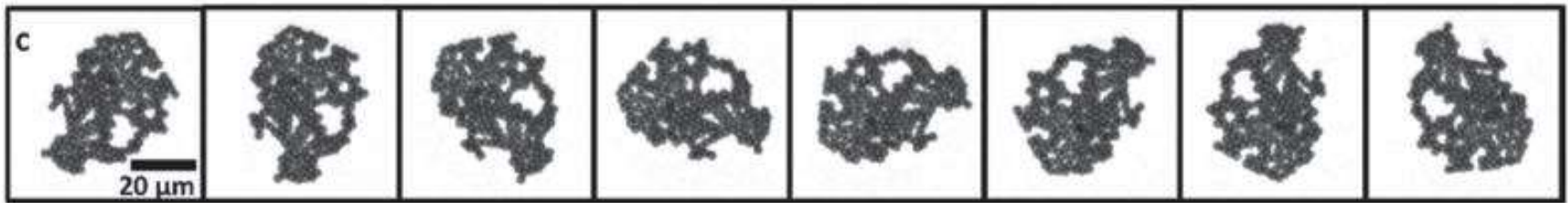
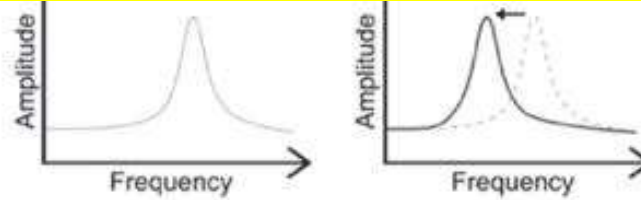
Bacteria + antibody coated magnetic beads



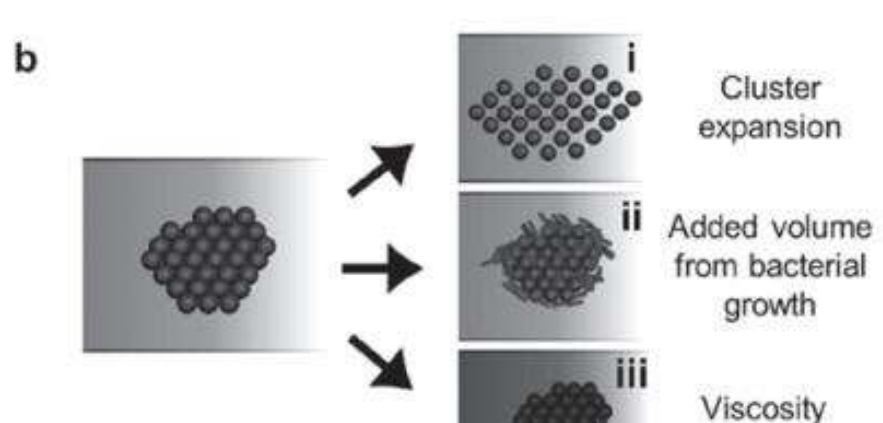
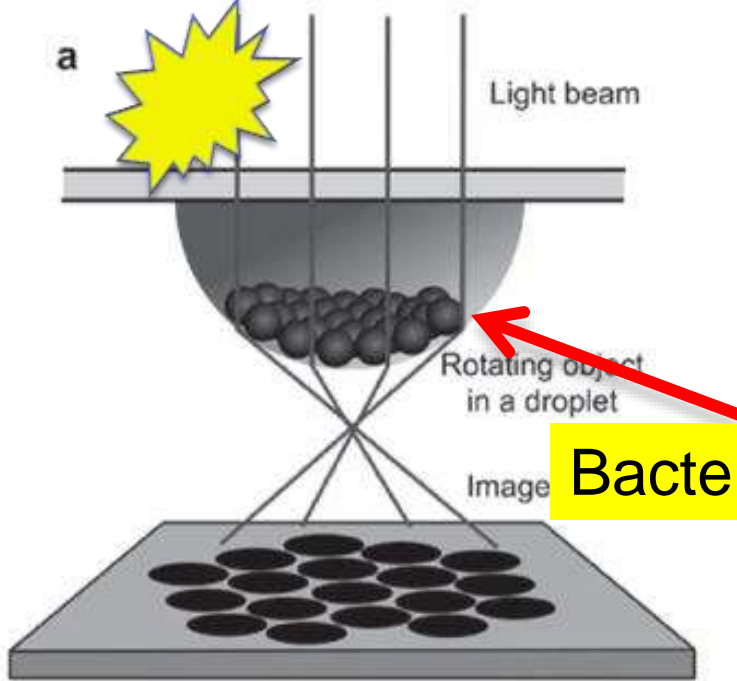




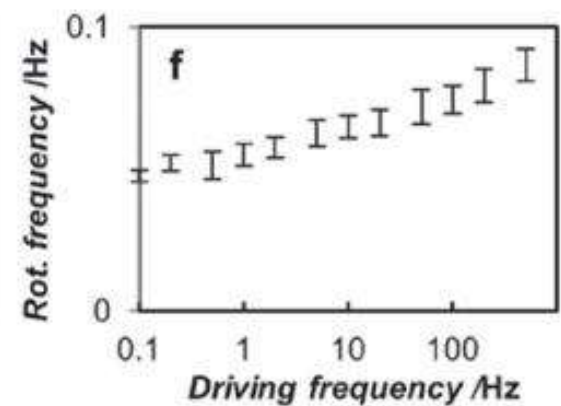
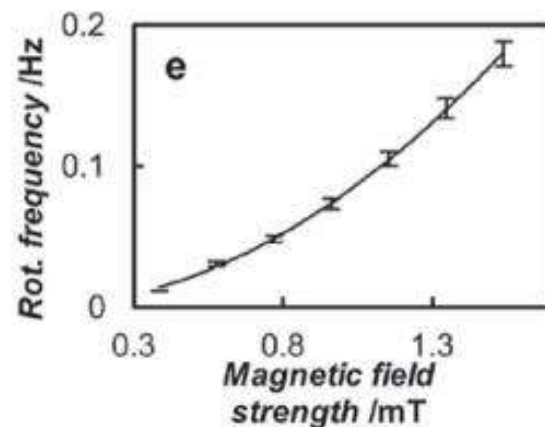
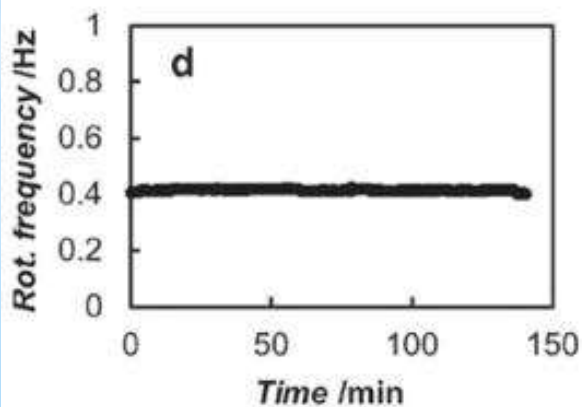
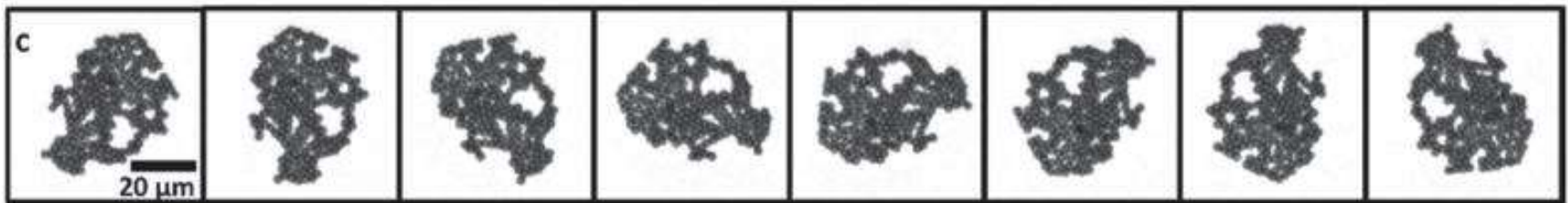
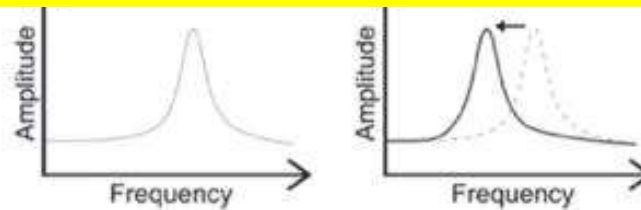
Bacteria + antibody coated magnetic beads

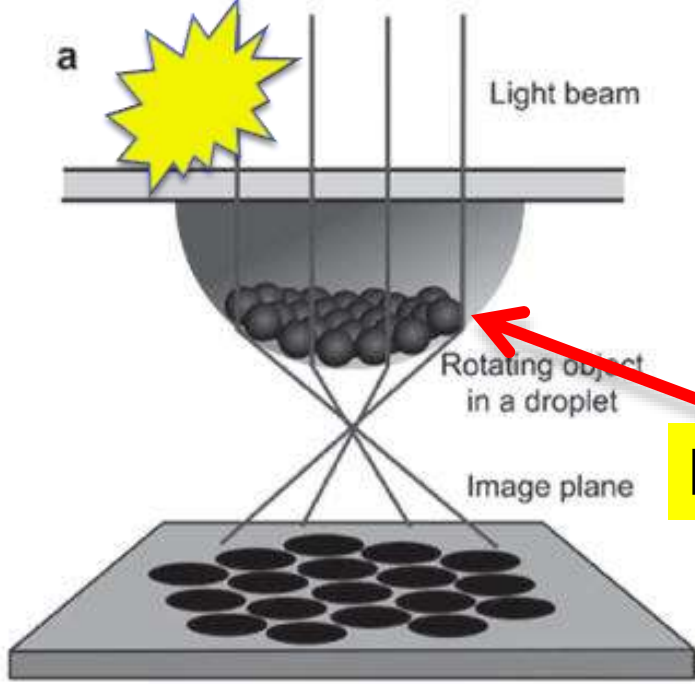




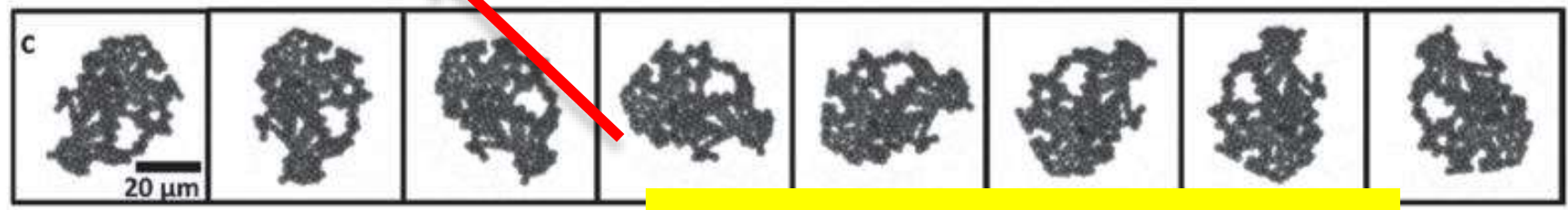
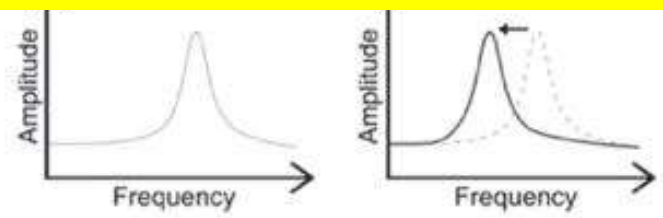
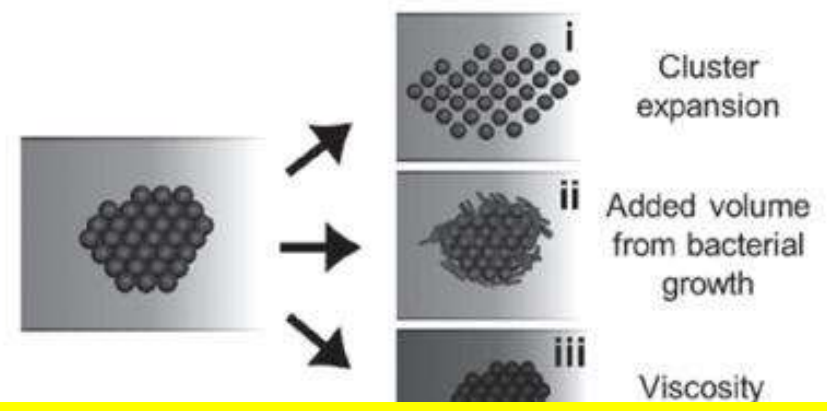


Bacteria + antibody coated magnetic beads

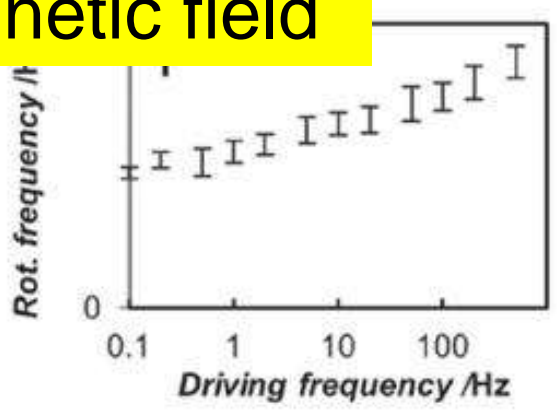
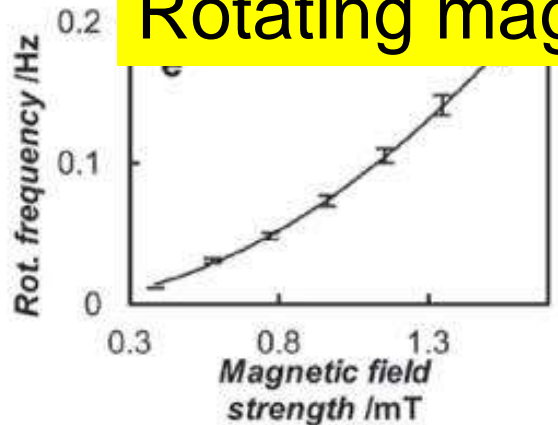
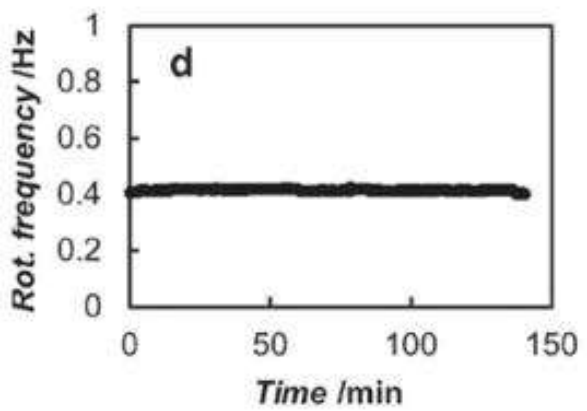


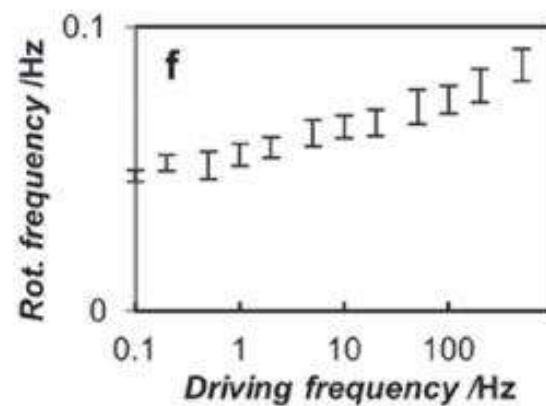
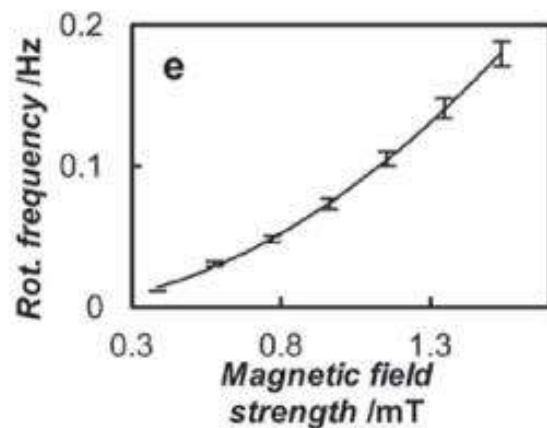
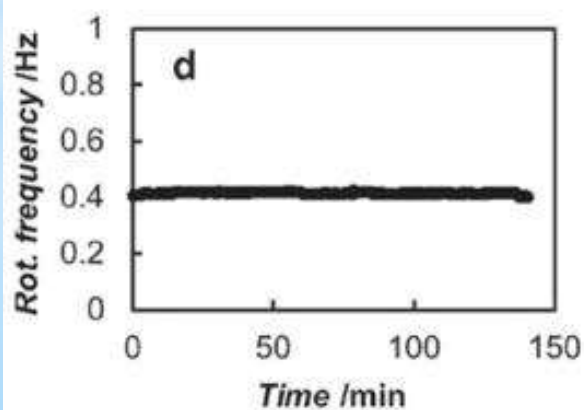
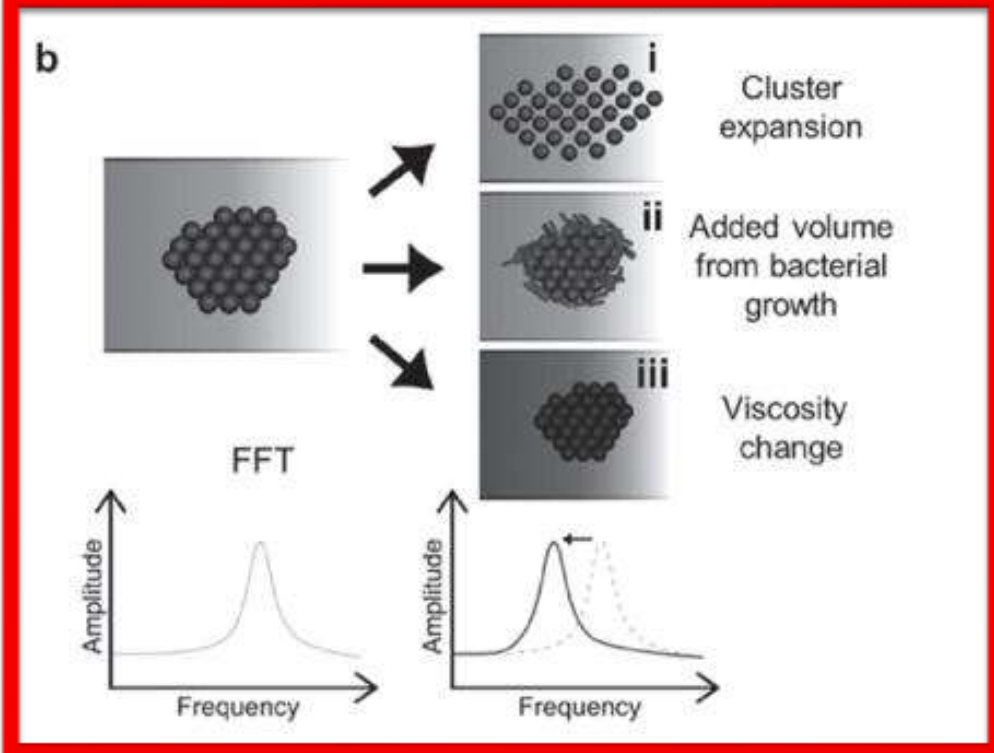
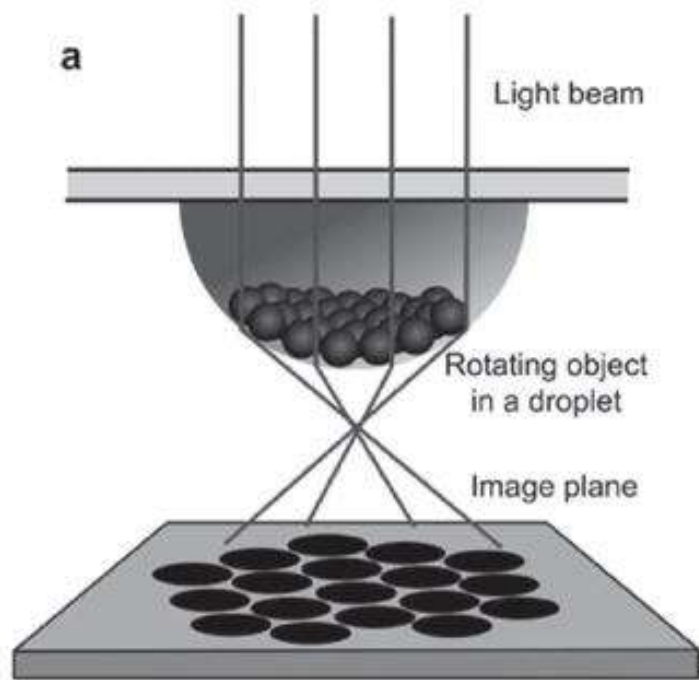


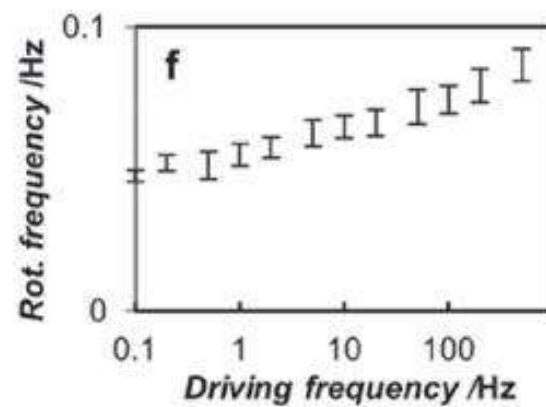
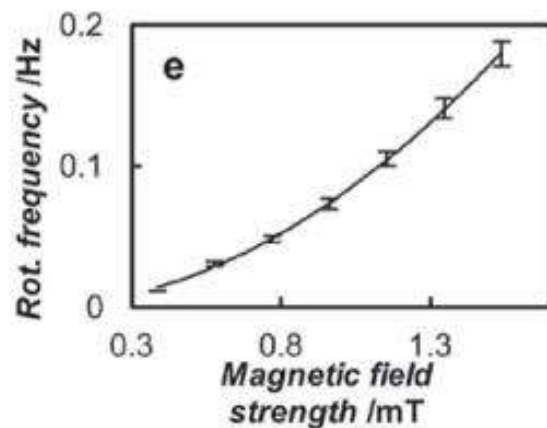
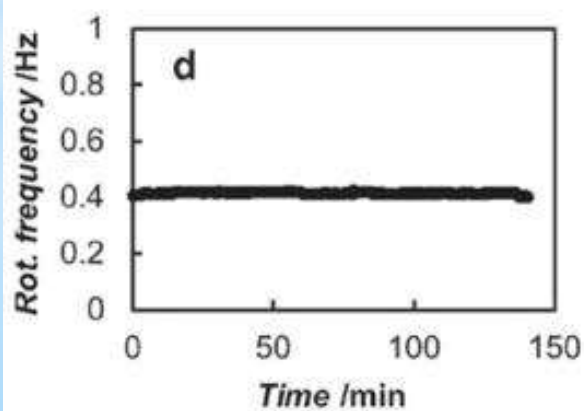
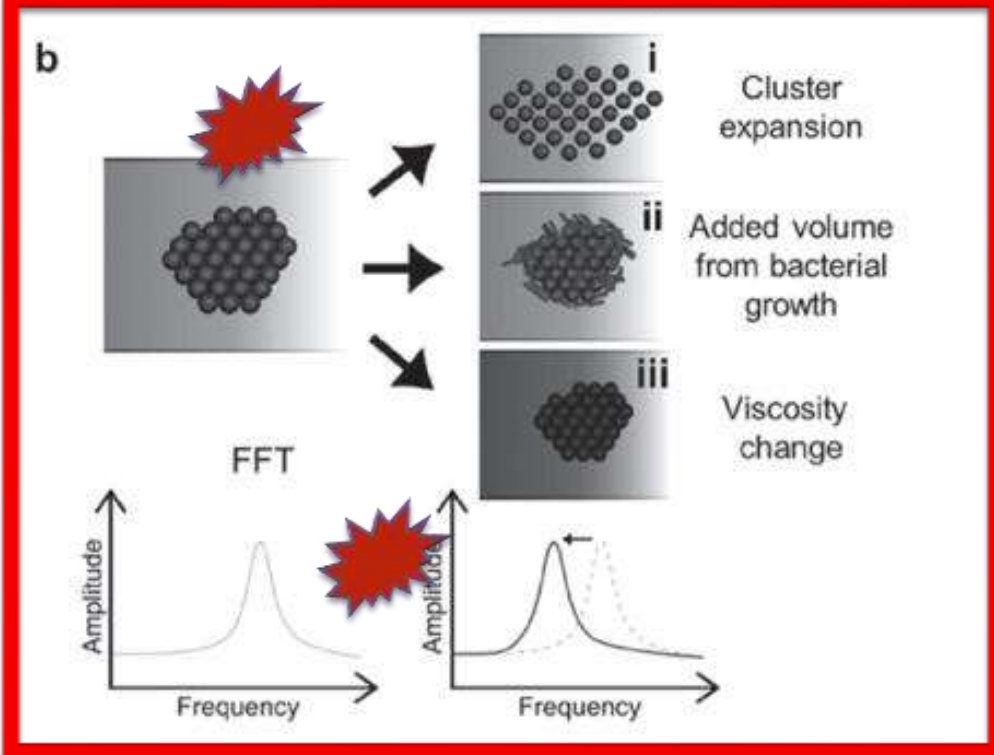
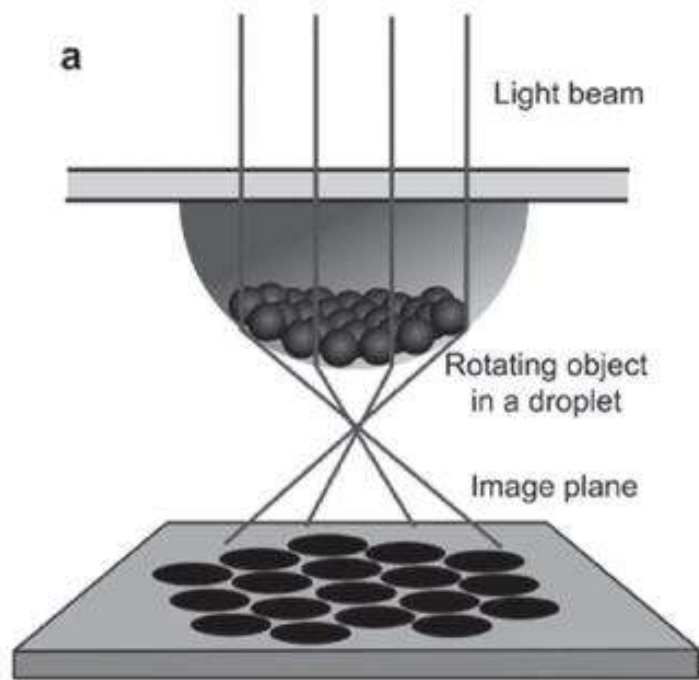
Bacteria + antibody + magnetic beads

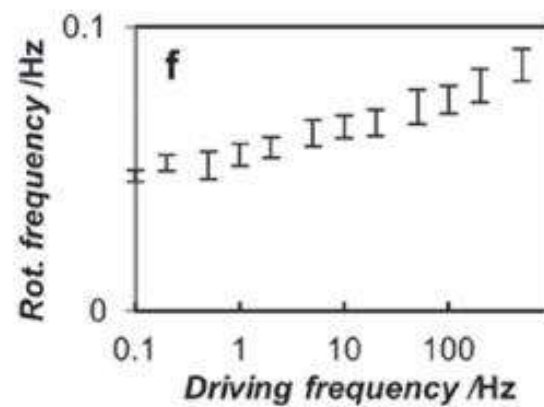
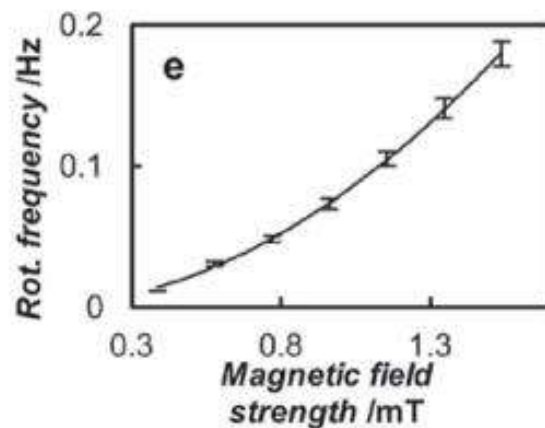
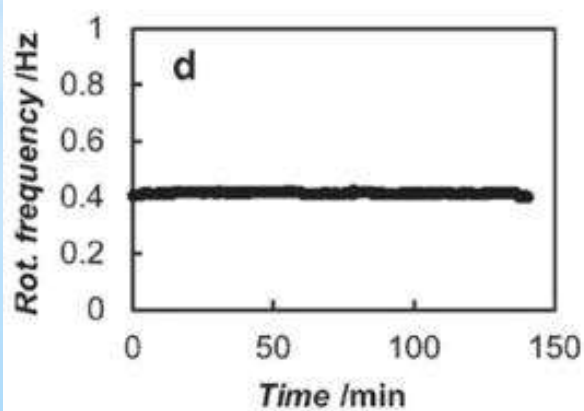
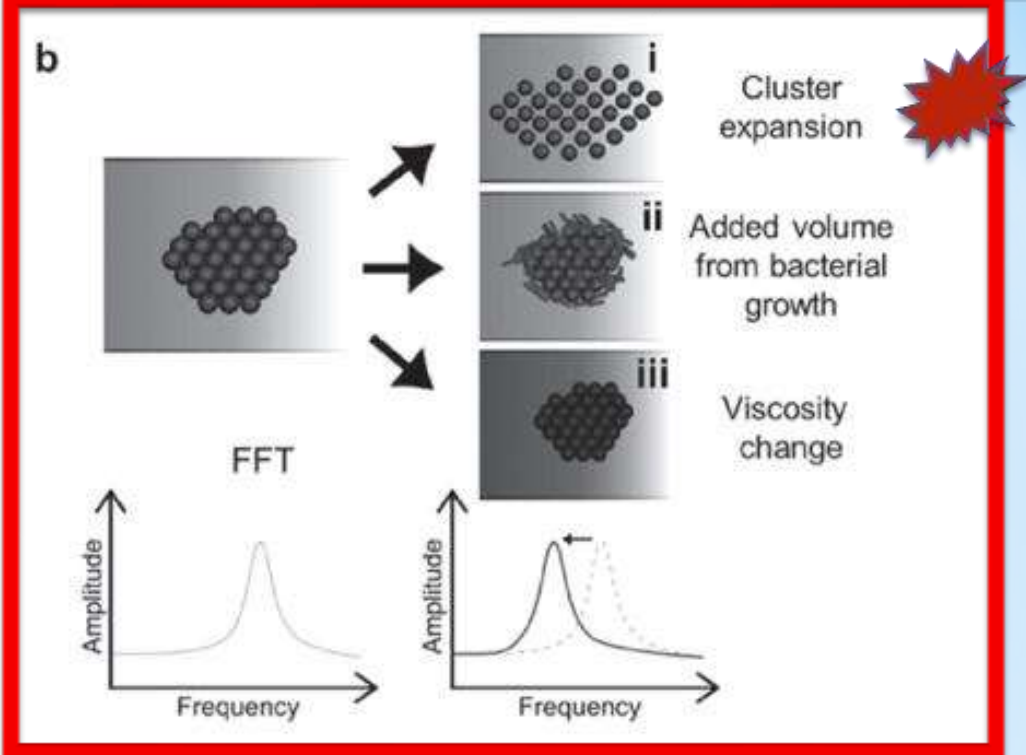
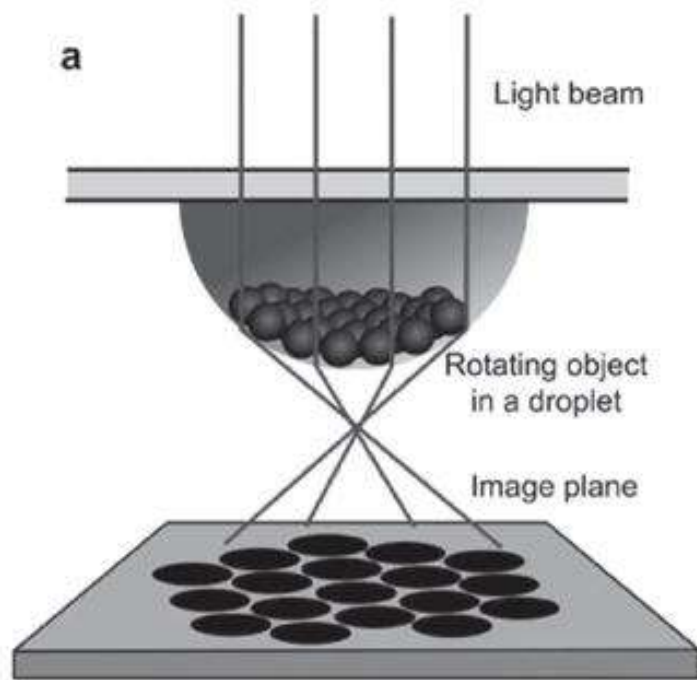


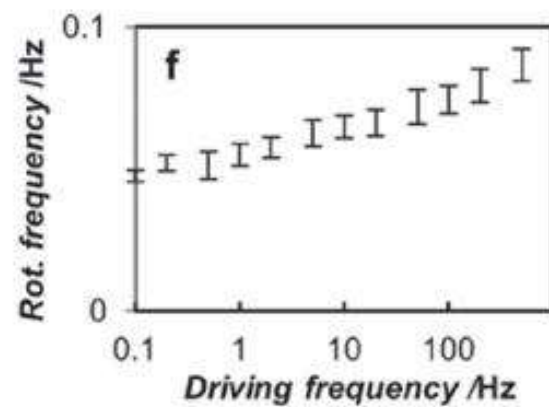
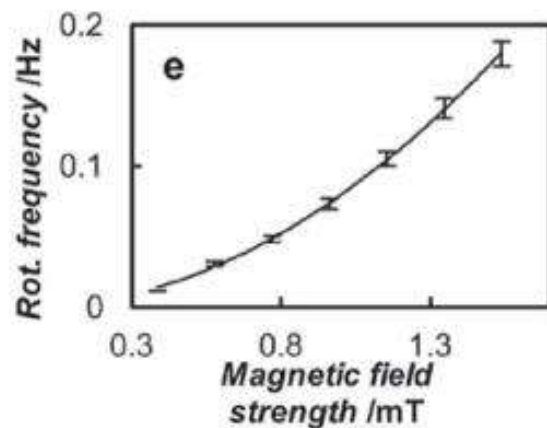
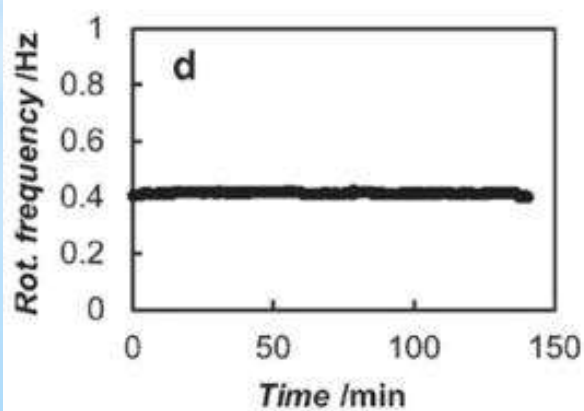
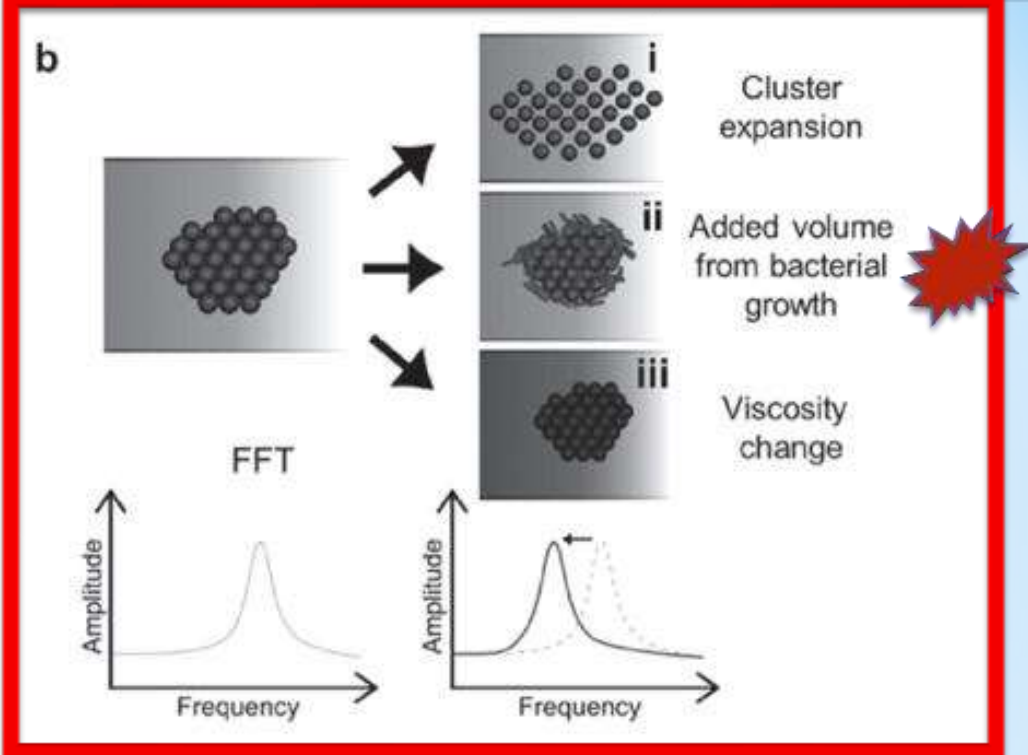
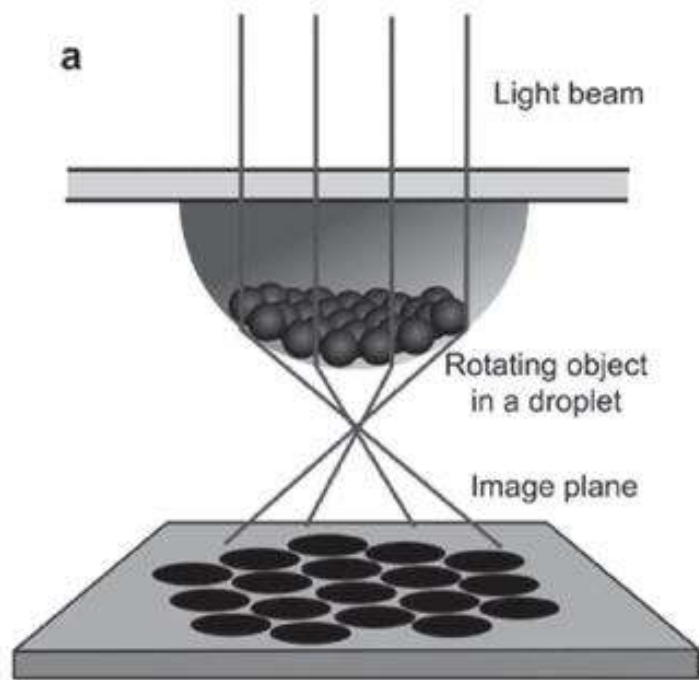
Rotating magnetic field

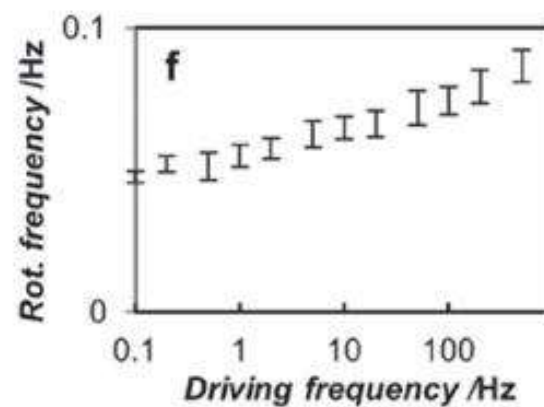
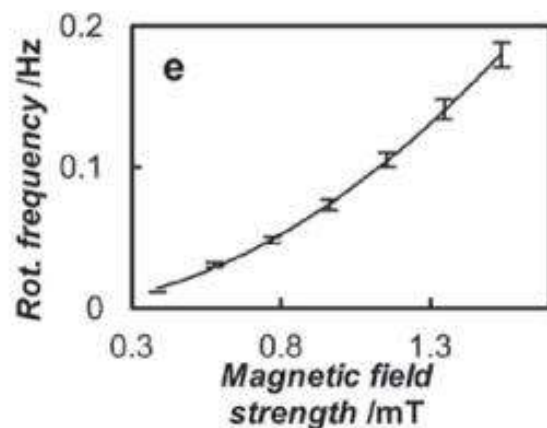
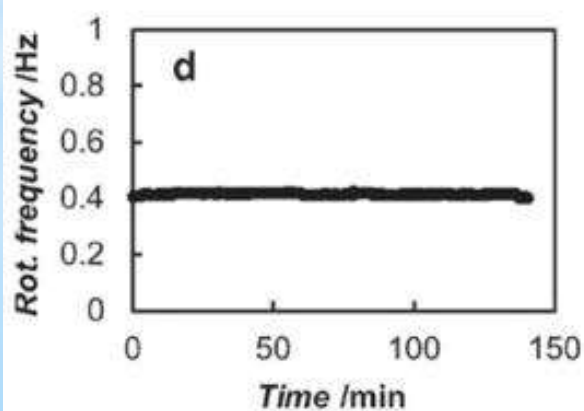
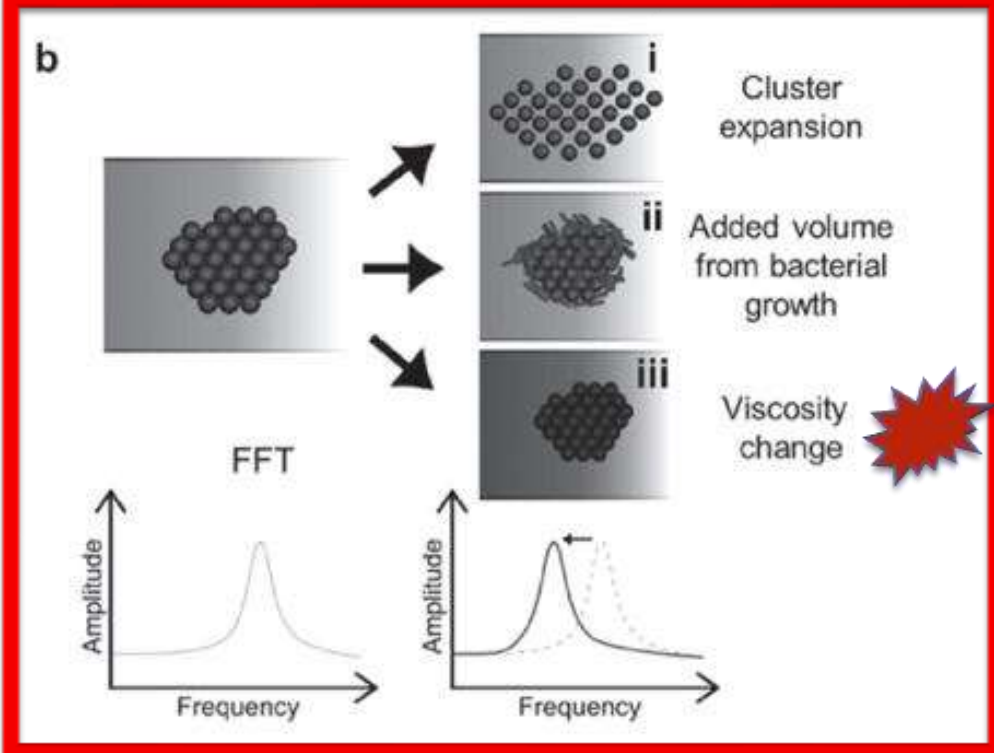
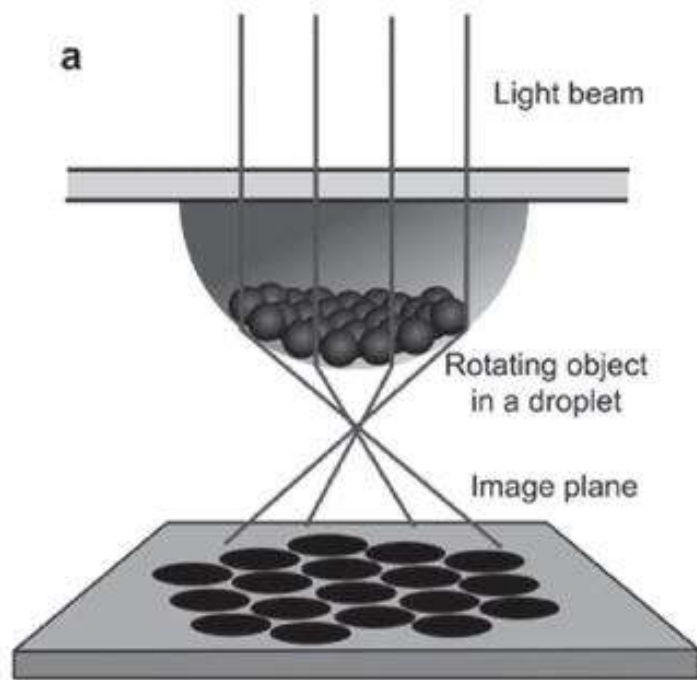


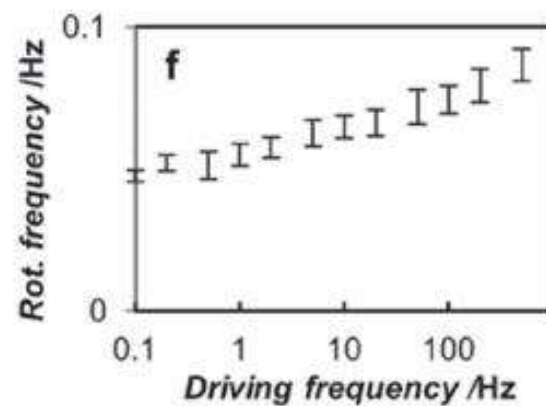
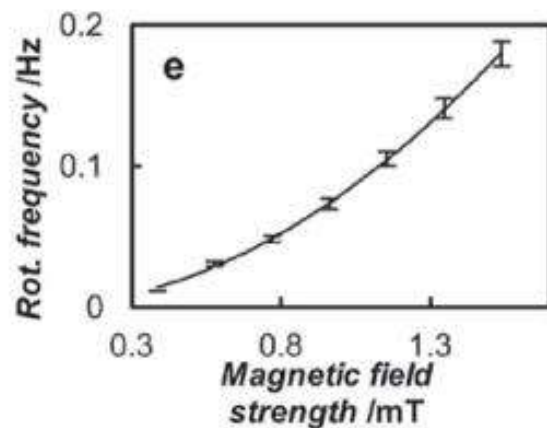
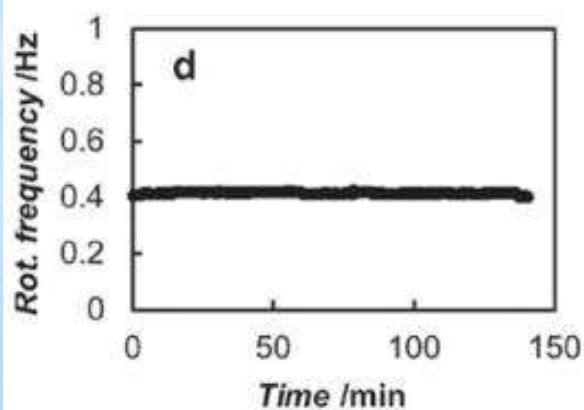
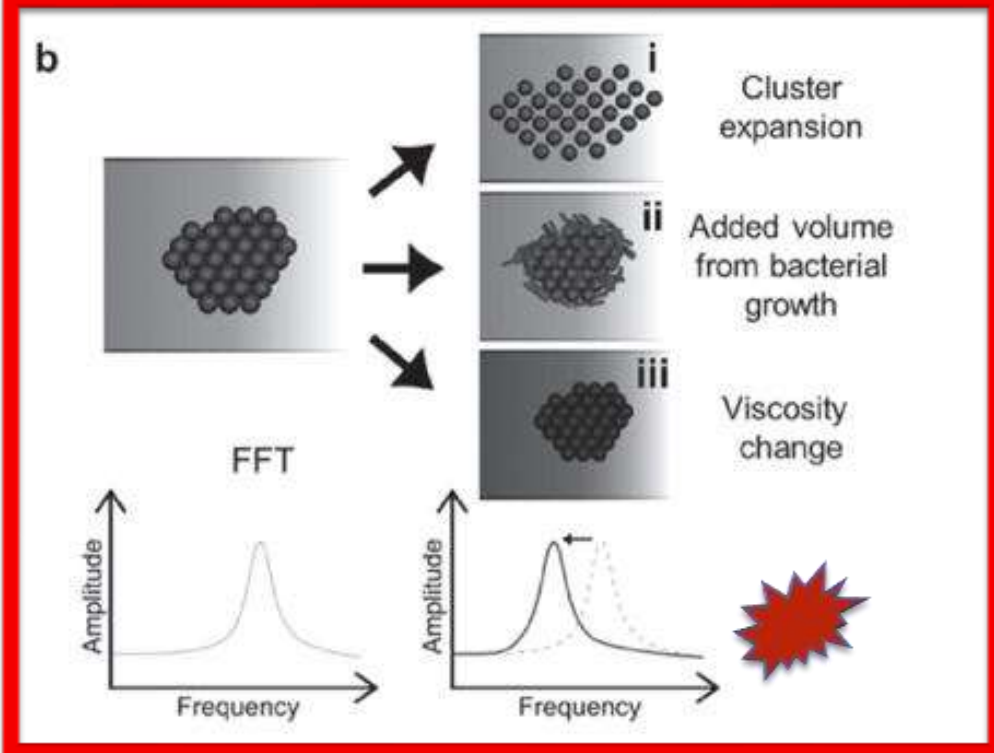
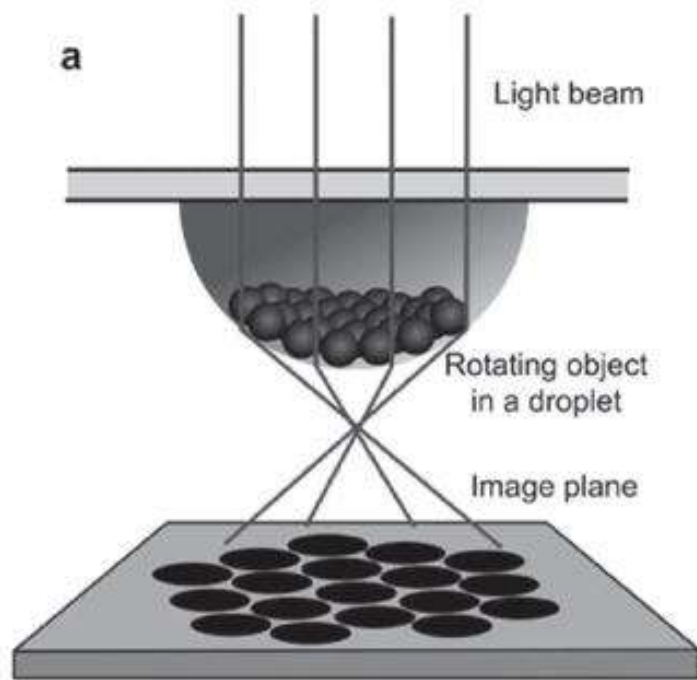






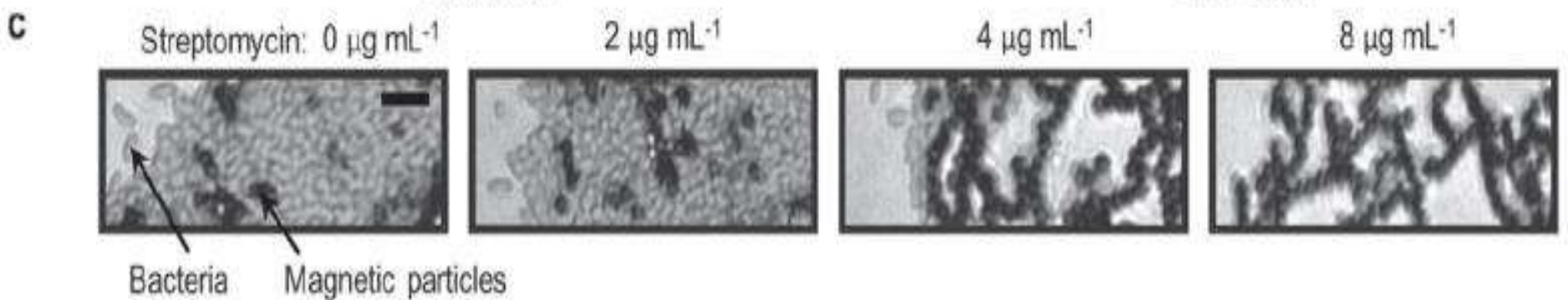
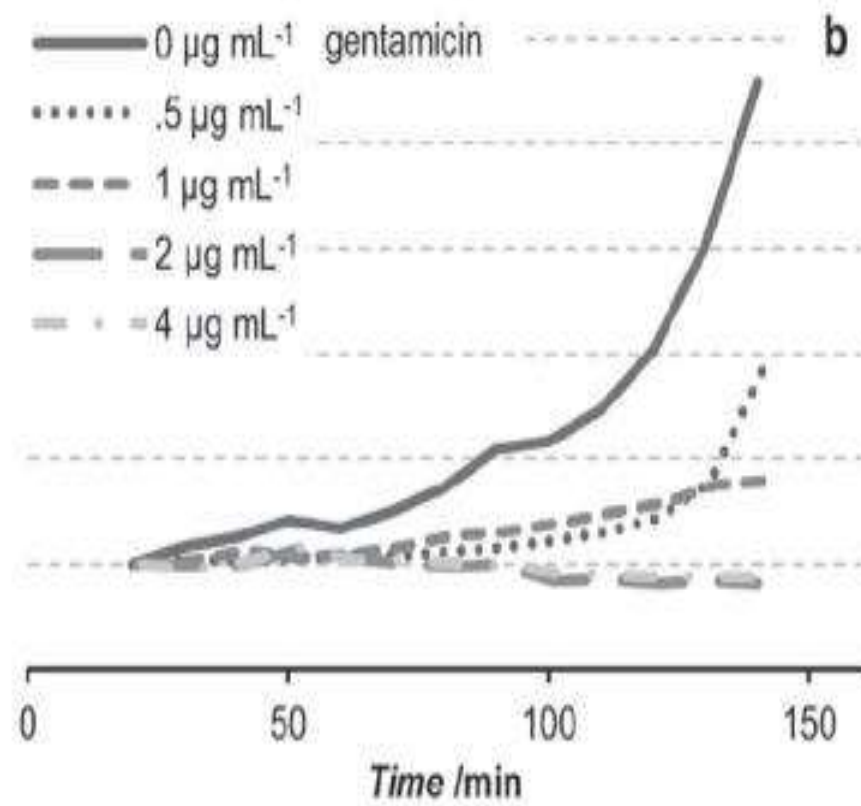
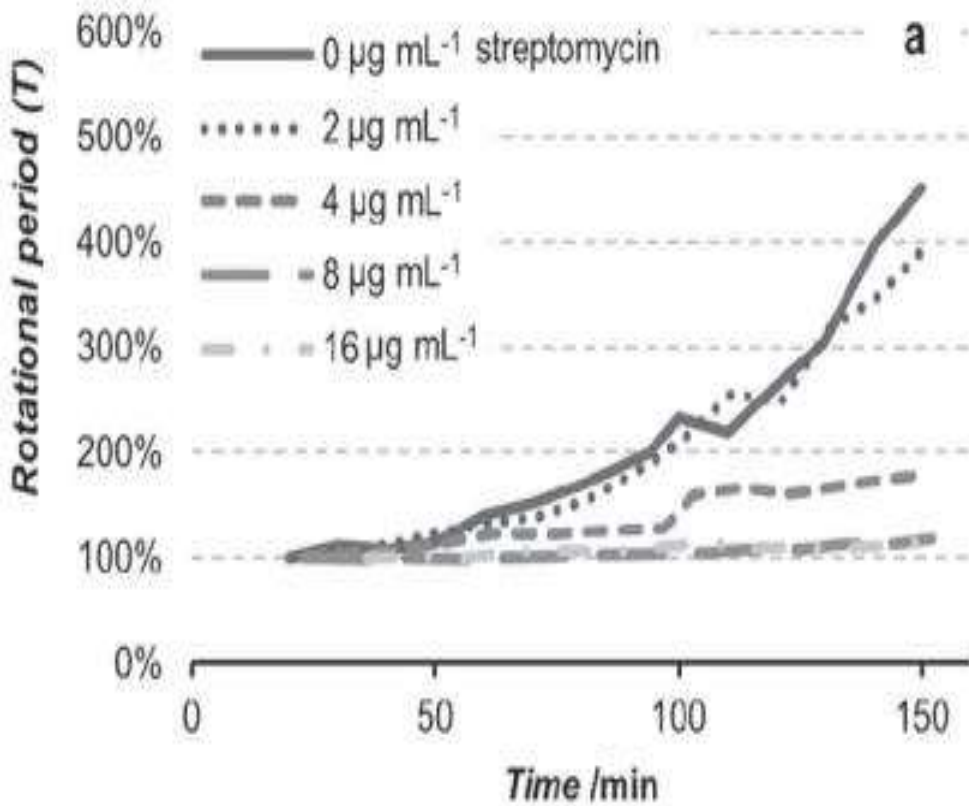




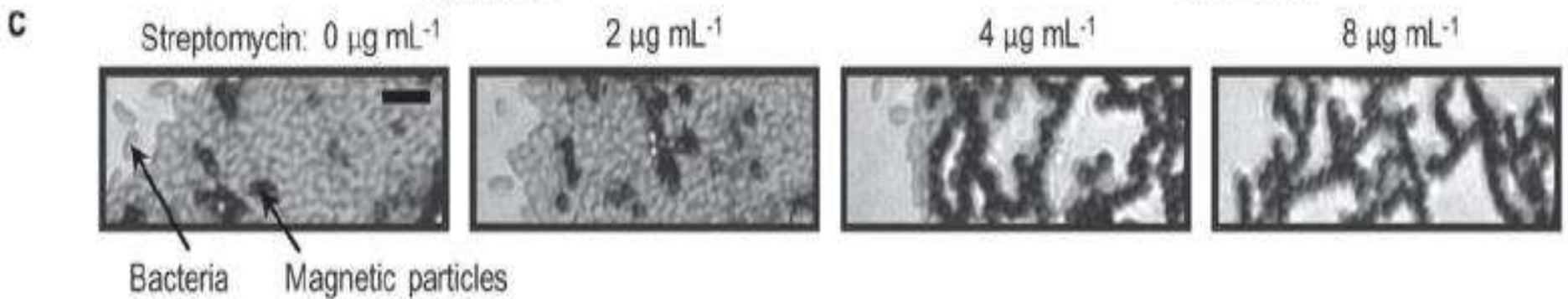
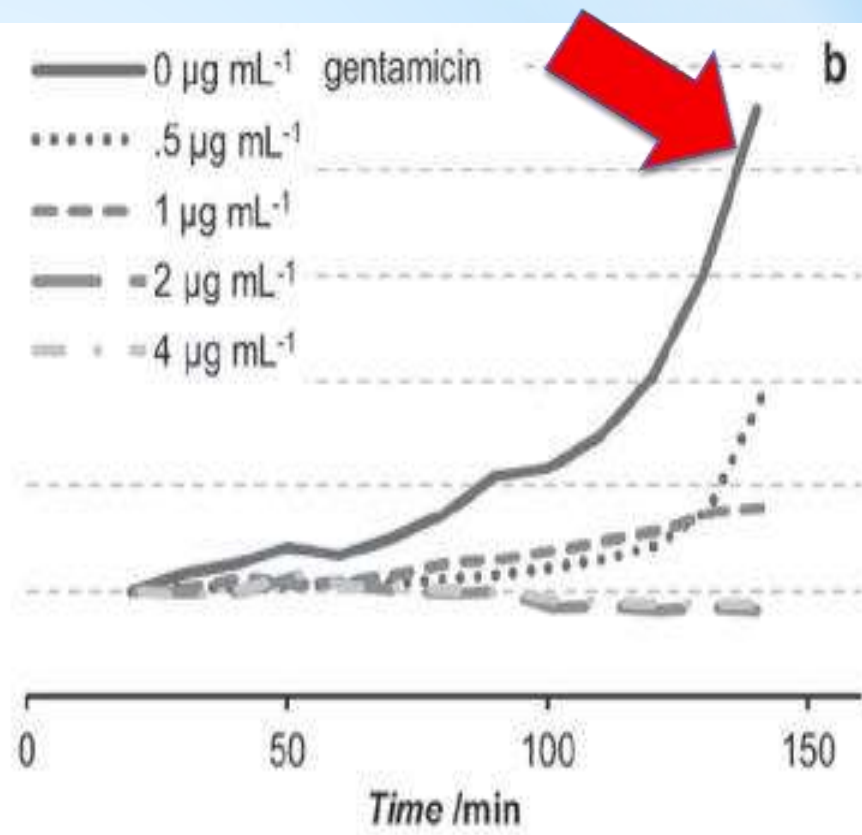
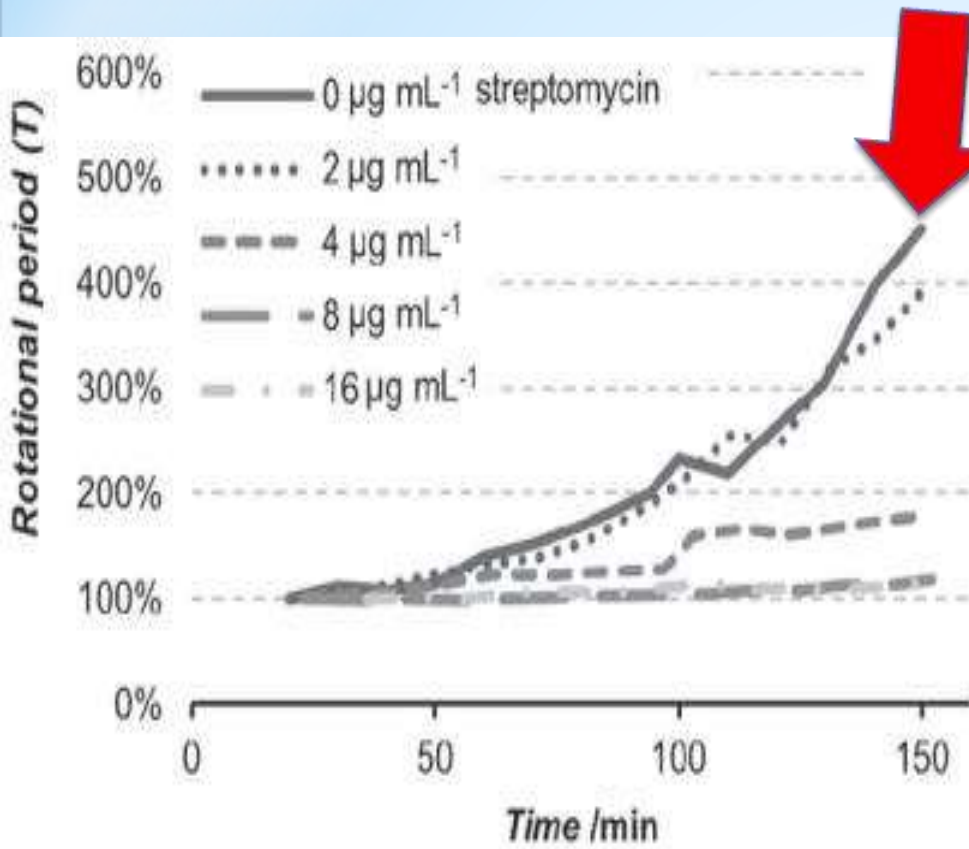




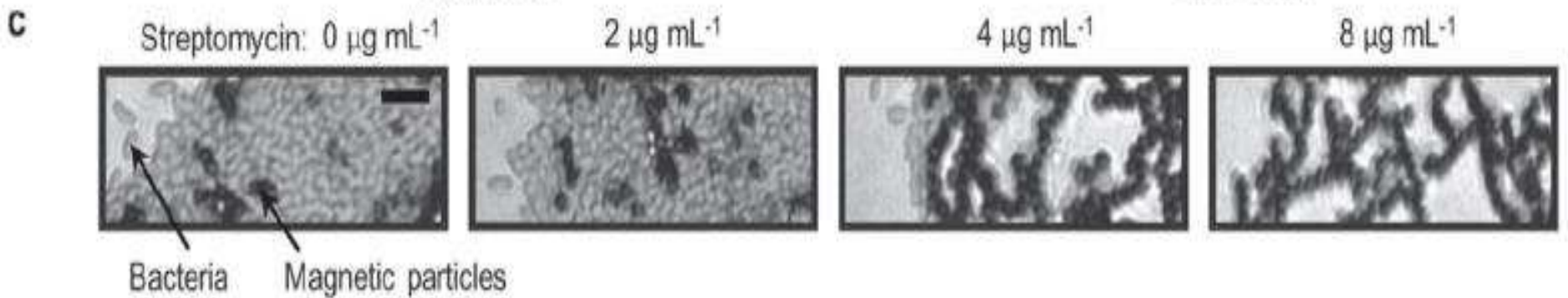
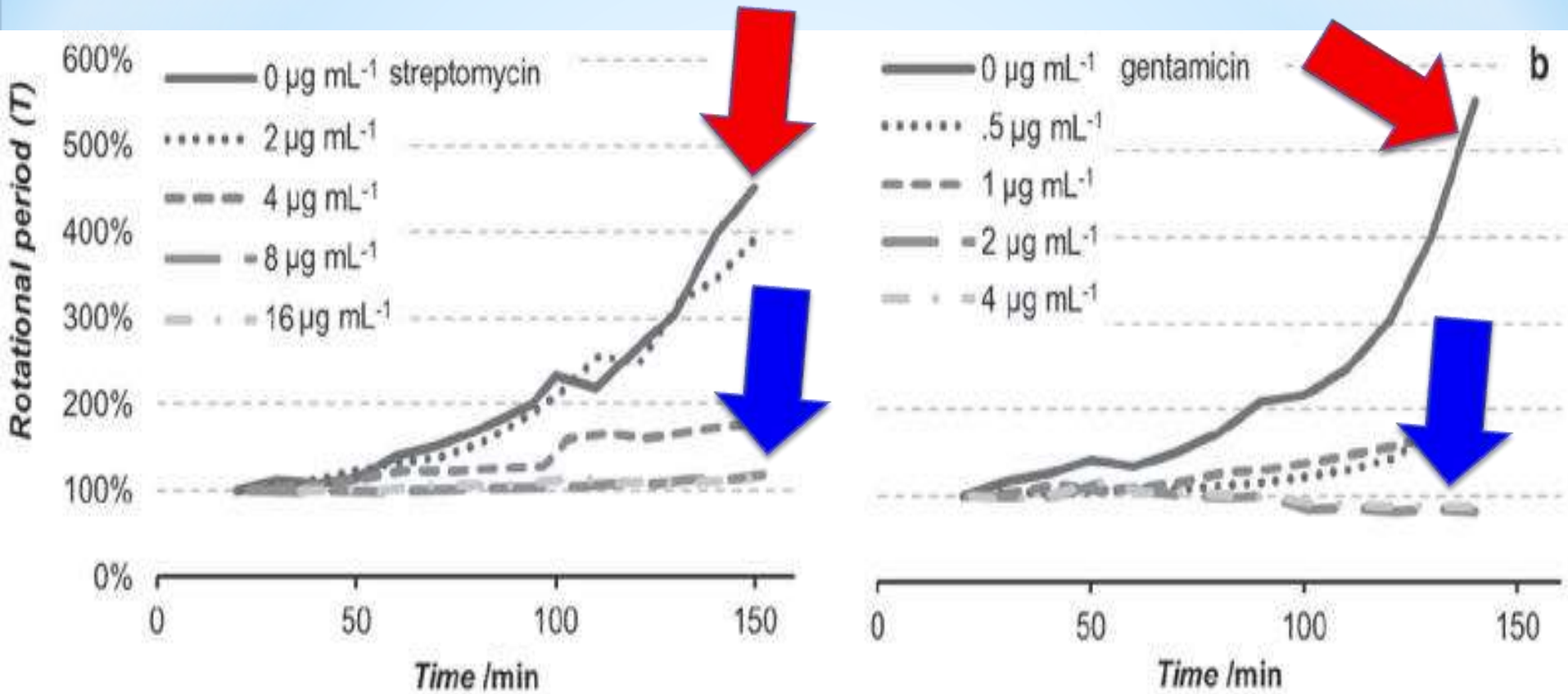
# Magnetic bead biosensor



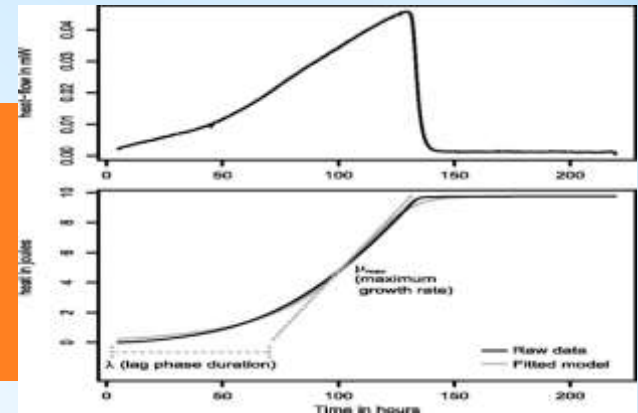
# Magnetic bead biosensor



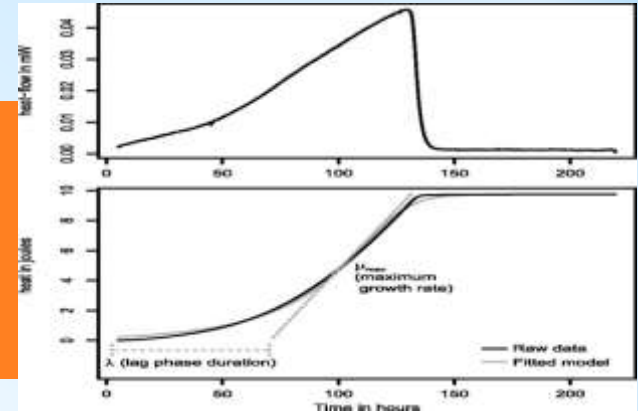
# Magnetic bead biosensor



# Isothermal microcalorimetry (IMC)

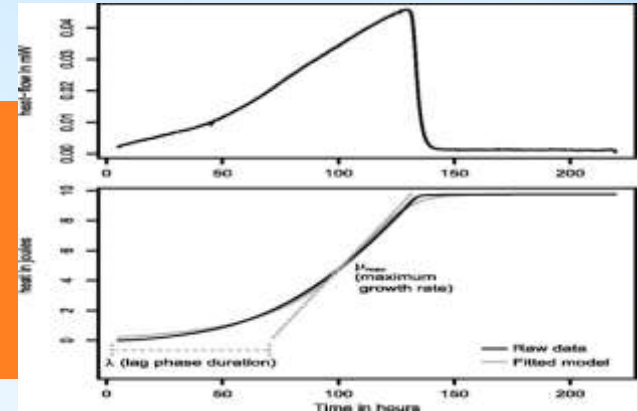


# Isothermal microcalorimetry (IMC)



***Chemical or physical processes either consume or produce heat***

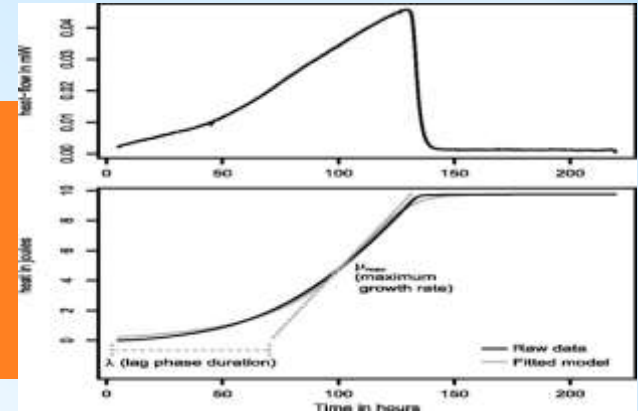
# Isothermal microcalorimetry (IMC)



***Chemical or physical processes  
either consume or produce heat***

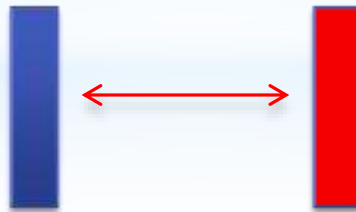


# Isothermal microcalorimetry (IMC)

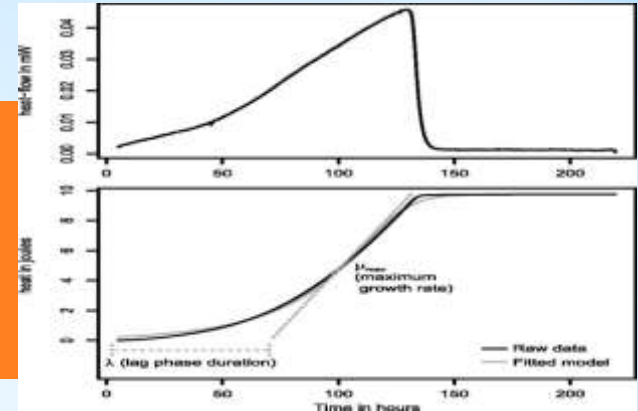


***Chemical or physical processes either consume or produce heat***

Rate of heat transferred (watts)

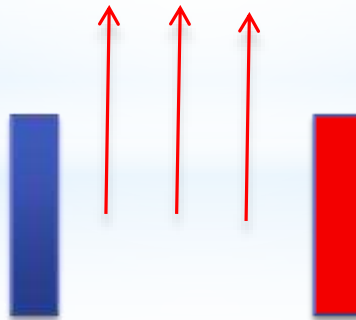


# Isothermal microcalorimetry (IMC)



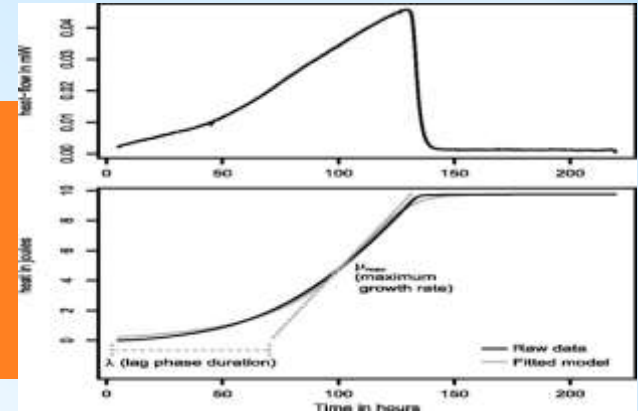
***Chemical or physical processes either consume or produce heat***

**Heat produced (joules)**





# Isothermal microcalorimetry (IMC)



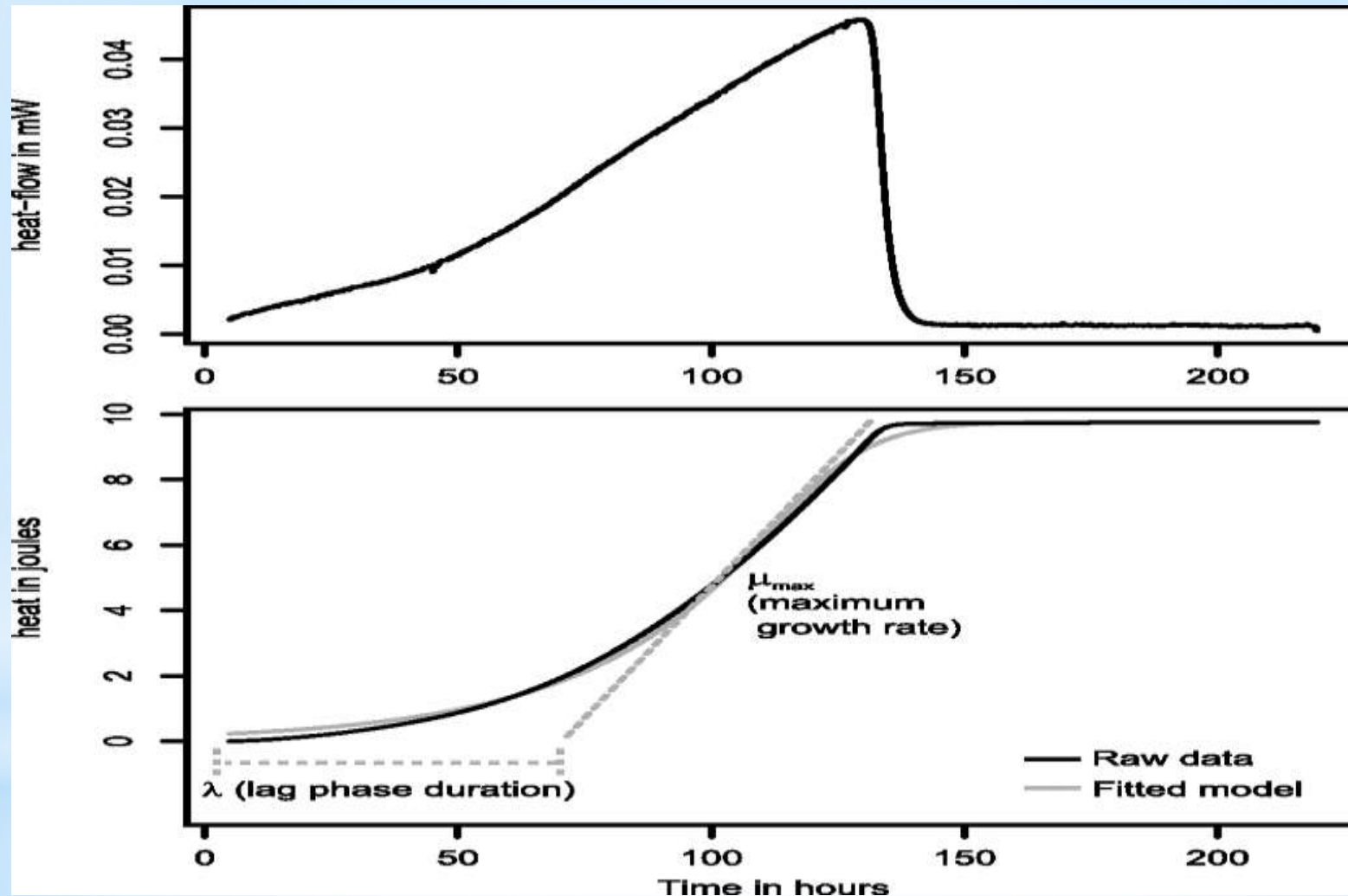
***Chemical or physical processes either consume or produce heat***

**Heat produced (joules)**



**Rate of heat transferred (watts)**

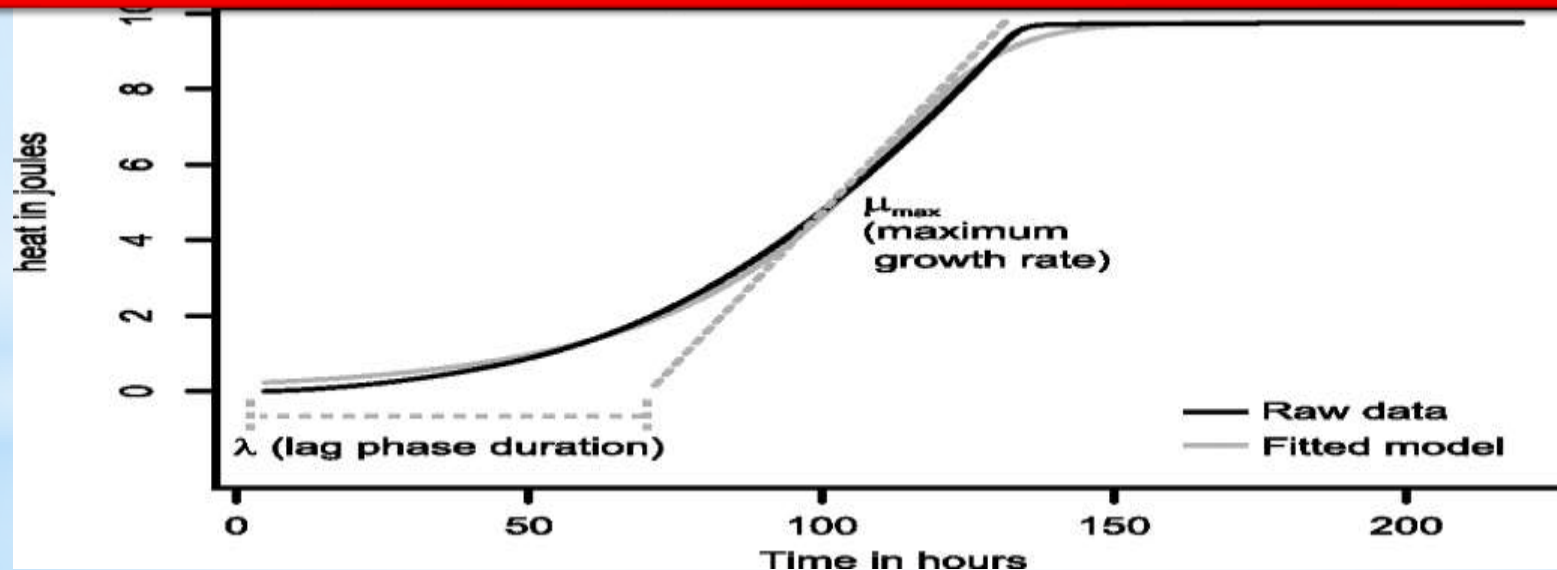
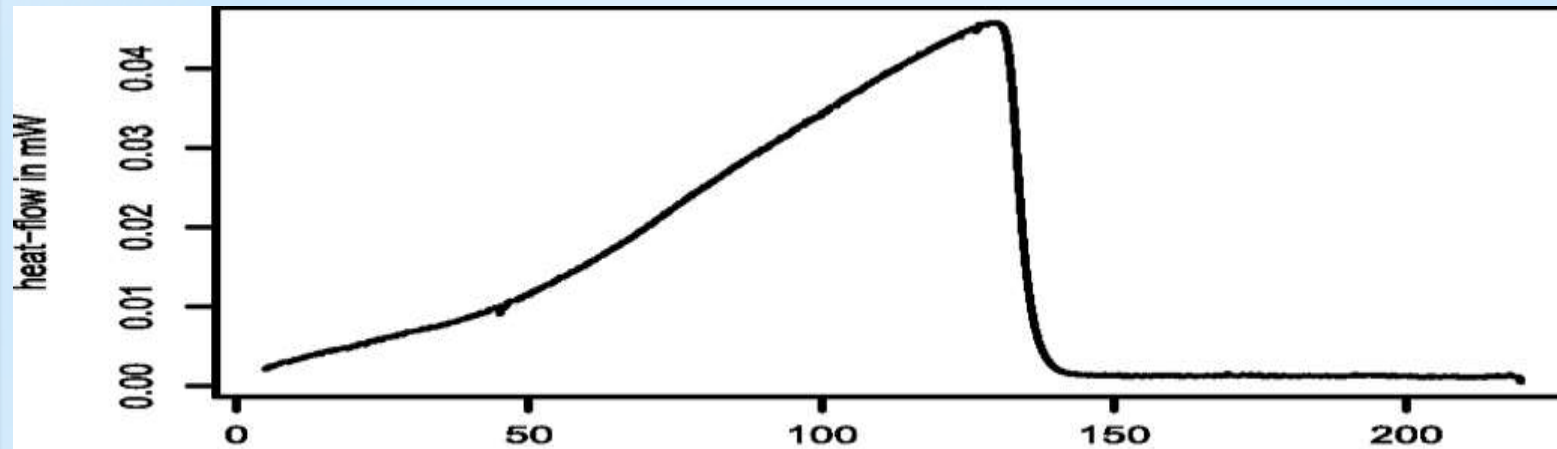
Growth parameters of *M. tuberculosis* analyzed using R (a control curve without antibiotic was used here as an example).



Howell M et al. J. Clin. Microbiol. 2012;50:16-20

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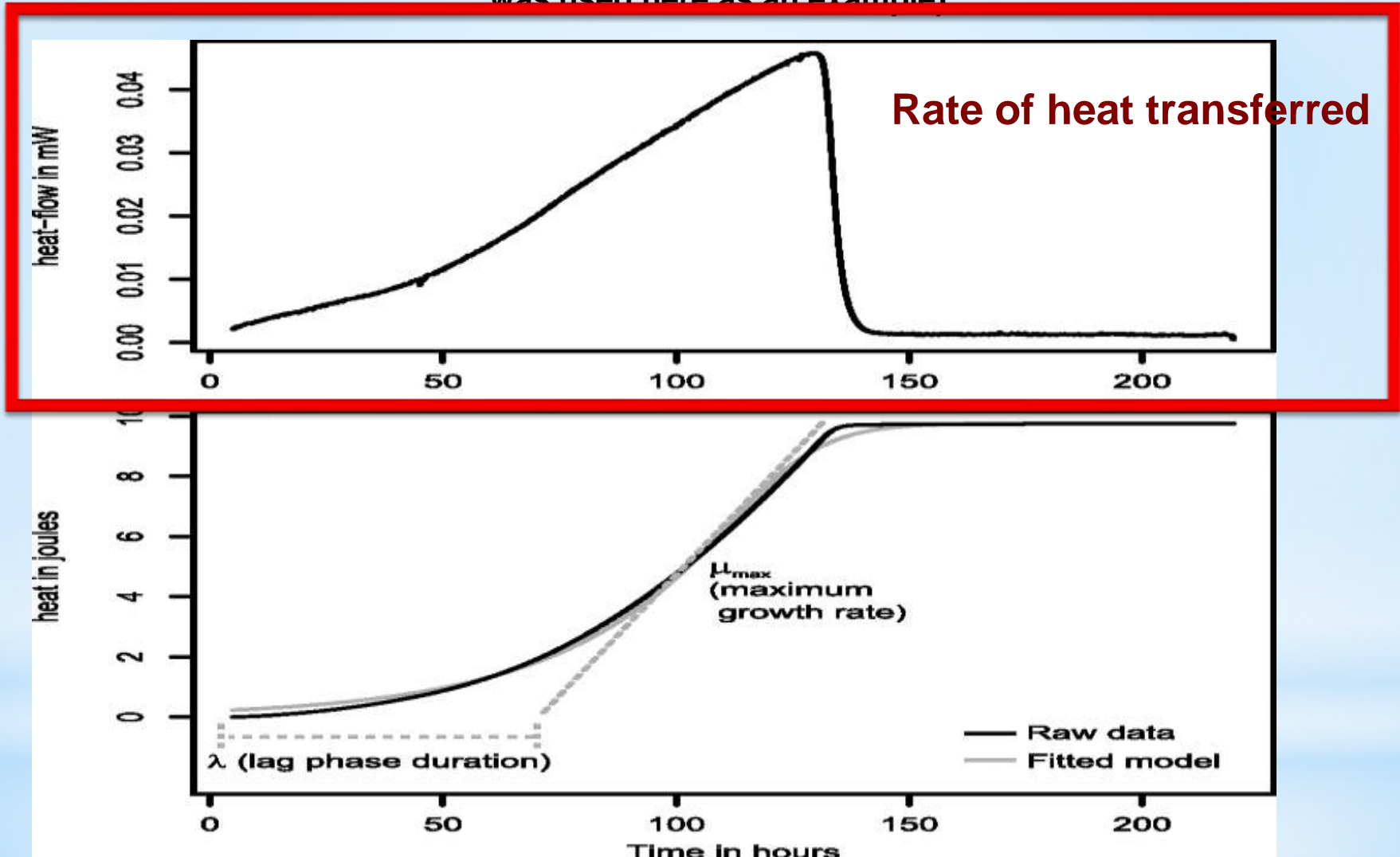
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Howell M et al. J. Clin. Microbiol. 2012;50:16-20

Journal of Clinical Microbiology

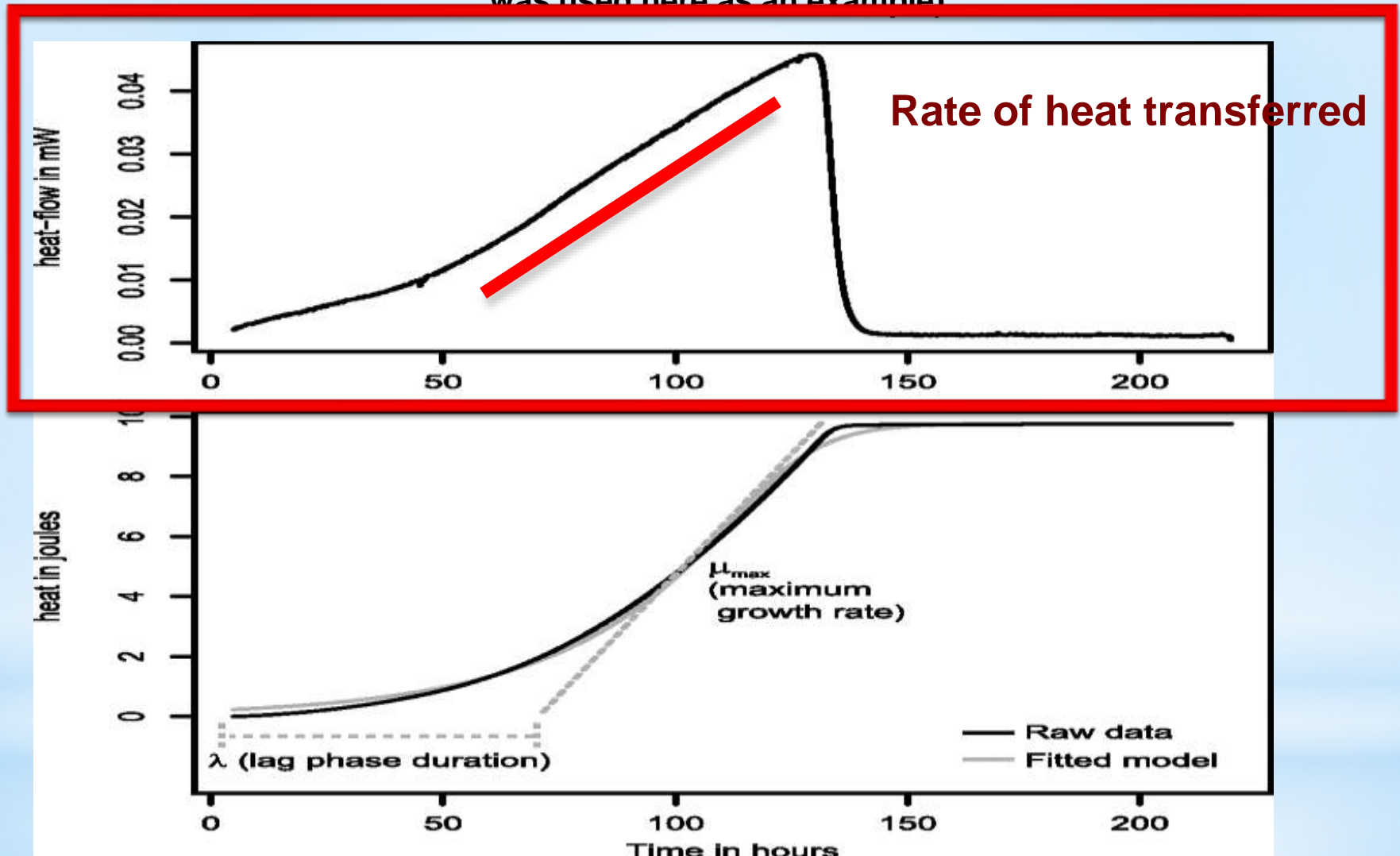
Growth parameters of *M. tuberculosis* analyzed using R (a control curve without antibiotic was used here as an example)



Howell M et al. J. Clin. Microbiol. 2012;50:16-20

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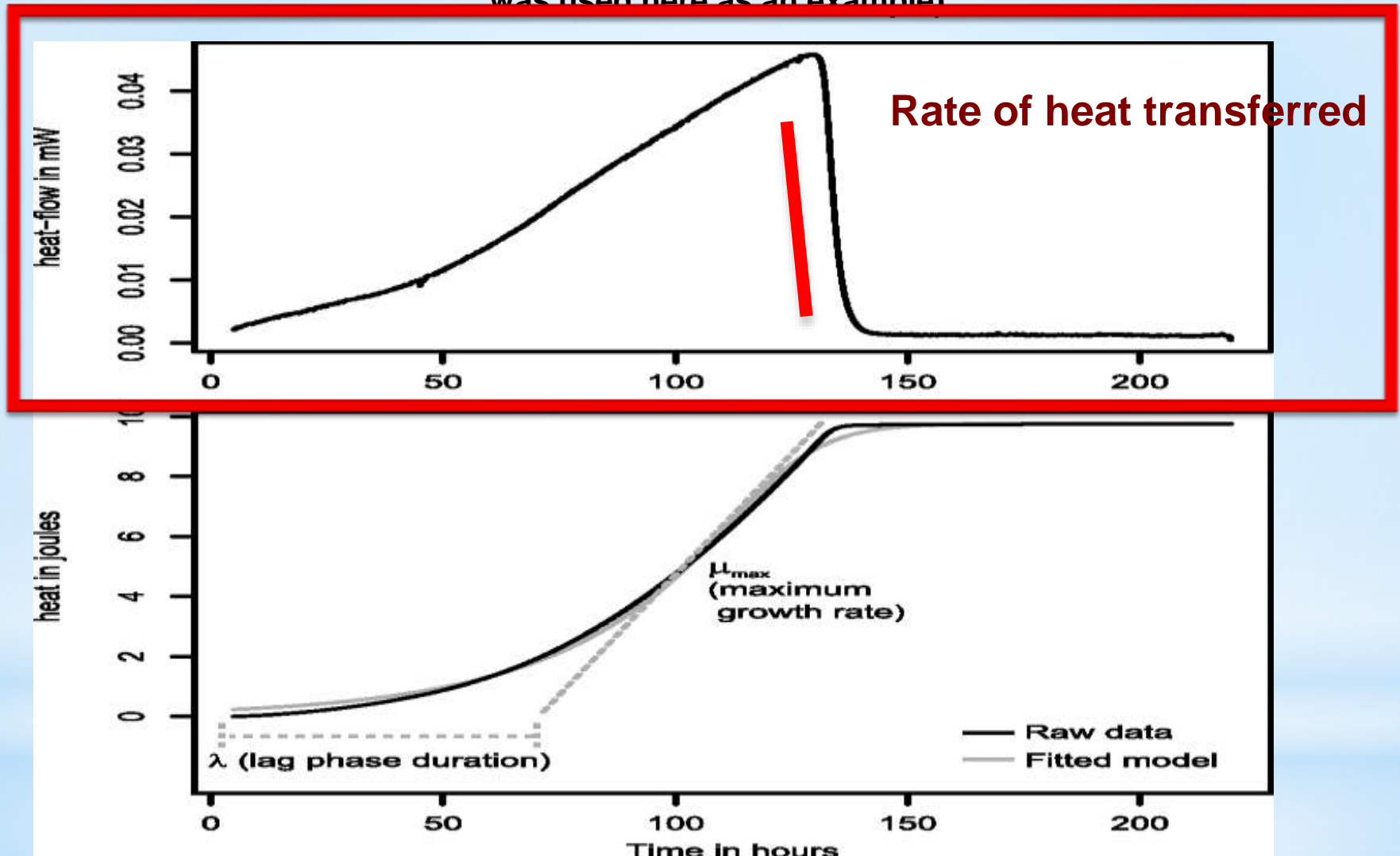
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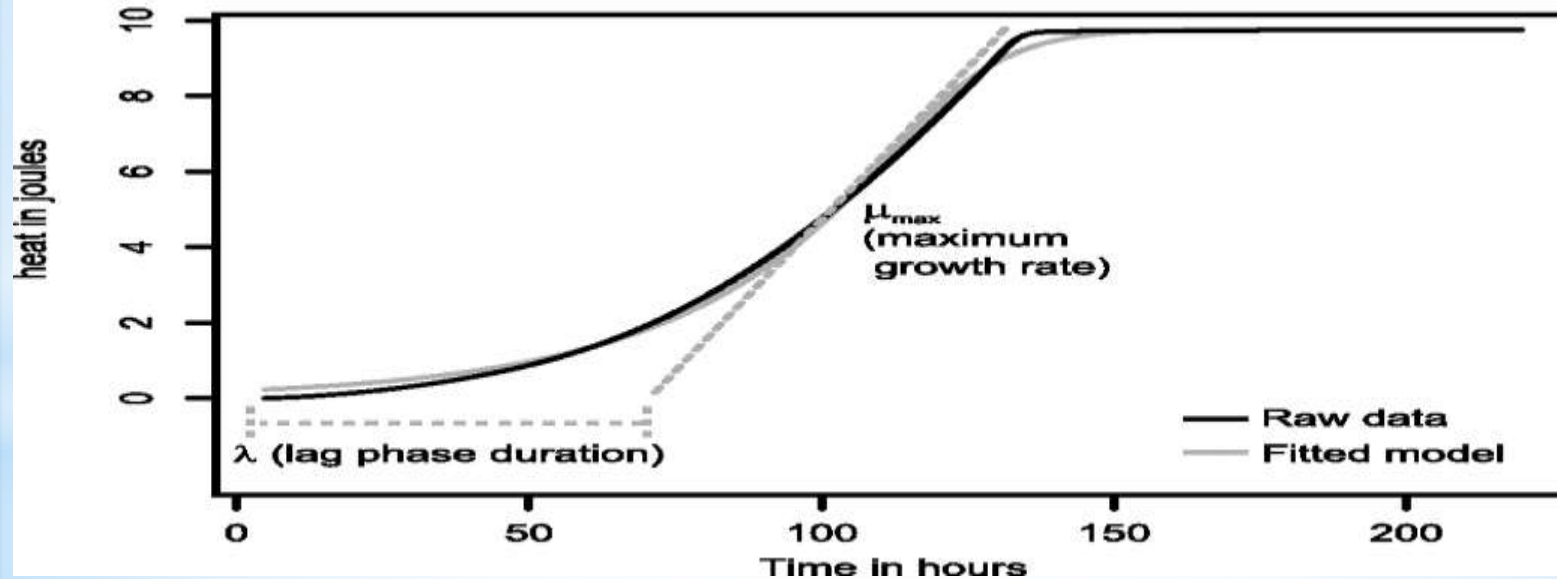
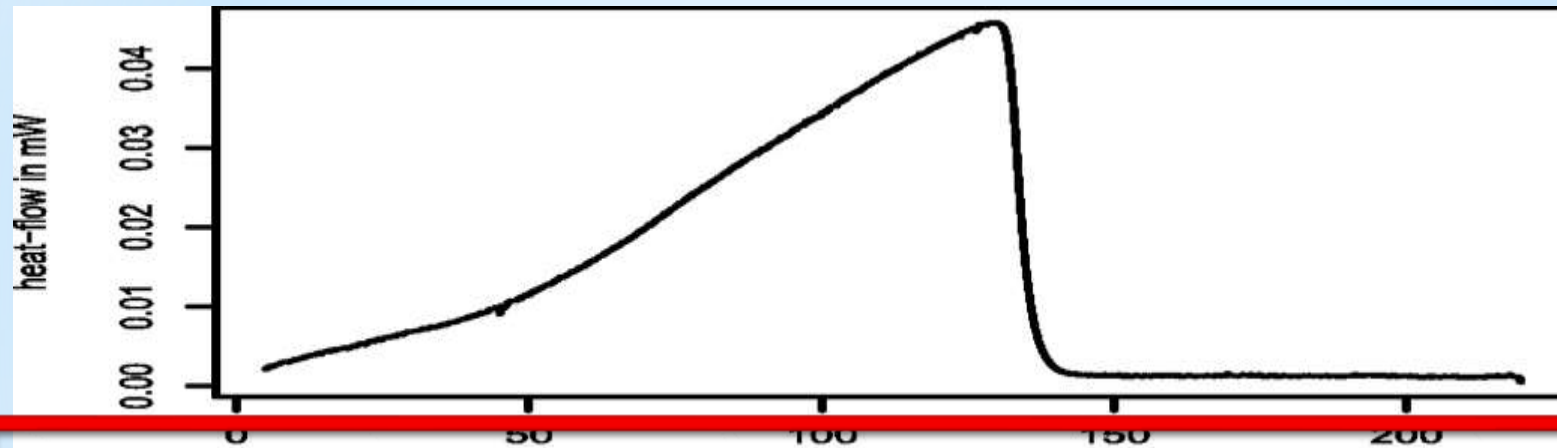
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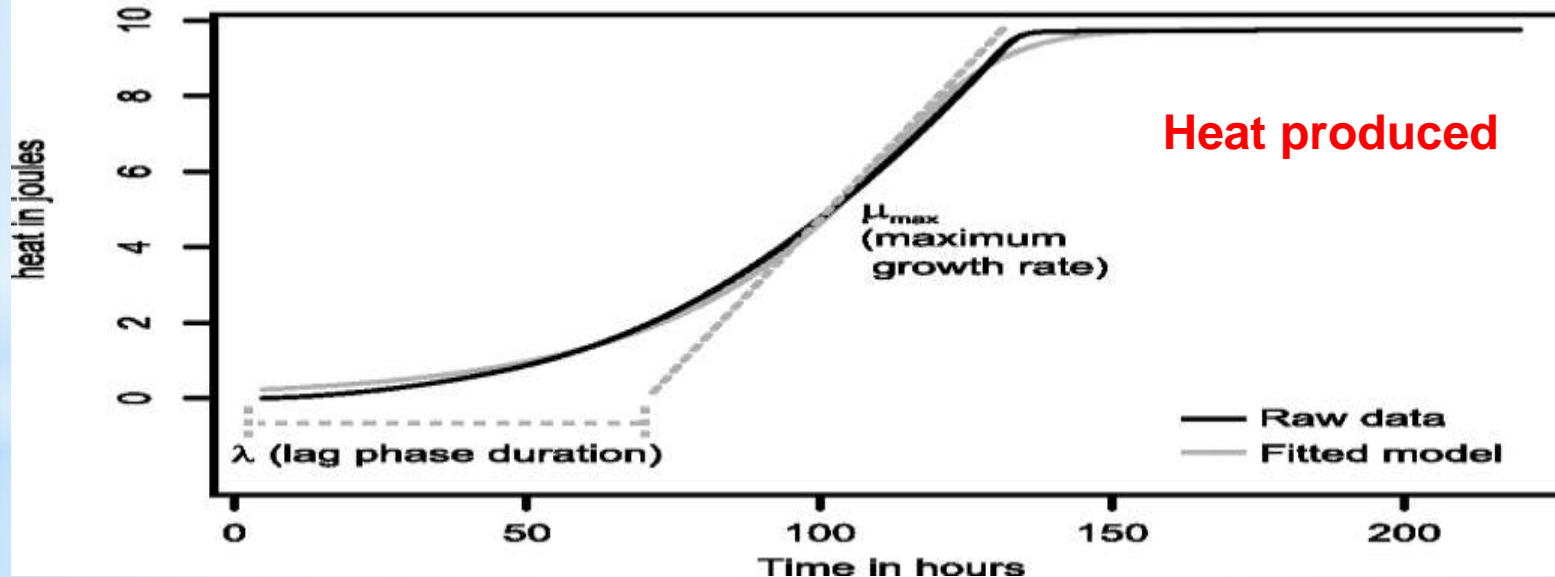
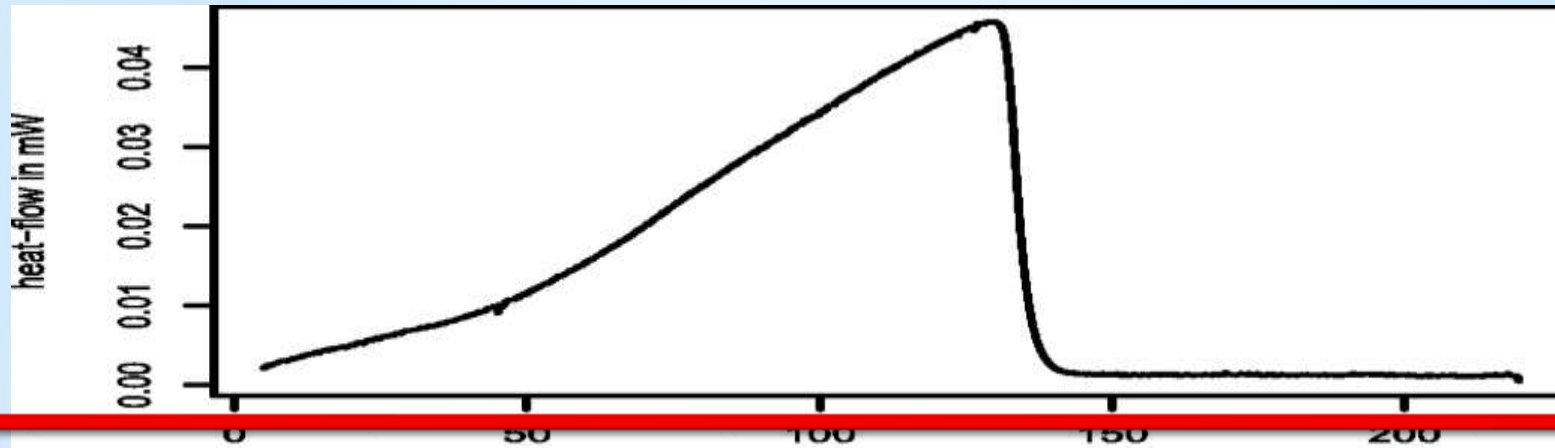
Journal of Clinical Microbiology

Growth parameters of *M. tuberculosis* analyzed using R (a control curve without antibiotic was used here as an example).



Howell et al. *J. Clin. Microbiol.* 2012;50:19-26

Growth parameters of *M. tuberculosis* analyzed using R (a control curve without antibiotic was used here as an example).

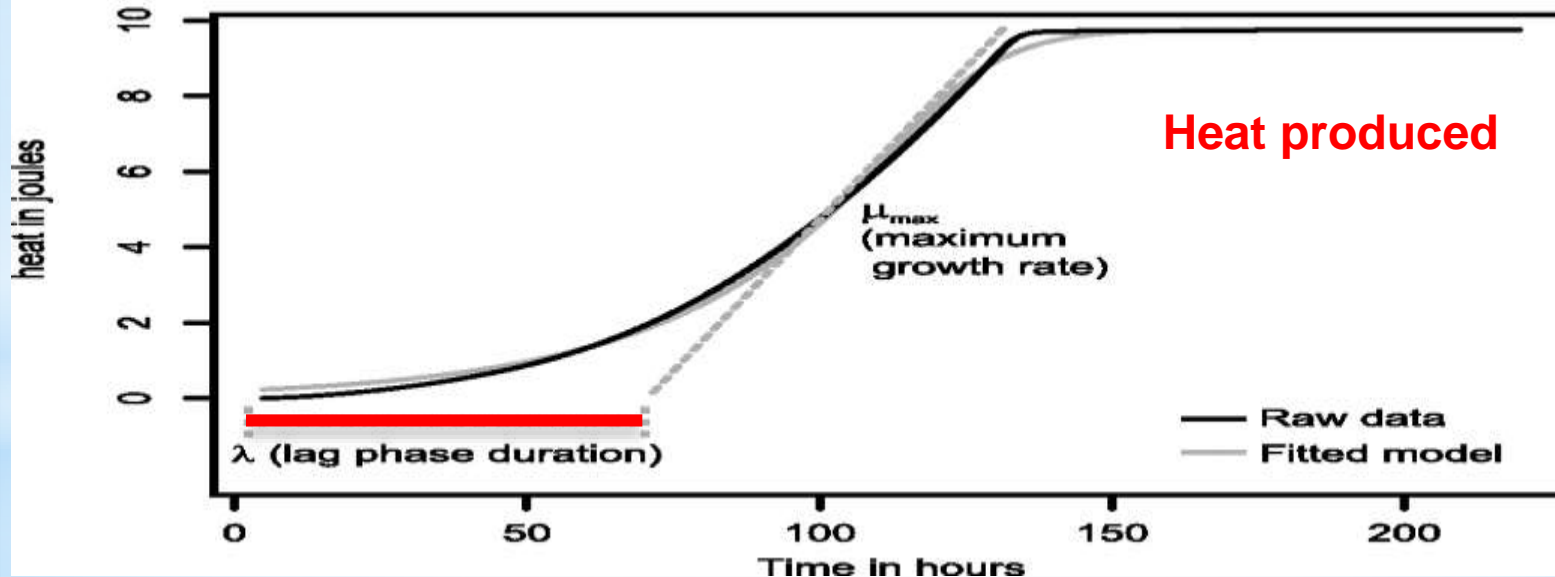
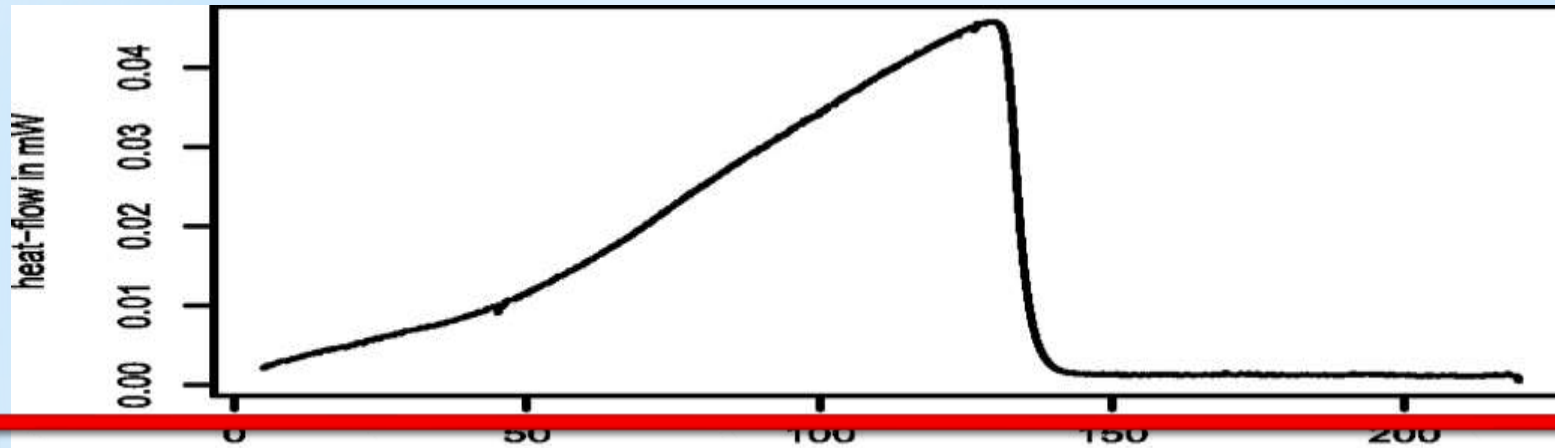


Howell et al. *J. Clin. Microbiol.* 2012;50:19-20

Journal of Clinical Microbiology

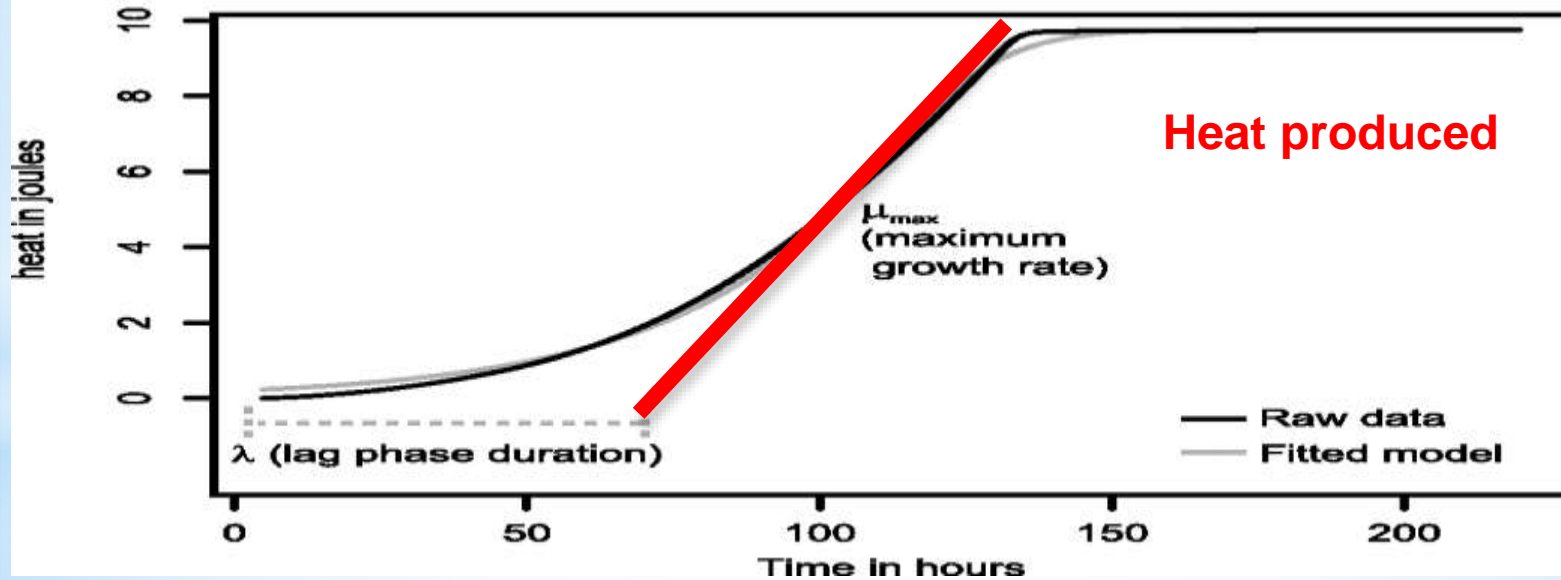
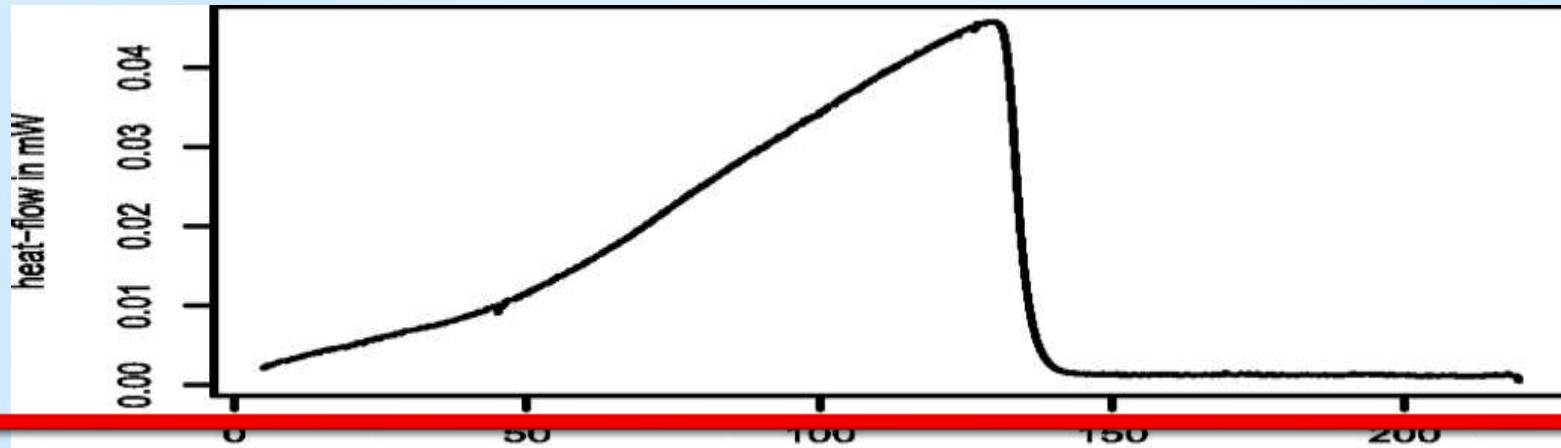


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Howell et al. *et al.* *J. Clin. Microbiol.* 2012;50:19-20

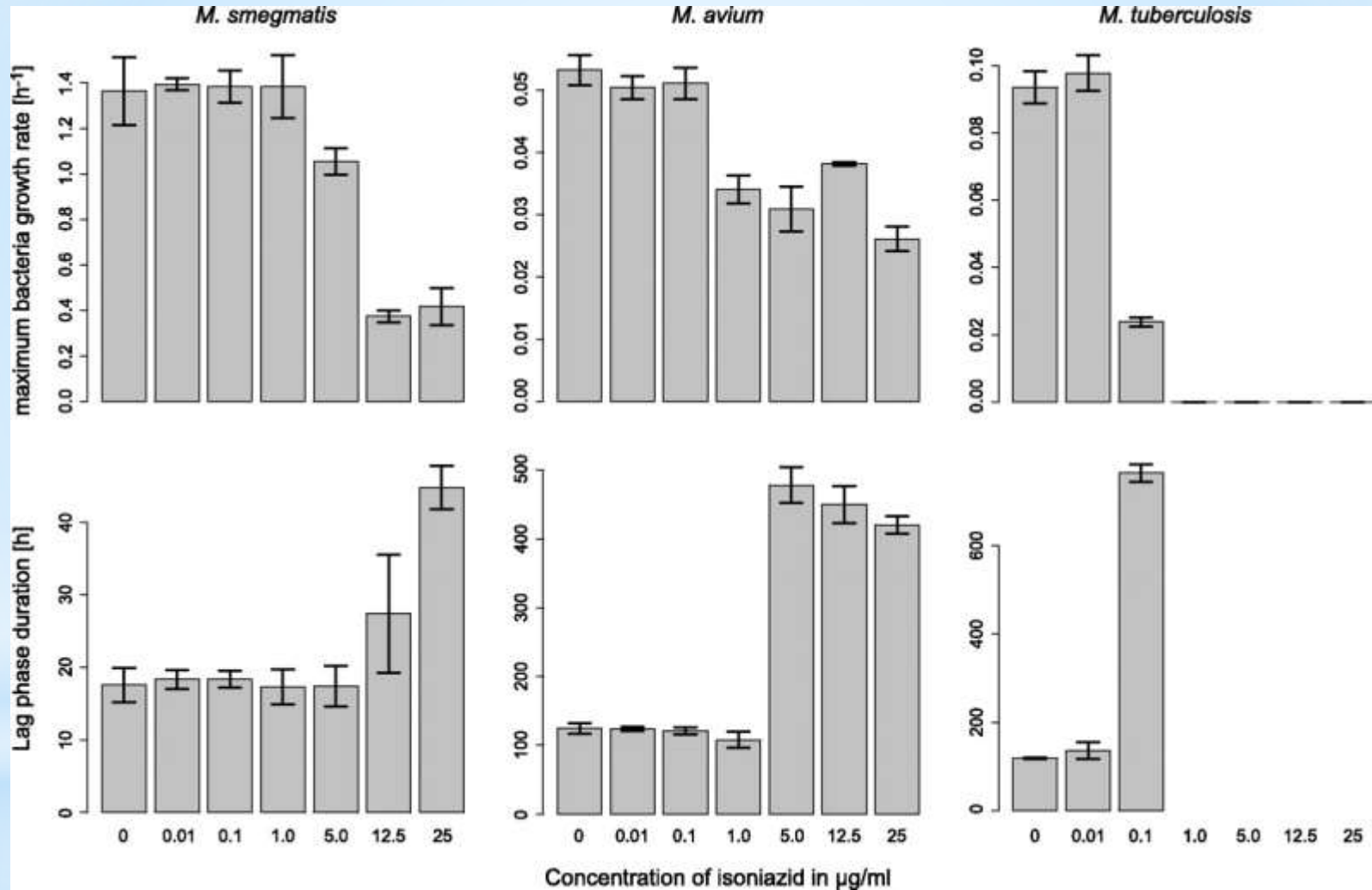
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Journal of Clinical Microbiology

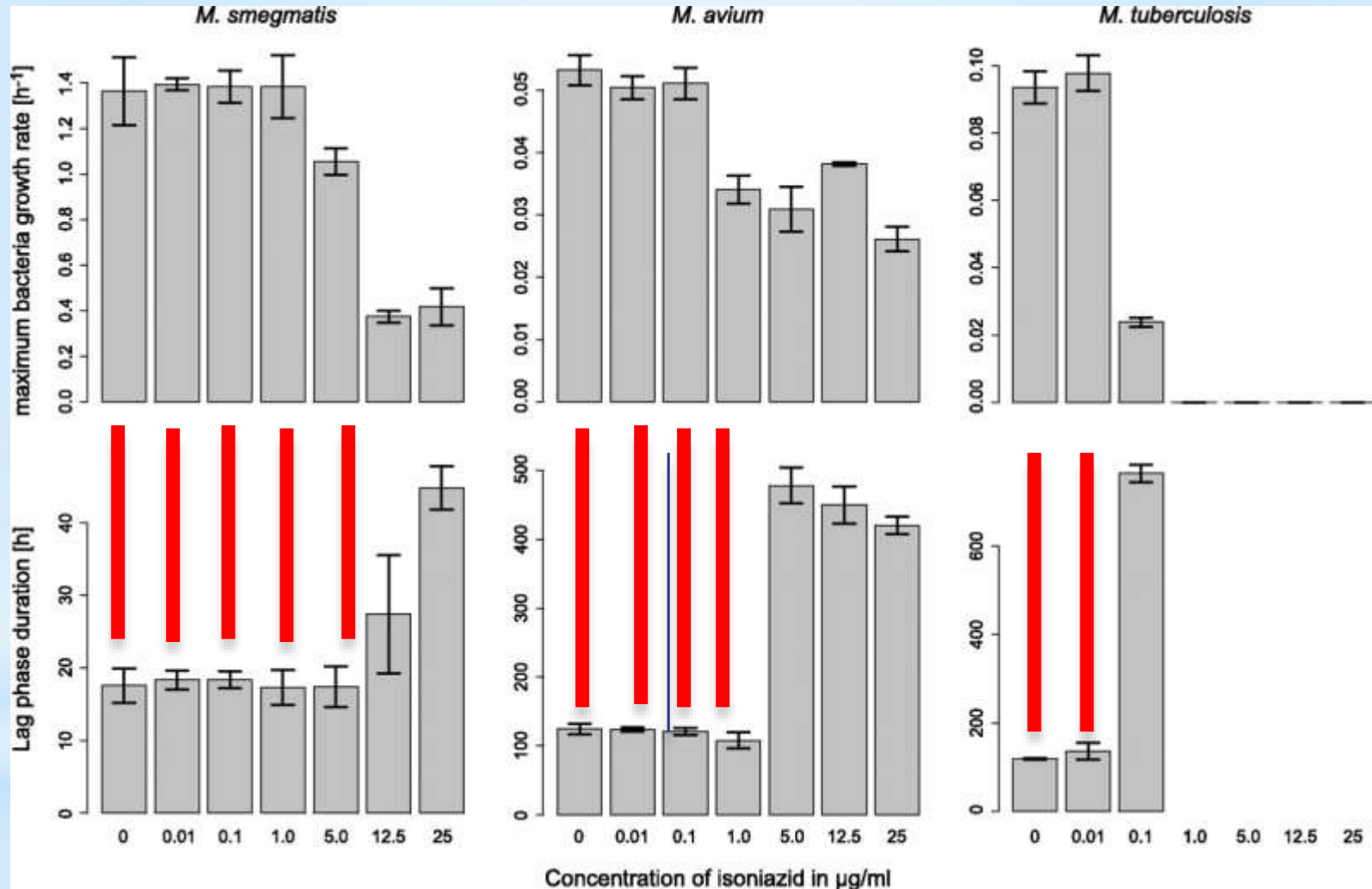
Mean and standard deviation (n = 3) of the growth rate and lag phase duration with increasing concentrations of isoniazid for *M. smegmatis*, *M. avium*, and *M. tuberculosis*.



Howell M et al. J. Clin. Microbiol. 2012;50:16-20

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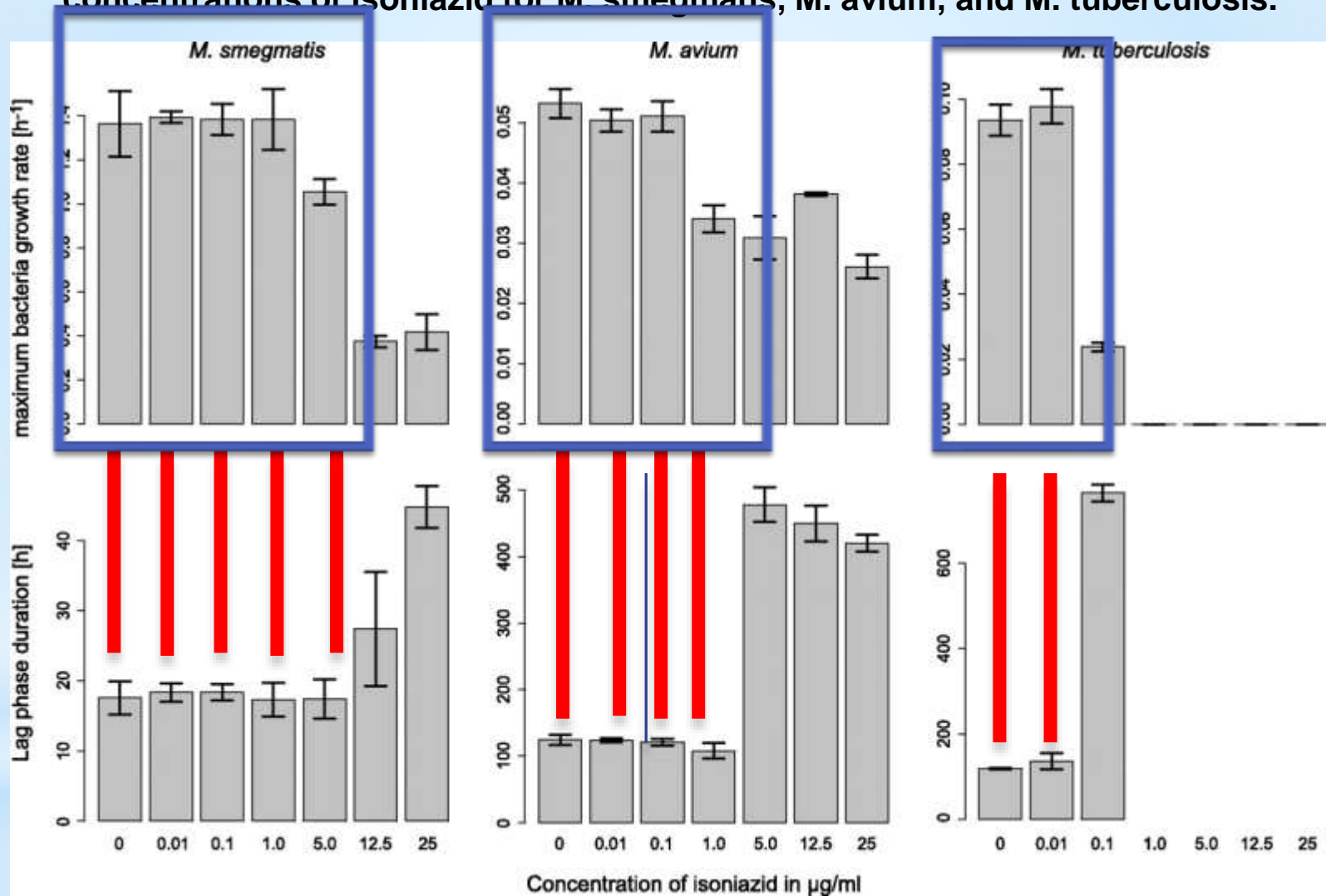
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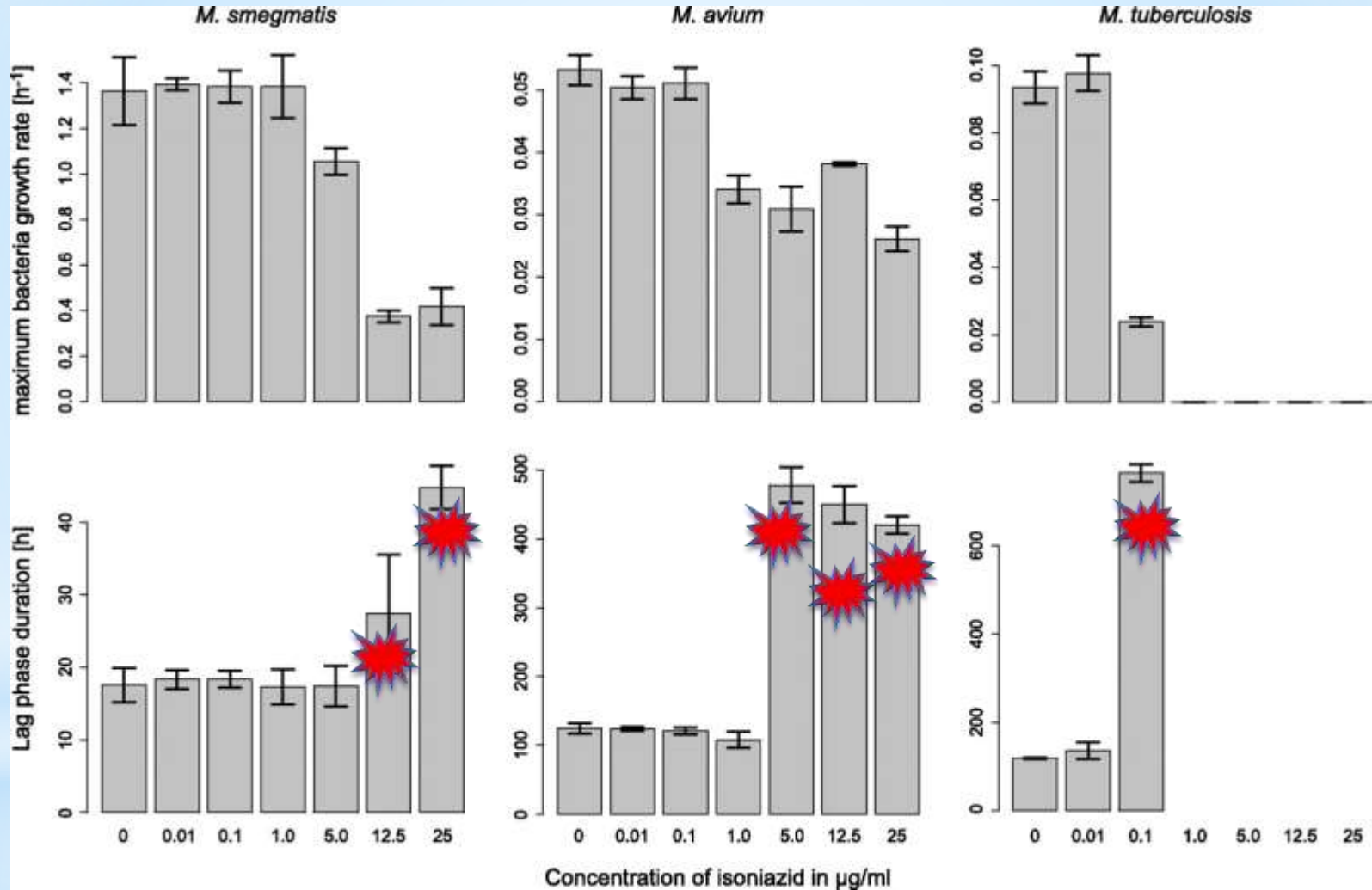
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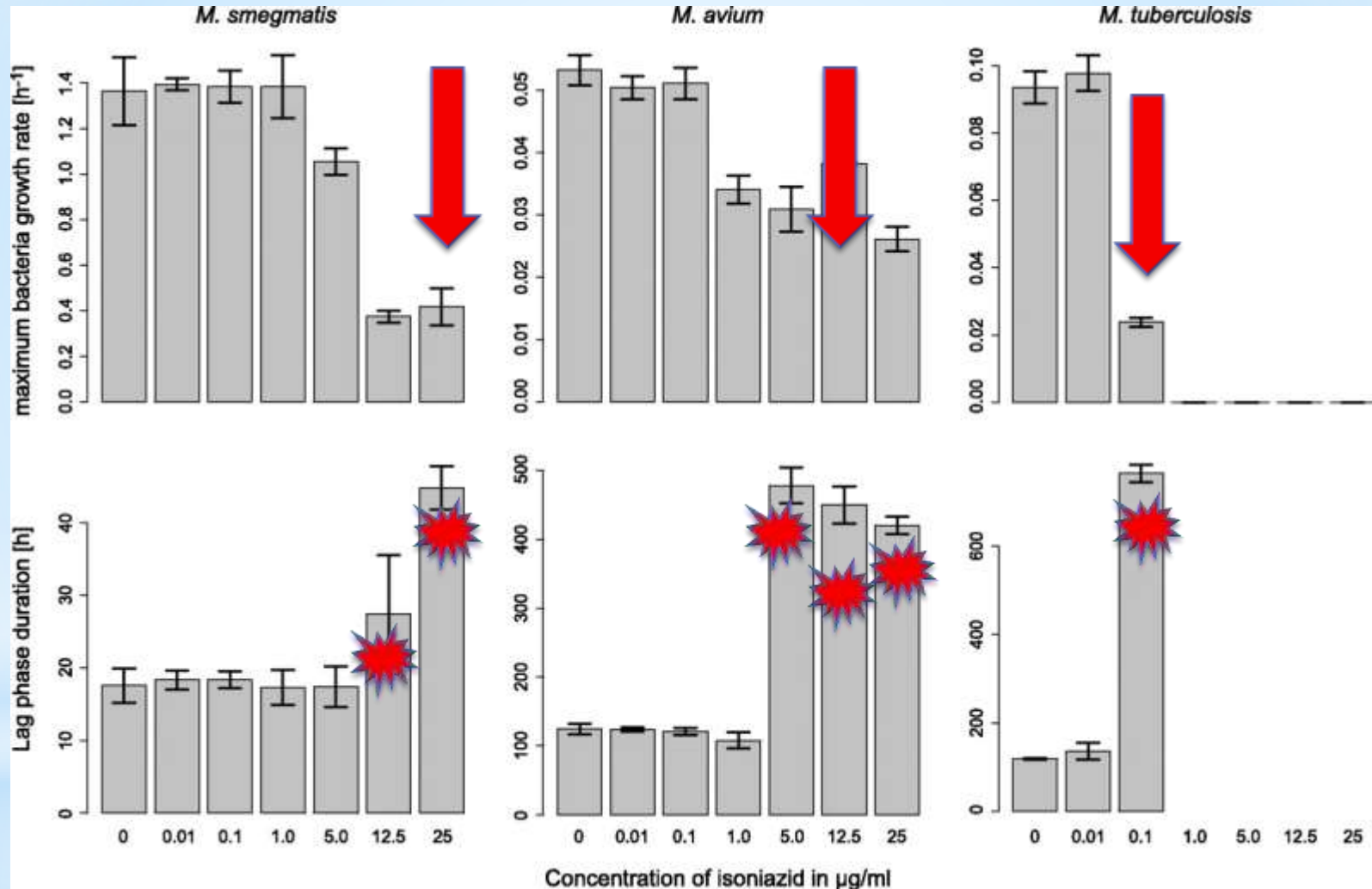
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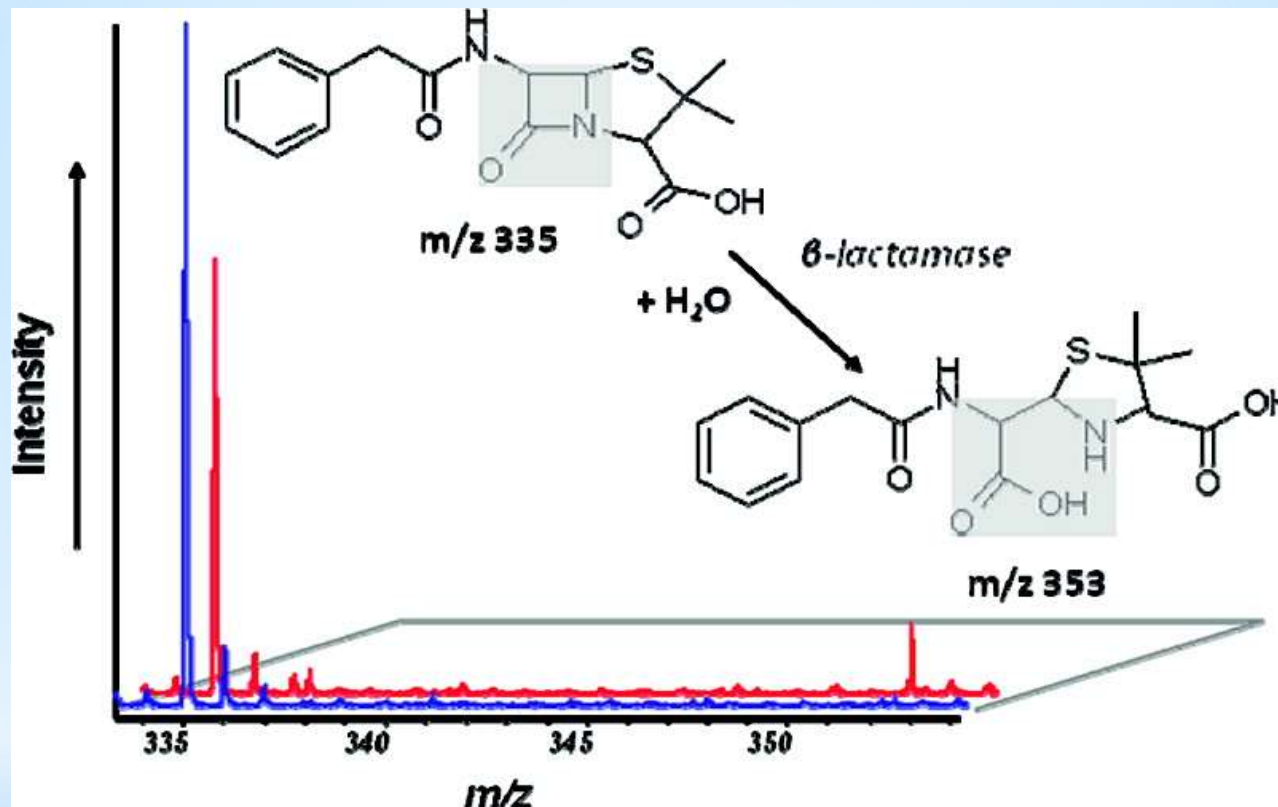
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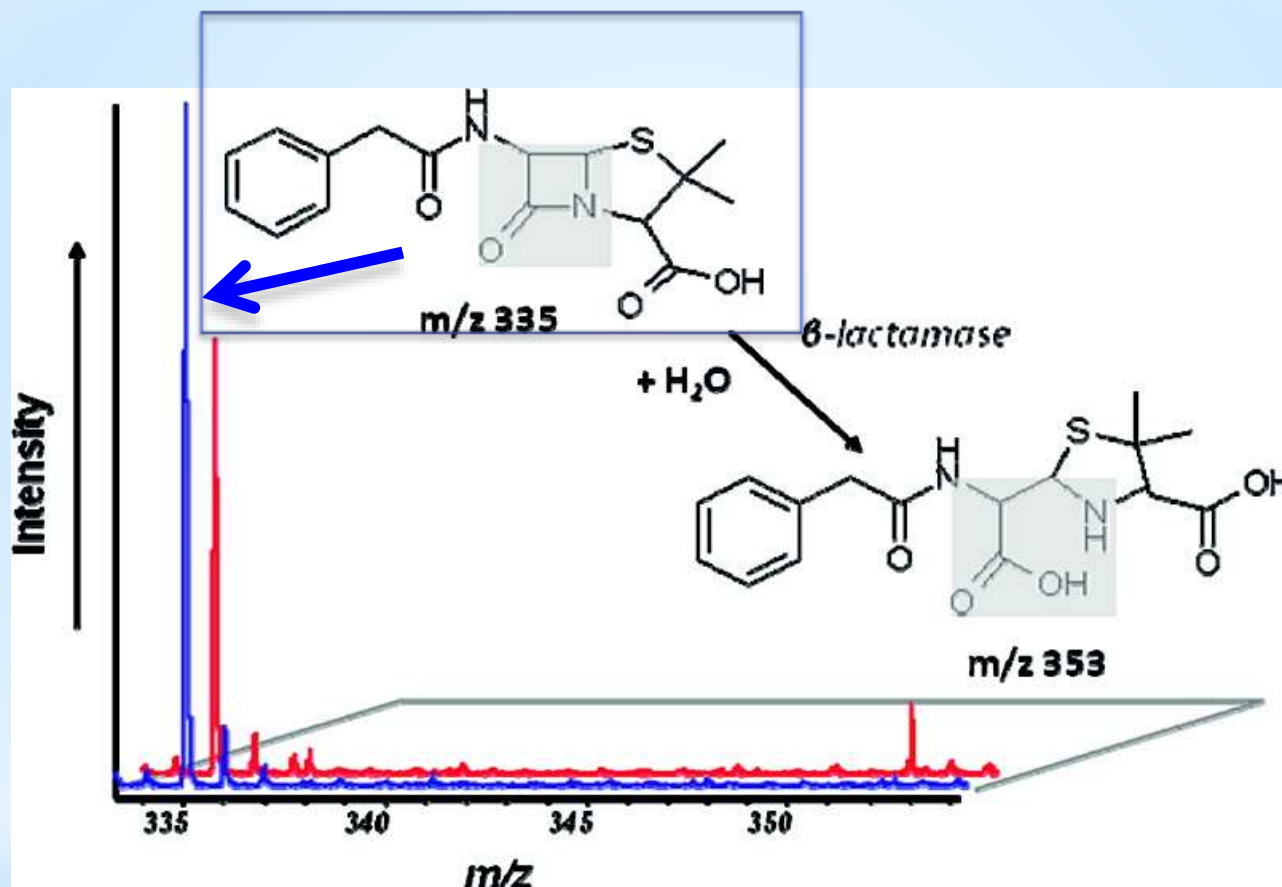
Howell M et al. J. Clin. Microbiol. 2012;50:16-20

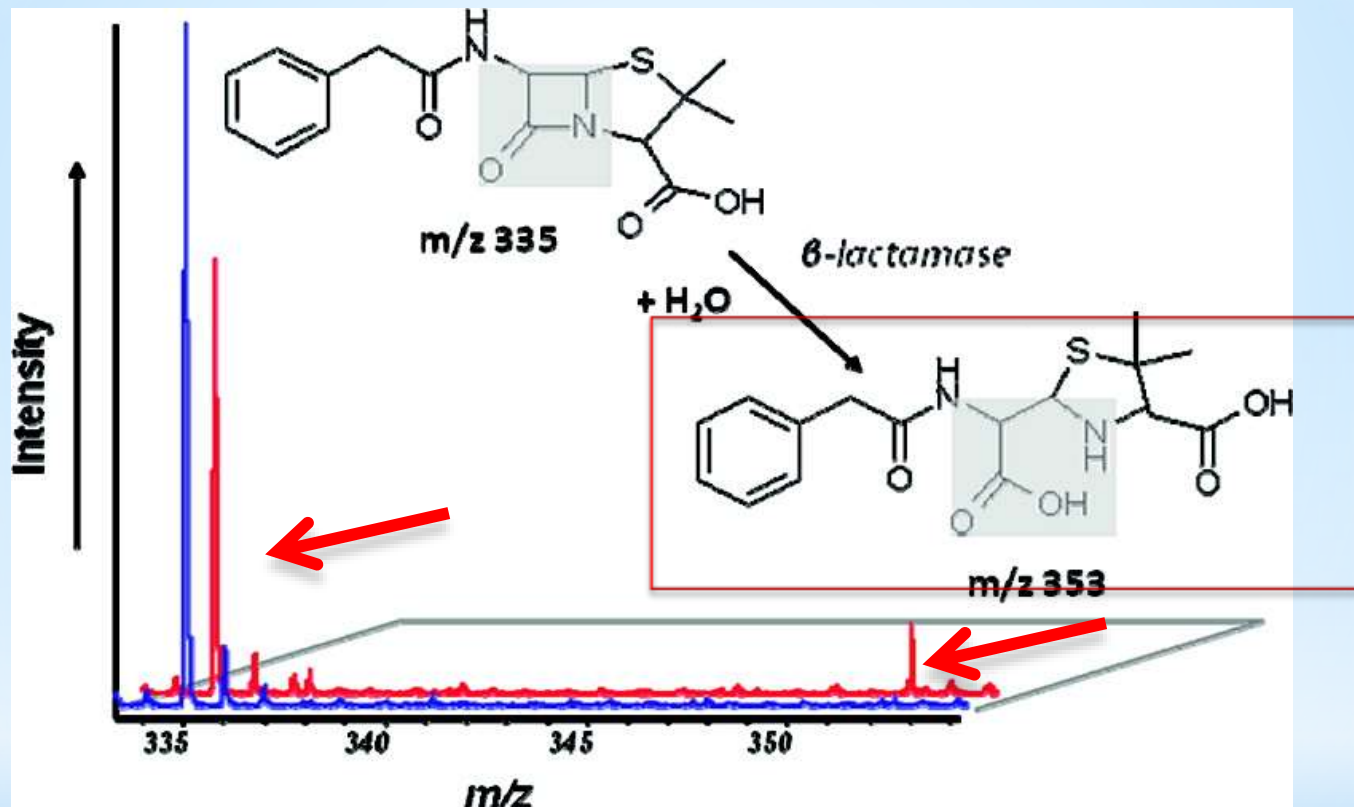
Journal of Clinical Microbiology

# MALDI-TOFF





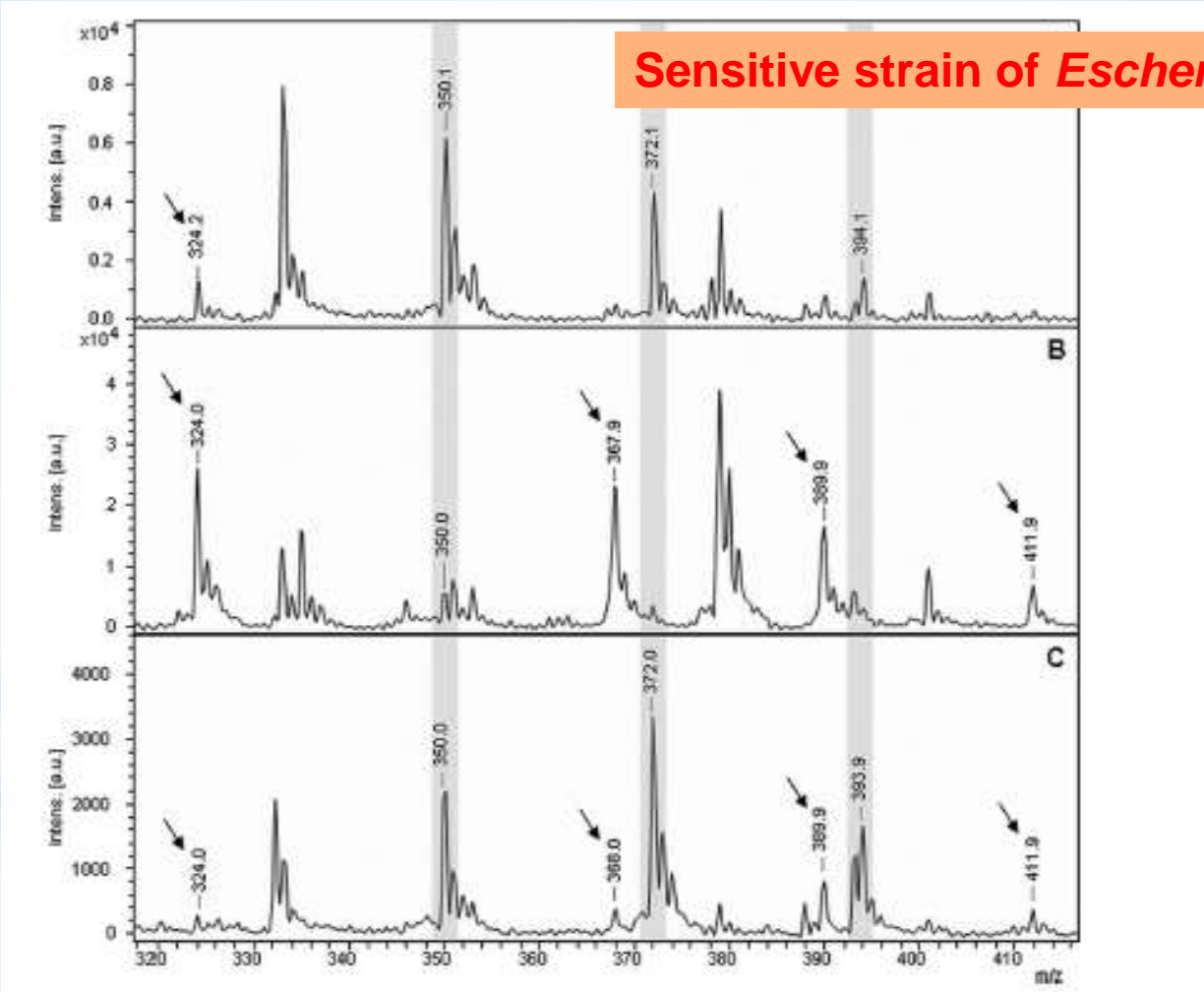




# MALDI-TOF - Ampicillin

## AMPICILLIN

**Sensitive strain of *Escherichia coli***



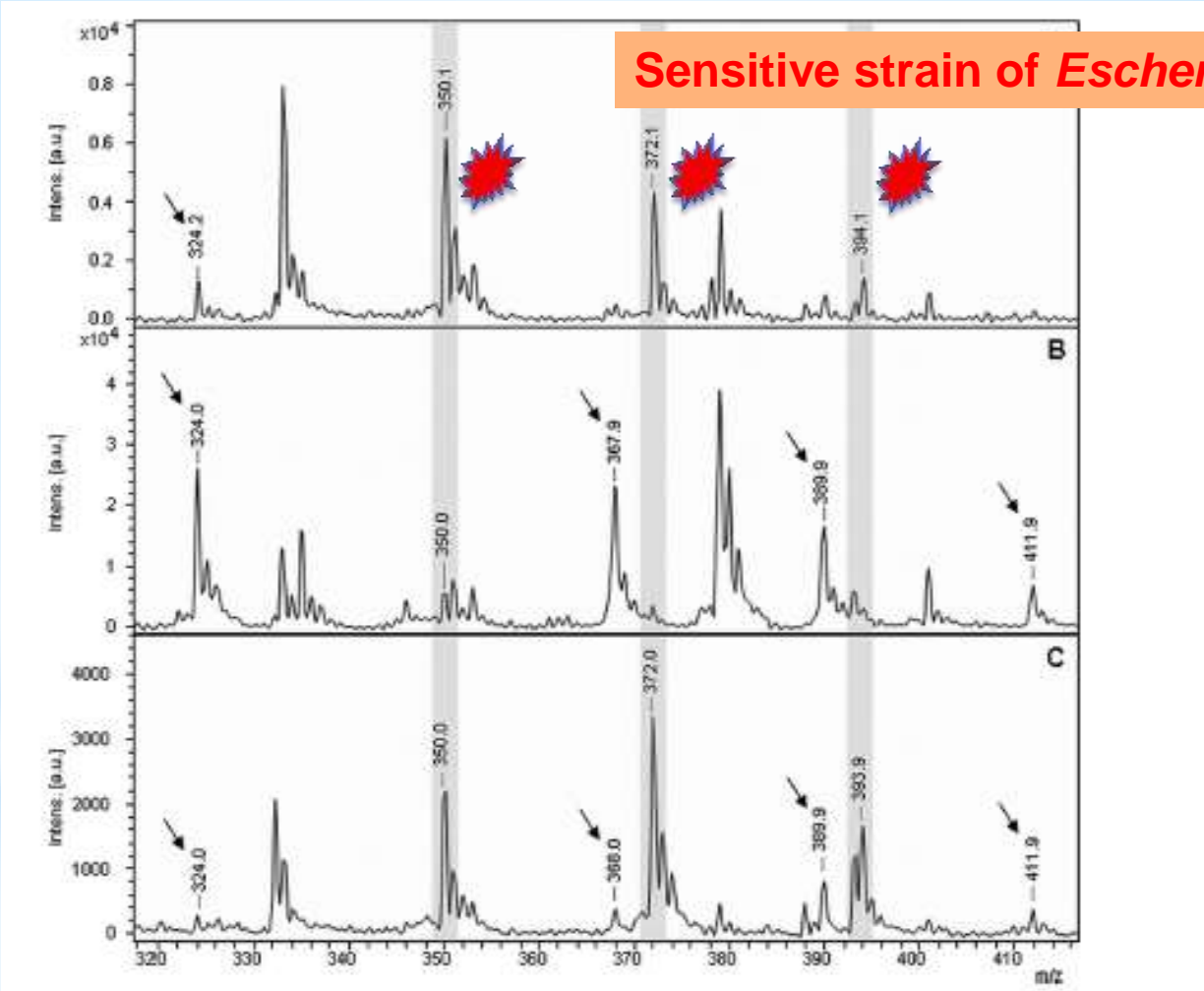
-----INTENSITY [a. u.]-----

-----MASS/CHARGE (m/z)-----

# MALDI-TOF - Ampicillin

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Sensitive strain of *Escherichia coli*

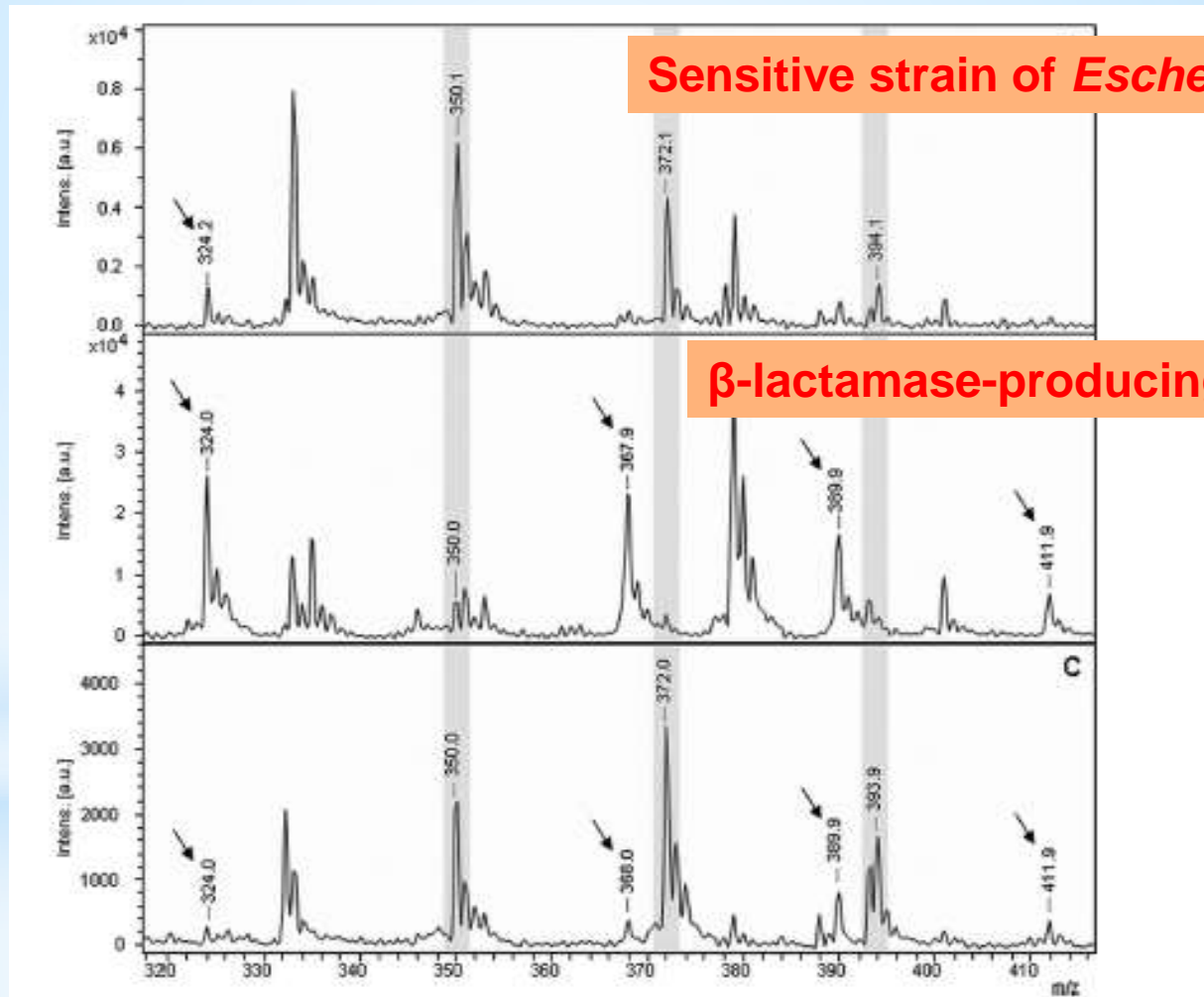


-----INTENSITY [a. u.]-----

-----MASS/CHARGE (m/z)-----

# MALDI-TOF - Ampicillin

## AMPICILLIN



**Sensitive strain of *Escherichia coli***

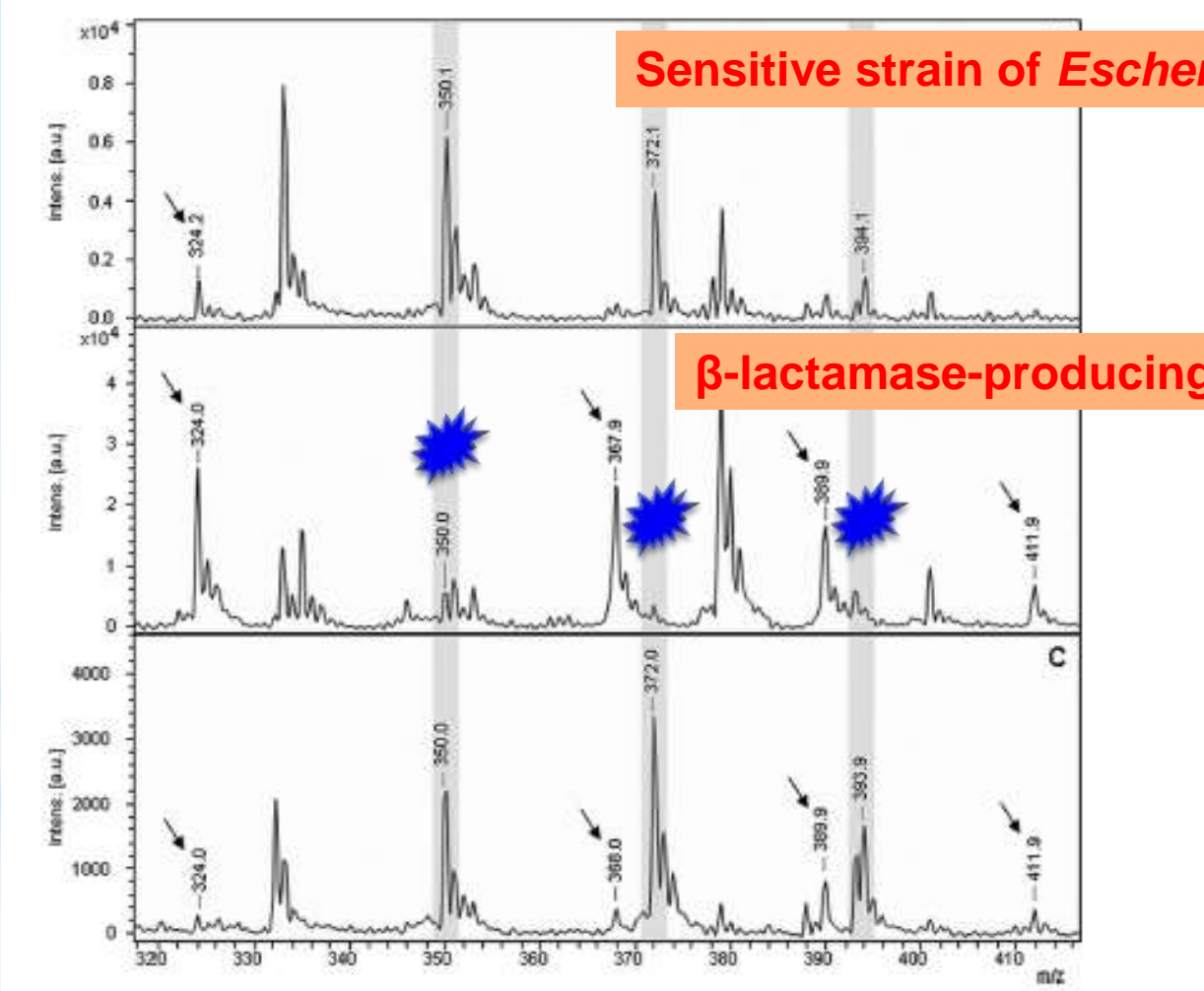
**$\beta$ -lactamase-producing strain**

-----INTENSITY [a. u.]-----

-----MASS/CHARGE (m/z)-----

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Sensitive strain of *Escherichia coli*

$\beta$ -lactamase-producing strain

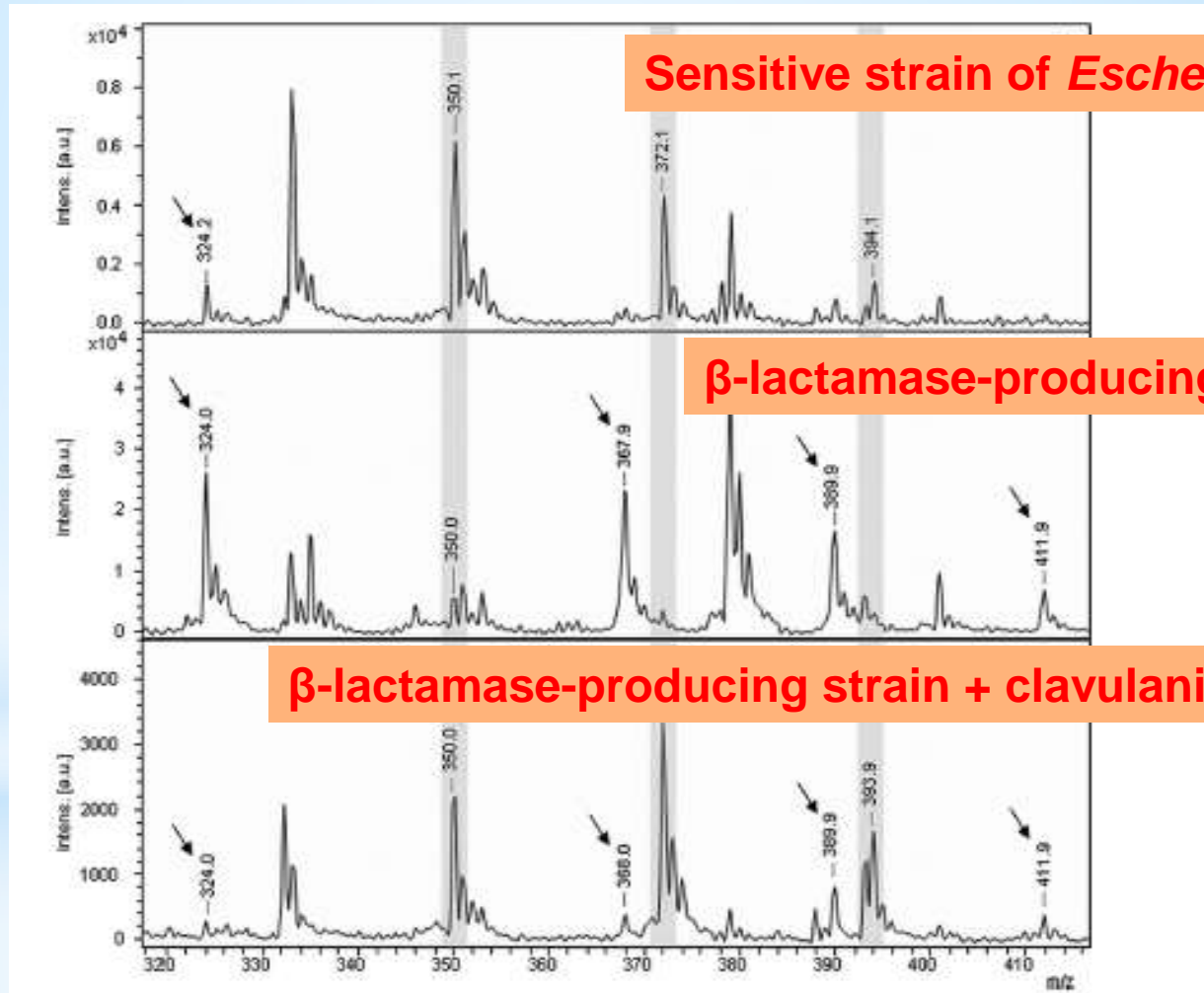
-----INTENSITY [a. u.]-----

-----MASS/CHARGE (m/z)-----

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-----INTENSITY [a. u.]-----



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**$\beta$ -lactamase-producing strain**

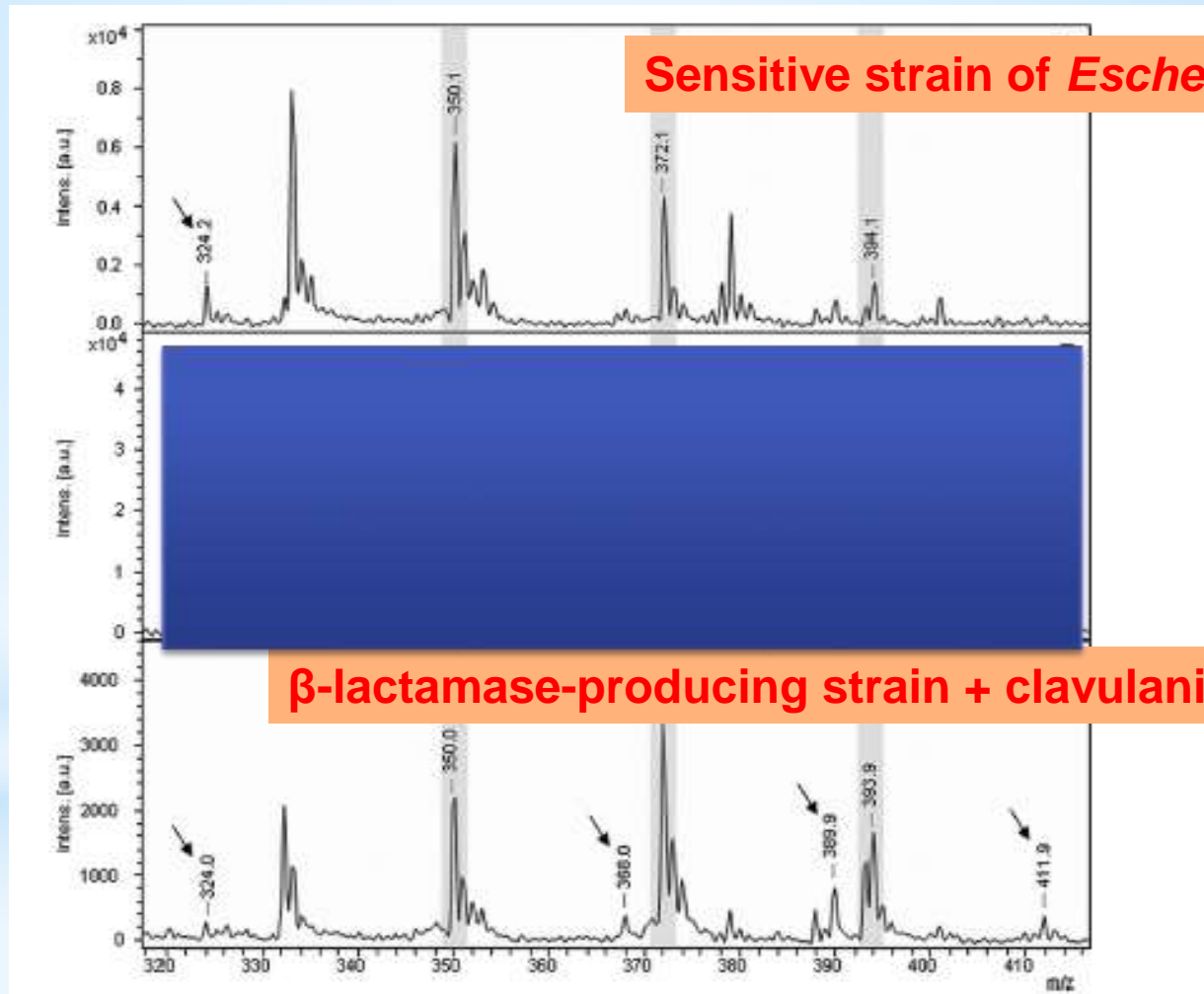
**$\beta$ -lactamase-producing strain + clavulanic acid**

-----MASS/CHARGE (m/z)-----

# MALDI-TOF - Ampicillin

## AMPICILLIN

-----INTENSITY [a. u.]-----



**Sensitive strain of *Escherichia coli***

**$\beta$ -lactamase-producing strain + clavulanic acid**

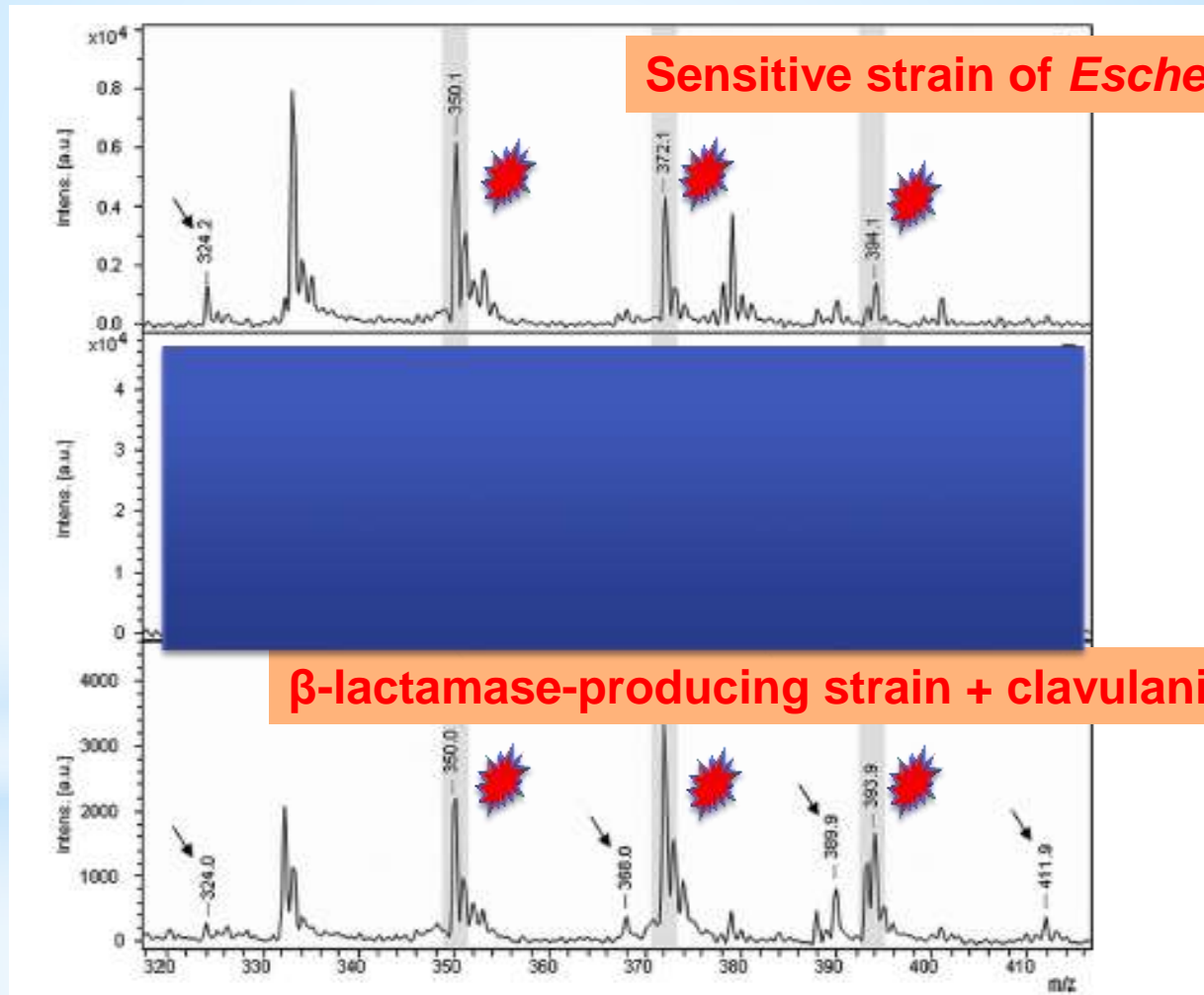
-----MASS/CHARGE (m/z)-----



# MALDI-TOF - Ampicillin

## AMPICILLIN

-----INTENSITY [a. u.]-----



**Sensitive strain of *Escherichia coli***

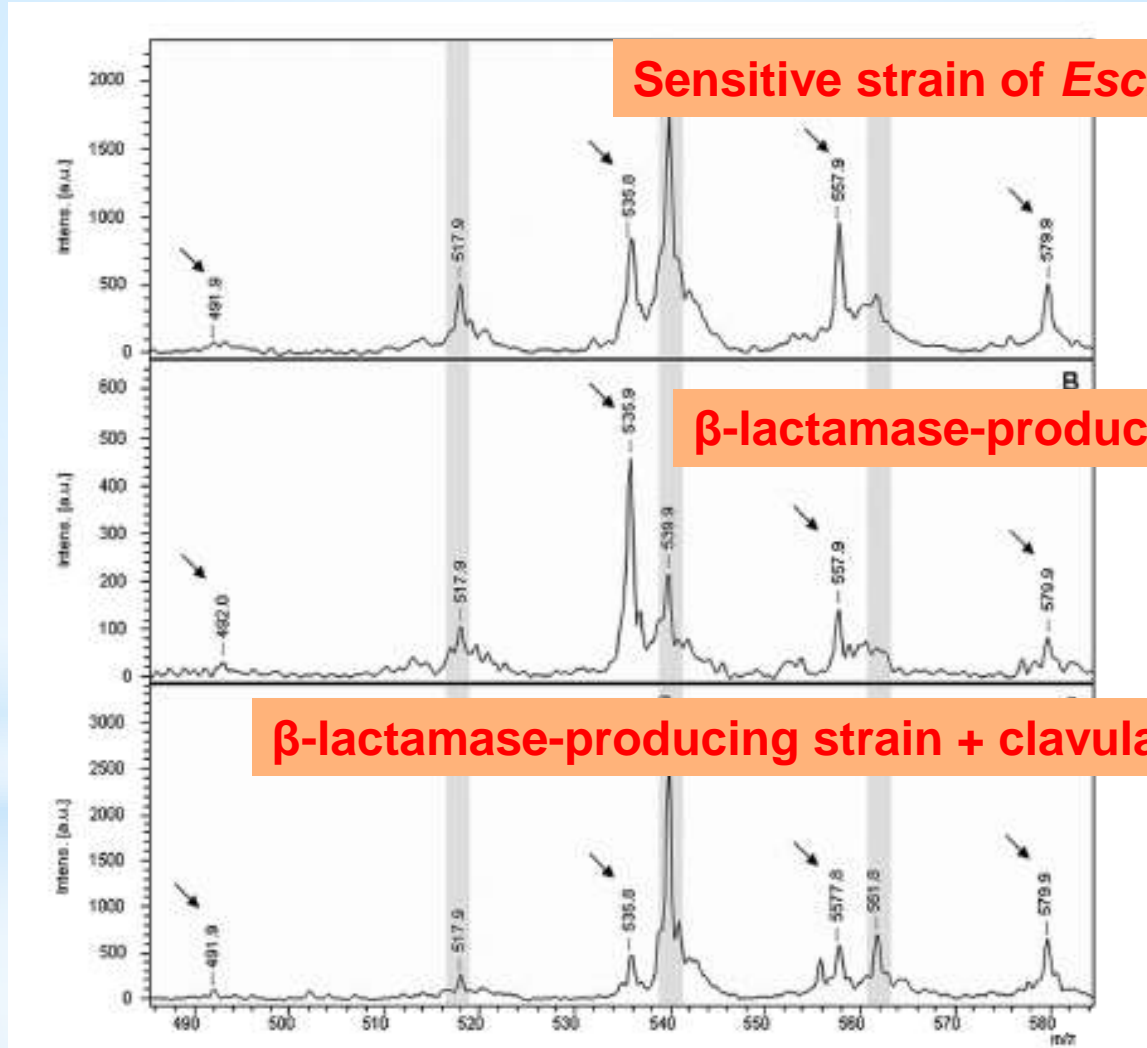
**$\beta$ -lactamase-producing strain + clavulanic acid**

-----MASS/CHARGE (m/z)-----

# MALDI-TOF

## PIPERACILLIN

-----INTENSITY [a. u.]-----



**Sensitive strain of *Escherichia coli***

**$\beta$ -lactamase-producing strain**

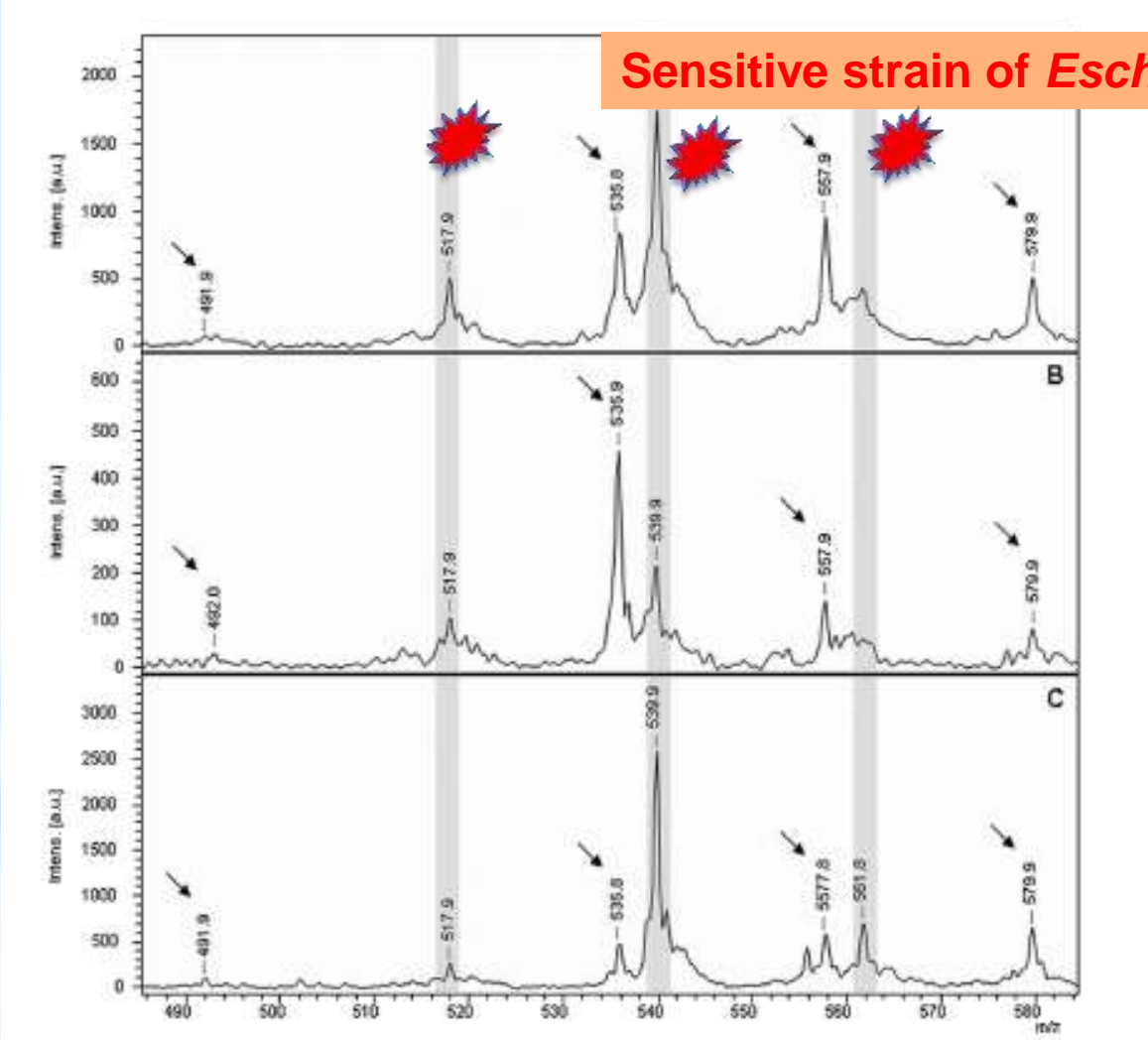
**$\beta$ -lactamase-producing strain + clavulanic acid**

-----MASS/CHARGE (m/z)-----

# MALDI-TOF

## PIPERACILLIN

**Sensitive strain of *Escherichia coli***



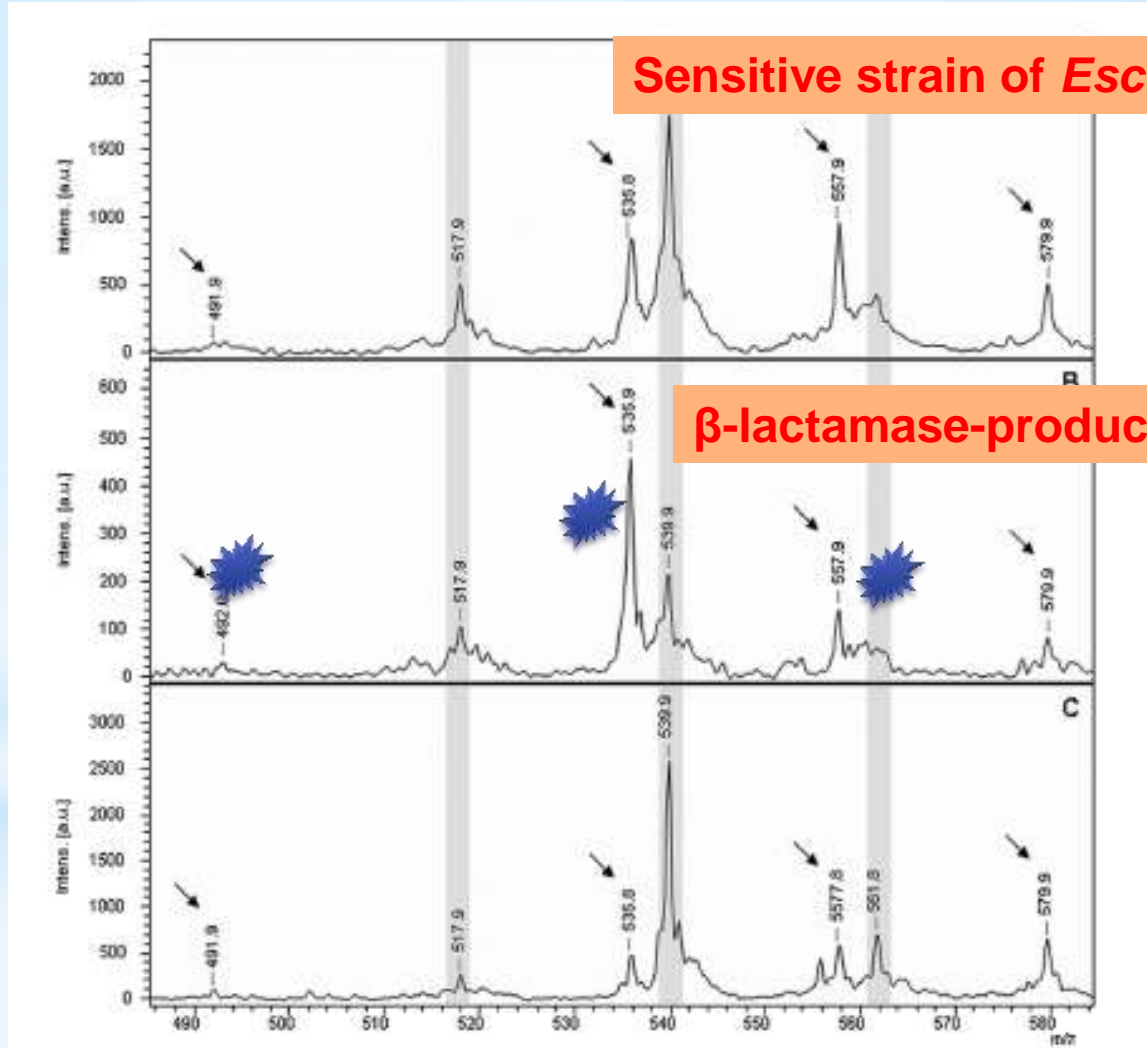
-----INTENSITY [a. u.]-----

-----MASS/CHARGE (m/z)-----

# MALDI-TOF

## PIPERACILLIN

-----INTENSITY [a. u.]-----

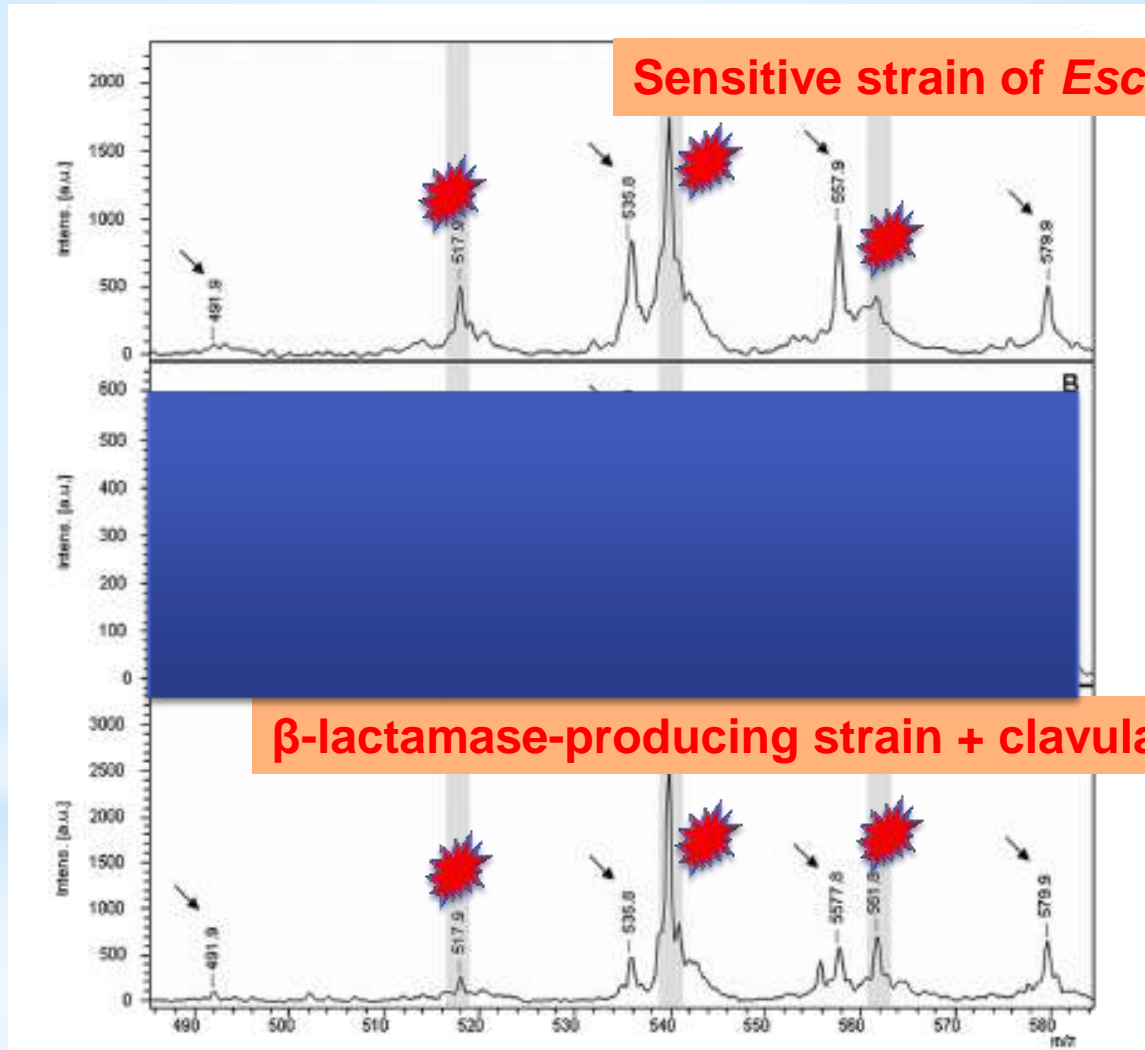


-----MASS/CHARGE (m/z)-----

# MALDI-TOF

## PIPERACILLIN

-----INTENSITY [a. u.]-----



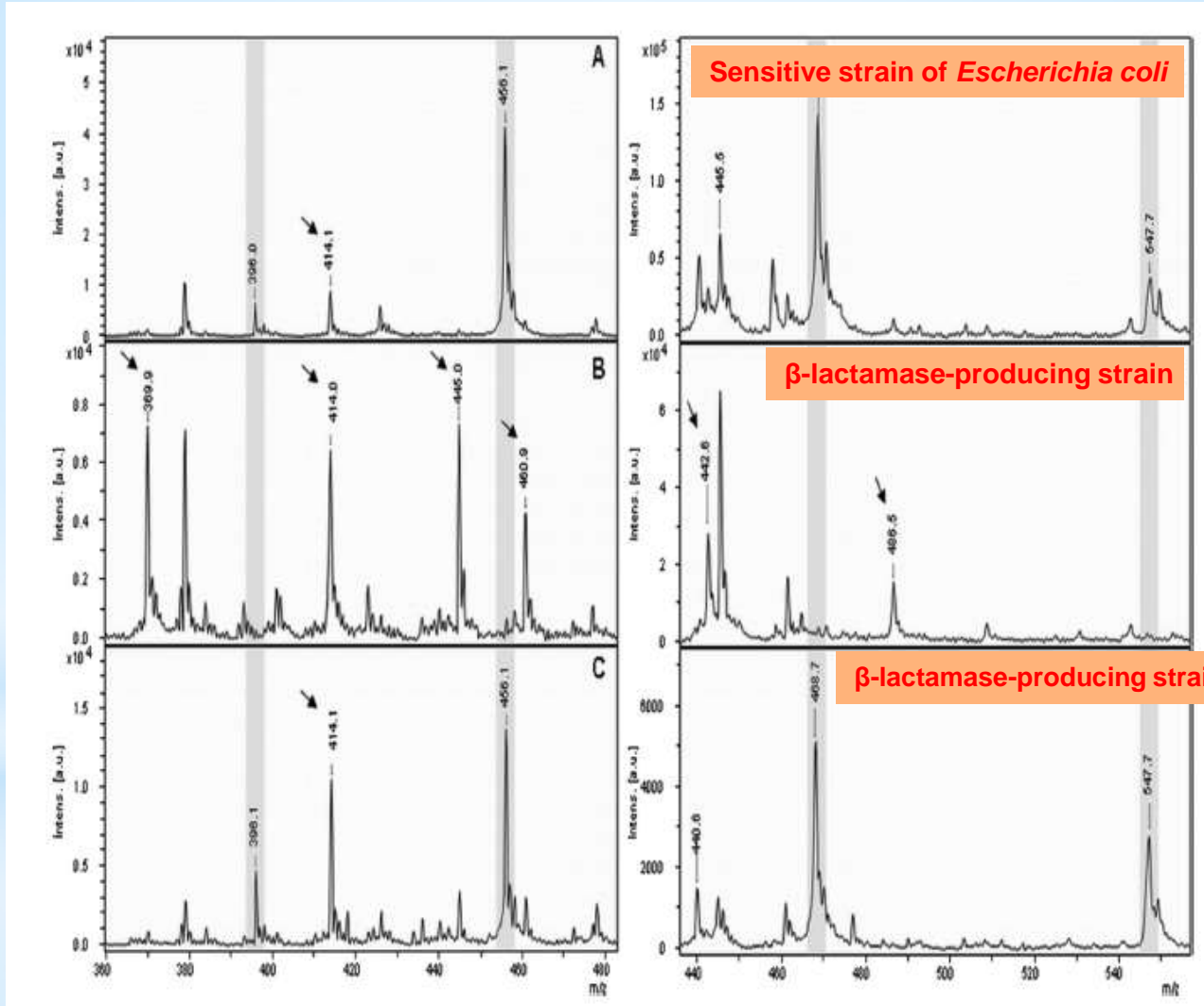
-----MASS/CHARGE (m/z)-----

# MALDI-TOF

## CEFOTAXIME

## CEFTAZIDIME

-----INTENSITY [a. u.]-----



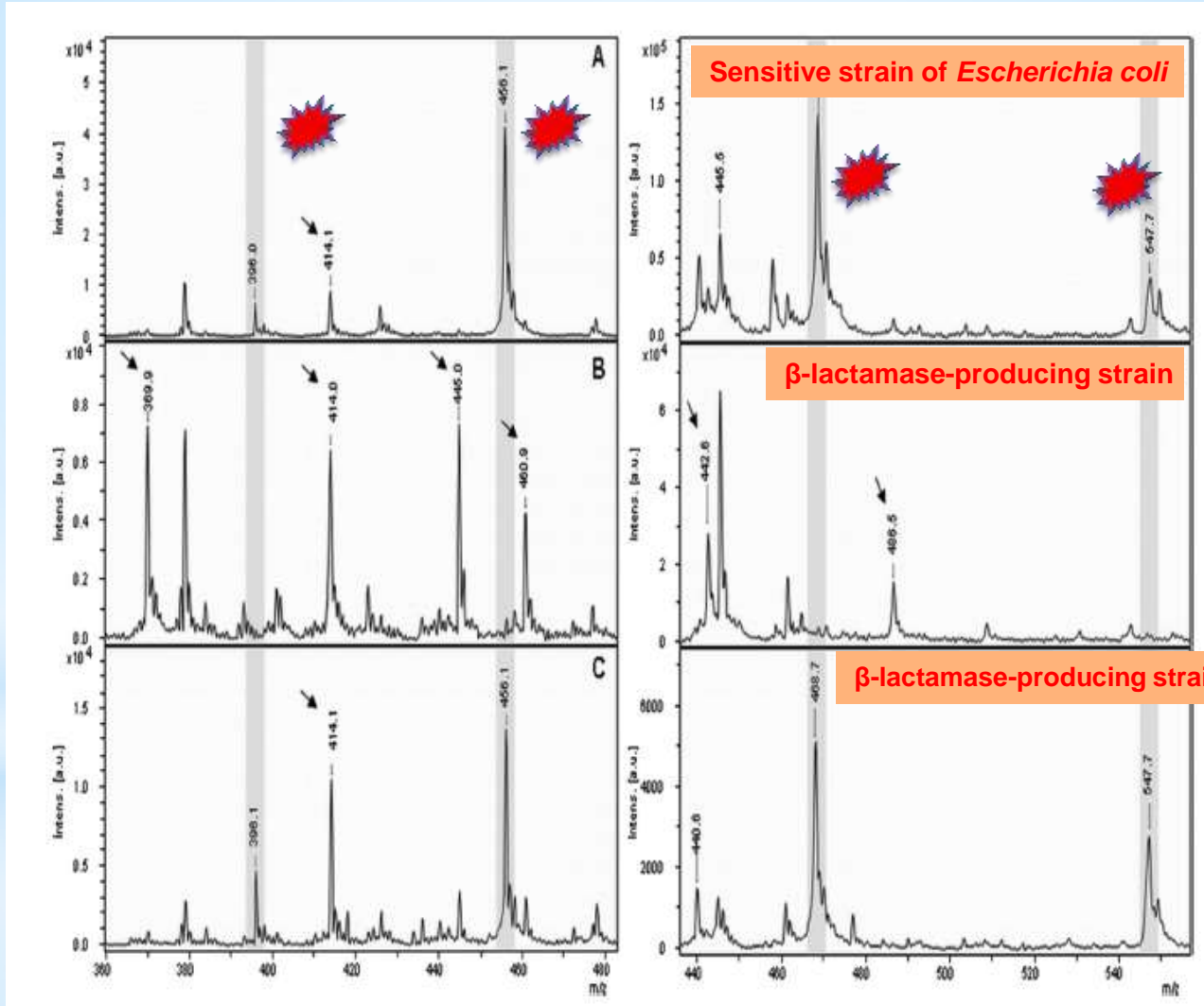
-----MASS/CHARGE (m/z)-----

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-----INTENSITY [a. u.]-----



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$\beta$ -lactamase-producing strain

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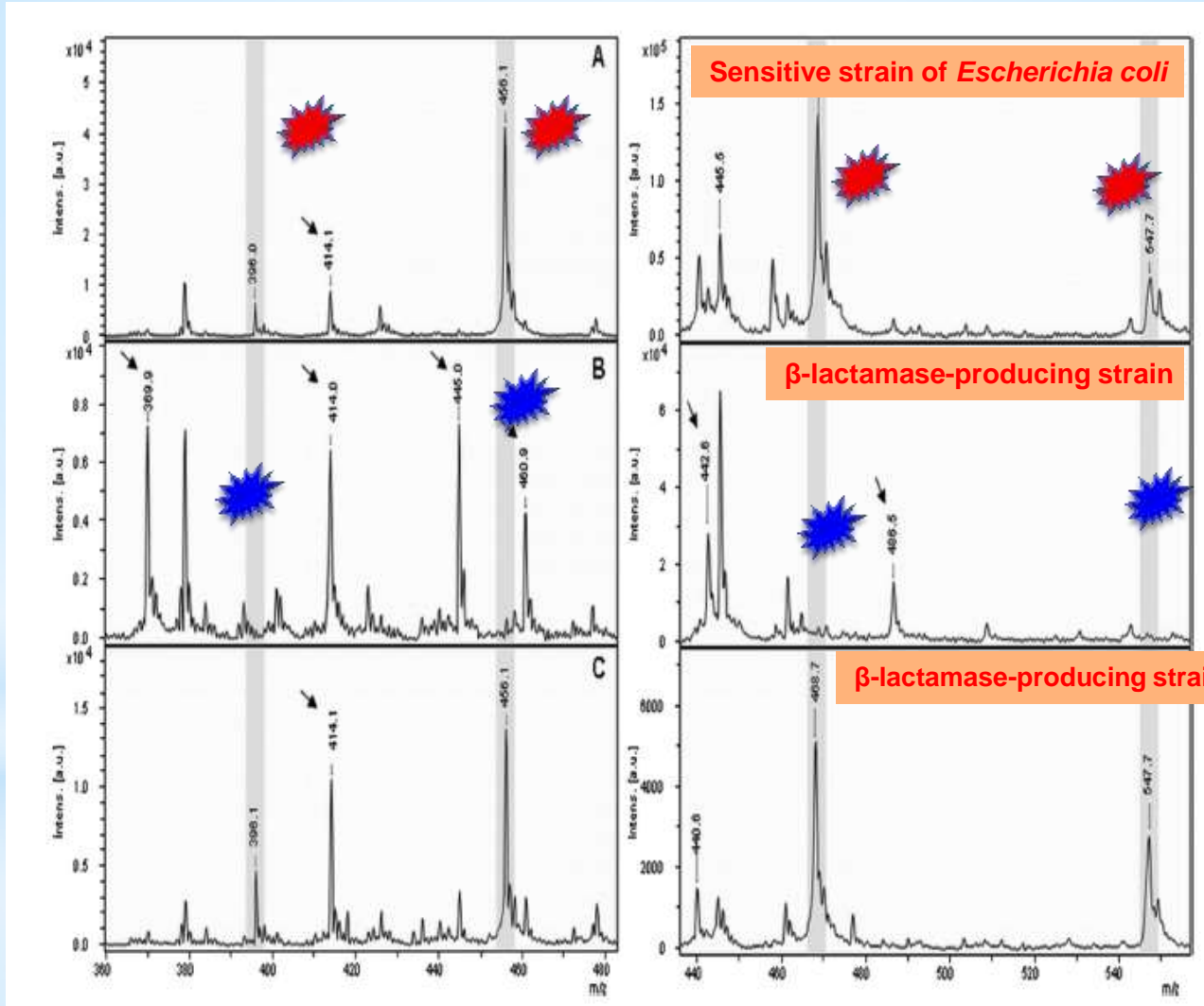
-----MASS/CHARGE (m/z)-----

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## CEFOTAXIME

## CEFTAZIDIME

-----INTENSITY [a. u.]-----



-----MASS/CHARGE (m/z)-----

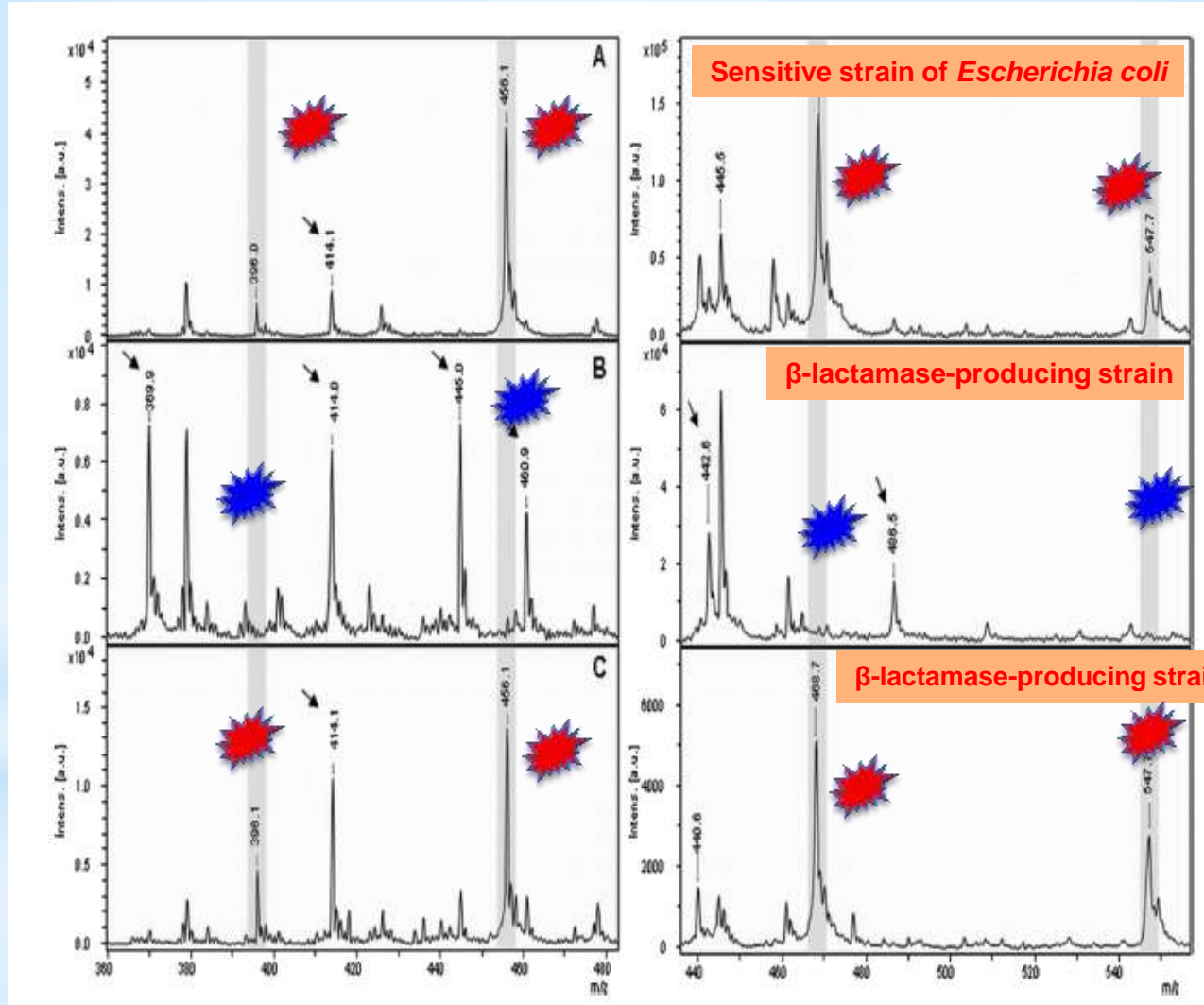


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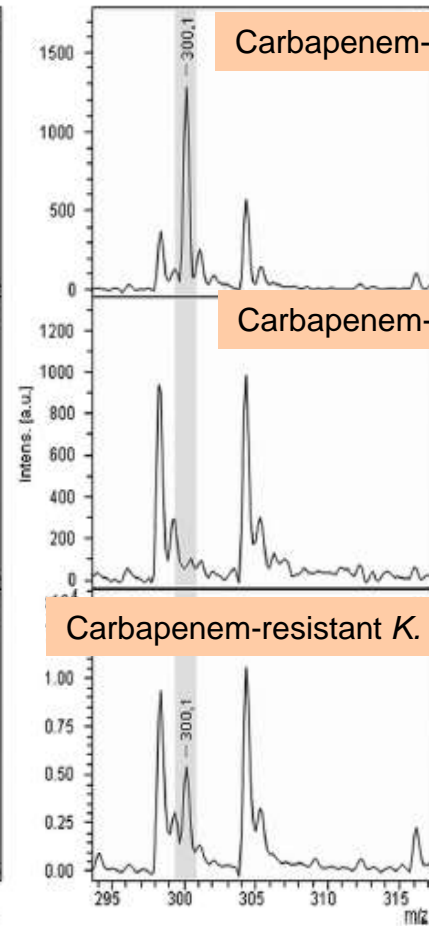
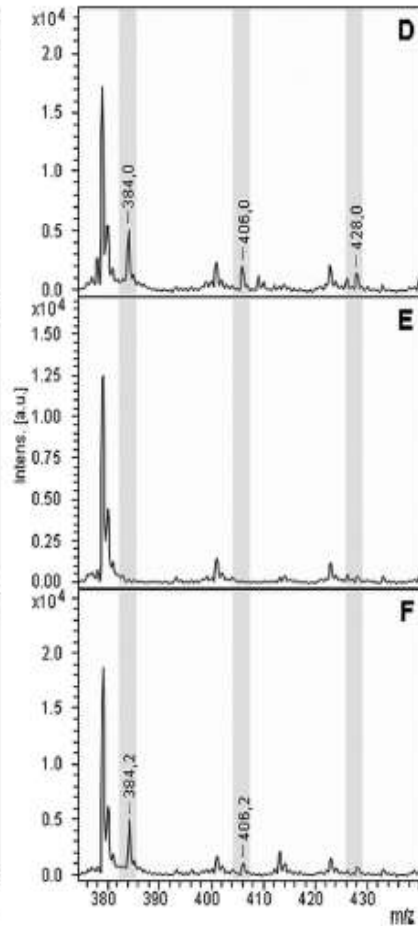
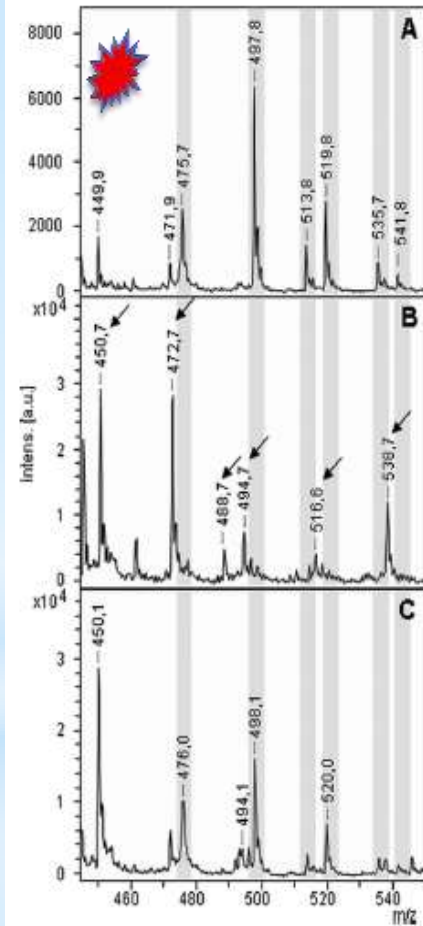
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ERTAPENEM

MEROPENEM

IMIPENEM

-----INTENSITY [a. u.]-----



-----MASS/CHARGE (m/z)-----

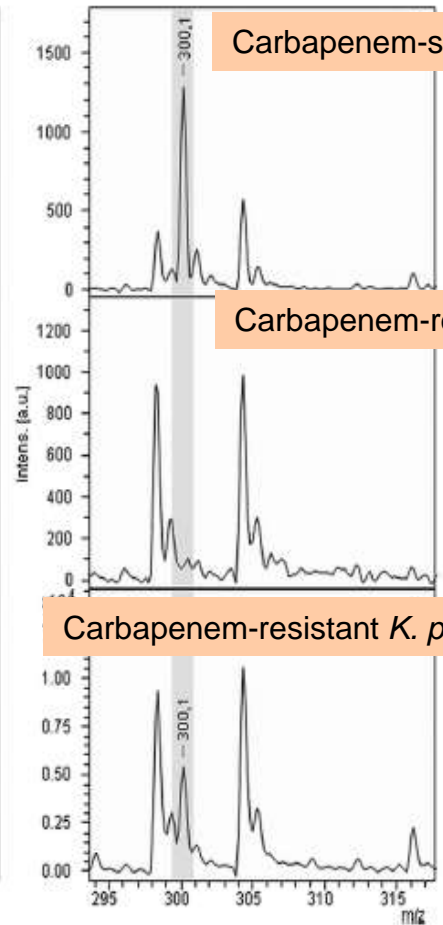
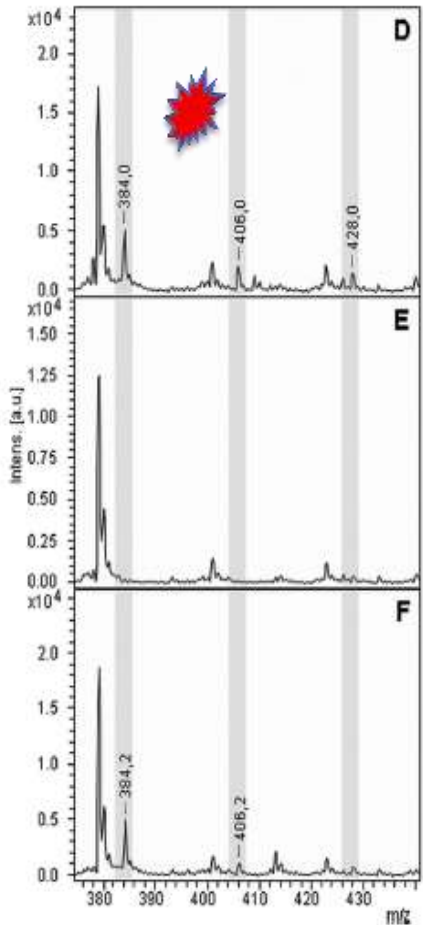
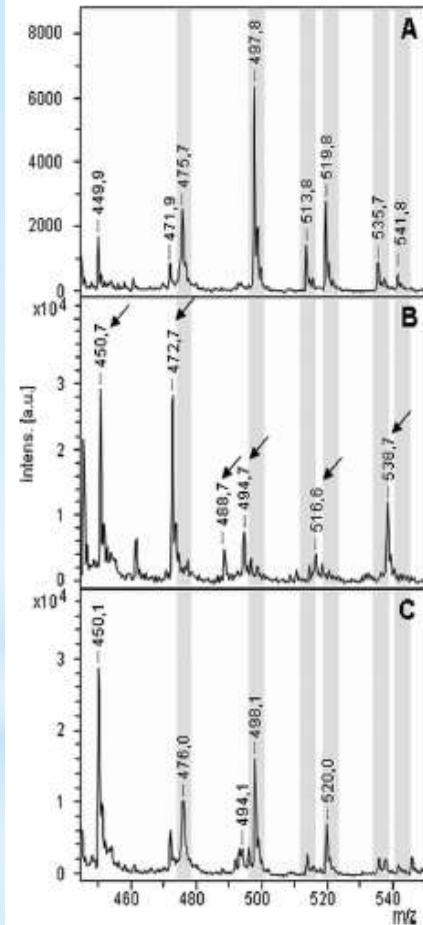
# MALDI-TOF

ERTAPENEM

MEROPENEM

IMIPENEM

-----INTENSITY [a. u.]-----



Carbapenem-sensitive *K. pneumoniae*

Carbapenem-resistant *K. pneumoniae*

Carbapenem-resistant *K. pneumoniae* + inhibitor

-----MASS/CHARGE (m/z)-----

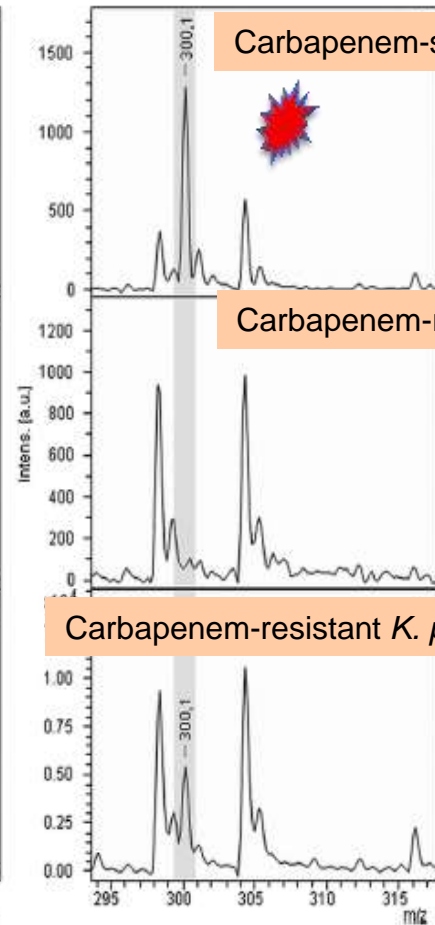
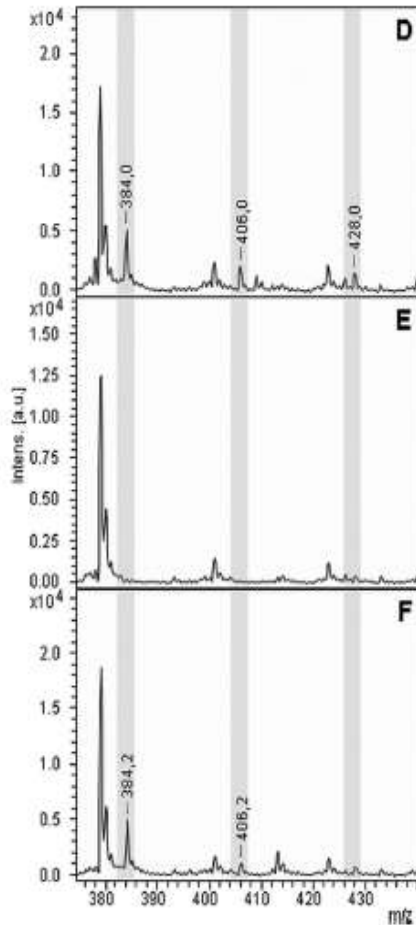
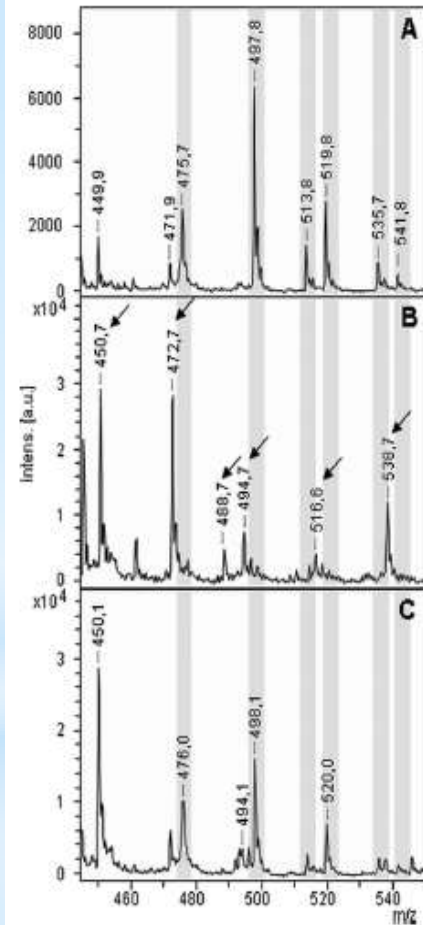
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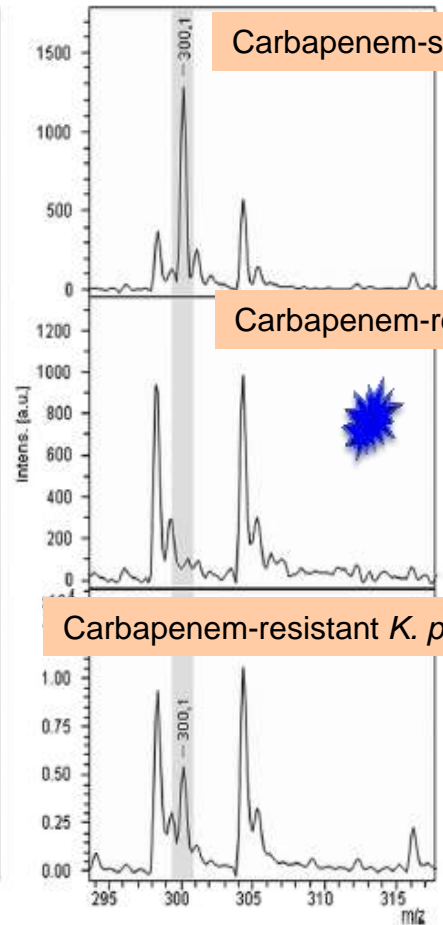
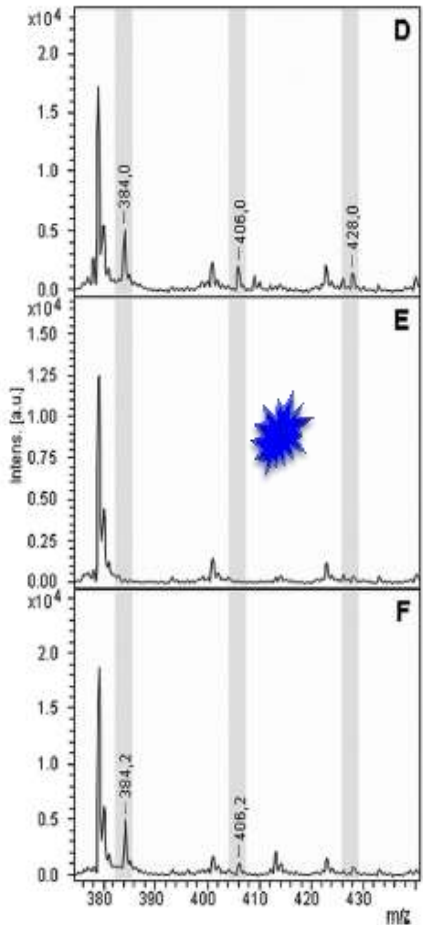
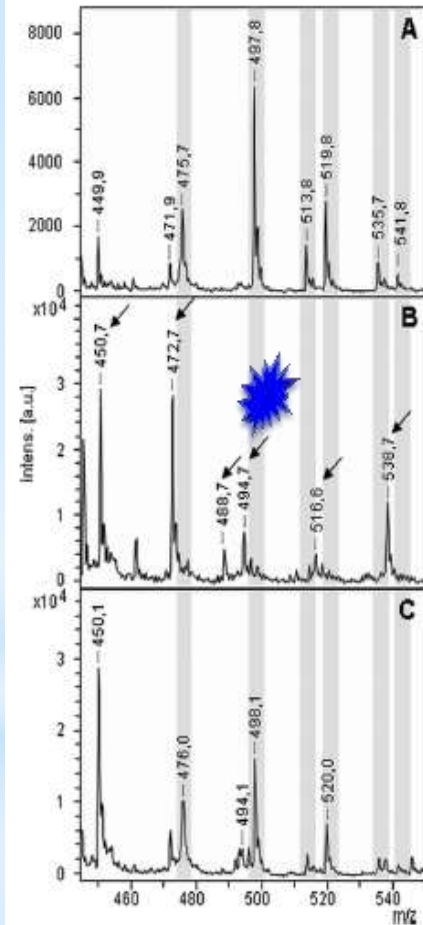
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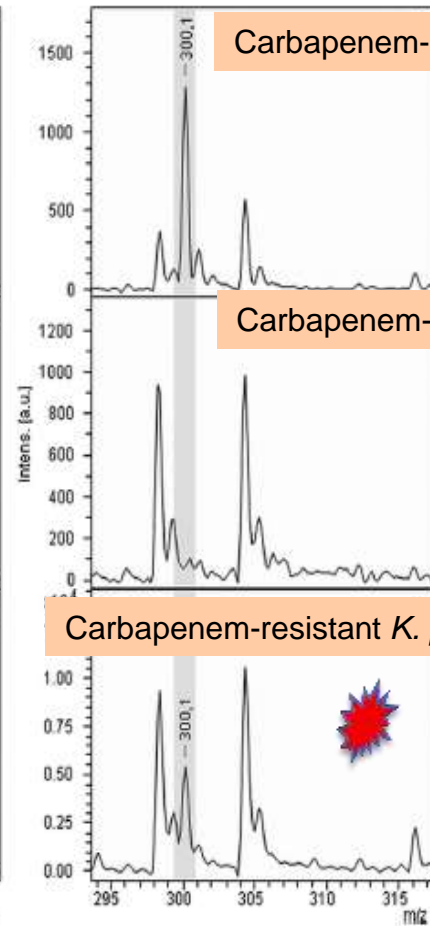
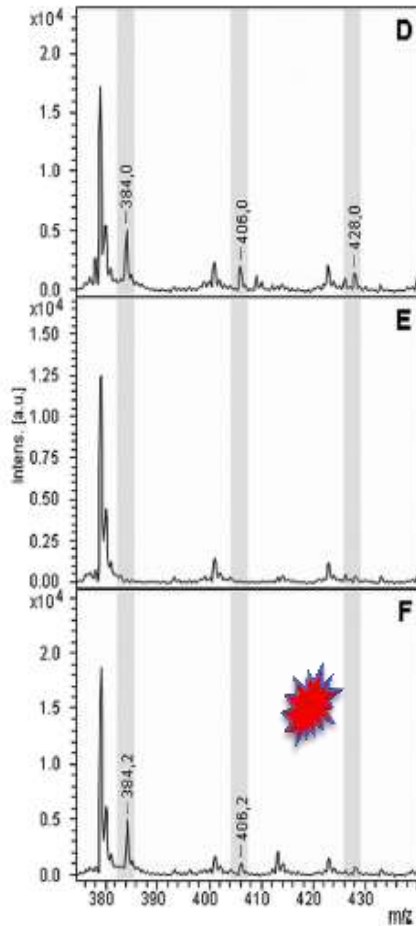
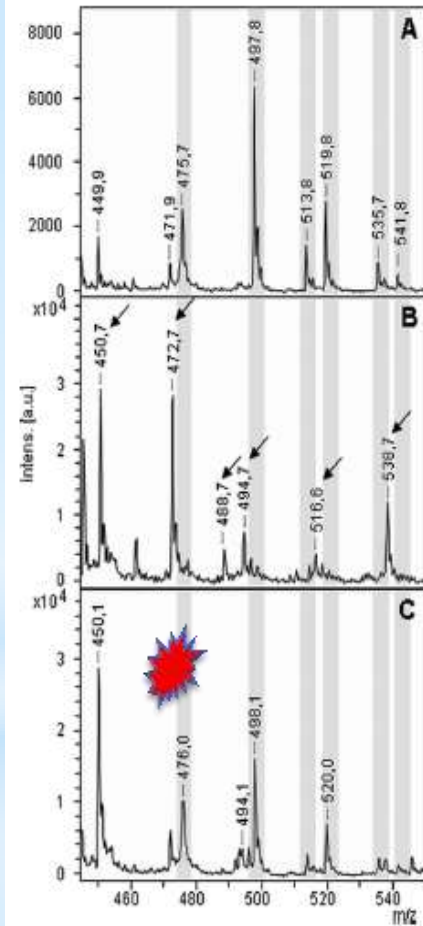
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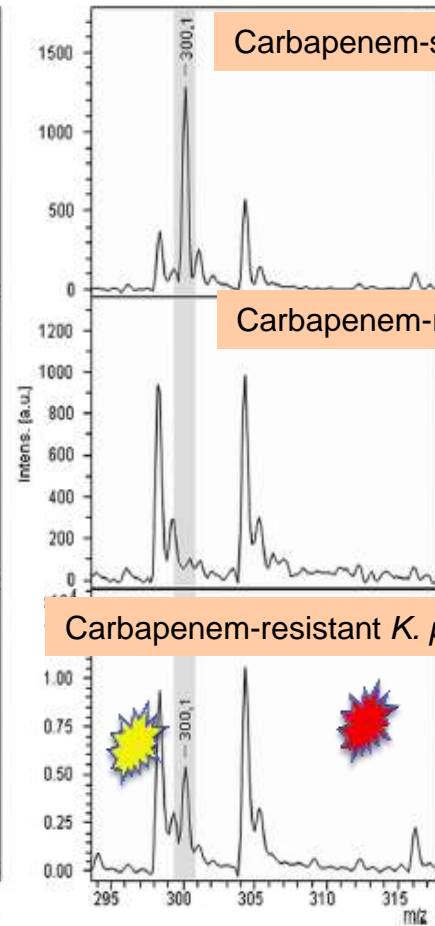
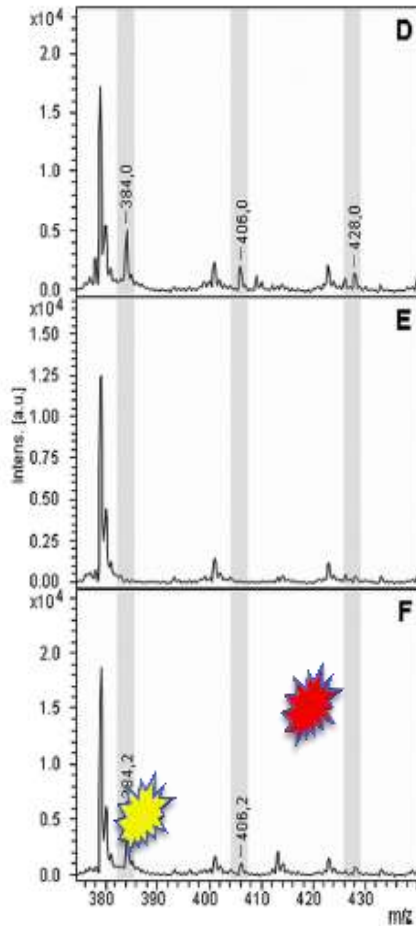
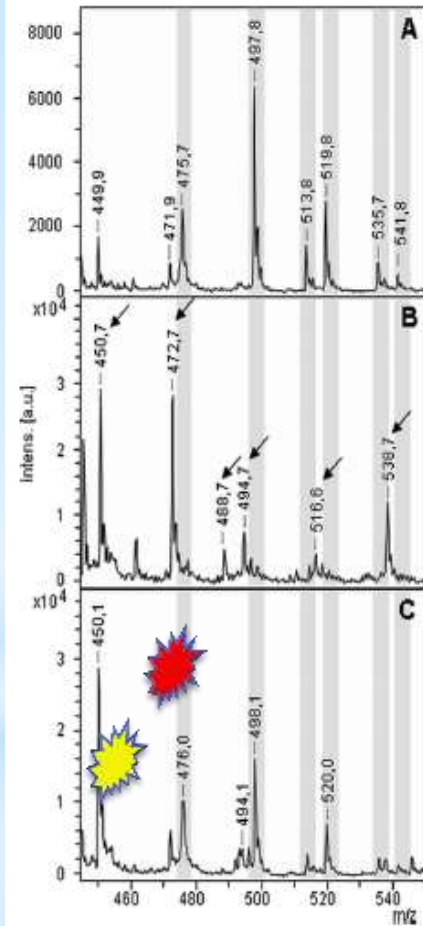
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# **IMPERATIVES FOR RAPID SUSCEPTIBILITY PROFILING**

**Judicious use antibiotics**

**Increasing costs of medical intervention**

**Increasing resistance to commonly used antimicrobial agents**

**Emerging resistance mechanisms**

**Stagnated development of antimicrobial agents**



## ***Concluding remarks***



Importance of organism identification

What happens when novel resistance occurs

Capacity to test a wide range and novel antibiotics

Phenotypic demonstration to predict *in vivo* response

Need for standardized method for validations

Need methods for resource limited laboratories

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