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Diagnostic de la résistance aux polymyxines

Aurélie Jayol

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Unité de Microbiologie Médicale et Moléculaire & INSERM LEA « Emerging Resistance to Antibiotics », Université de Fribourg, Suisse

Introduction

- Increase use of colistin
- No optimal method for polymyxin susceptibility testing
- Difficulties in testing the susceptibility to colistin :
  - Poor diffusion of the colistin into the agar
  - Cationic properties of the colistin
  - Occurence of heteroresistance to colistin in many species
  - Lack of an easy and reliable reference method

- Dilution methods
  - Broth microdilution method
  - Agar dilution method
- Routine test susceptibility methods
  - Non automatic systems
    - Disk diffusion test
      - E-test strips
      - UMIC system (Biocentric)
  - Automatic systems
    - Vitek-2 system (bioMérieux)
    - Phoenix system (Becton Dickinson)
    - MicroScan Walk away (Beckman Coulter)
    - TREK Sensititre (TREK Diagnostic)
- Qualitative detection techniques
  - Rapid Polymyxin NP test
  - SuperPolymyxin medium

- **Dilution methods**
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Broth microdilution method

Only method recommended by EUCAST and CLSI for colistin antimicrobial susceptibility testing.

Recommendations for MIC determination of colistin
As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group

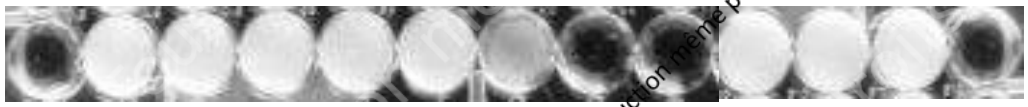
Reference testing of Gram negative rods is the broth microdilution method.

- Cation-adjusted Mueller-Hinton Broth
- No polysorbate-80
- Trays made of plain polystyrene and not treated
- Sulphate salts of polymyxins (the methanesulfonate derivative of colistin must not be used)

Published on www.eucast.org 22 March 2016

Broth microdilution method (2)

- Laborious and manual preparation of colistin solutions ➔ risk of errors
- Non-reproducible and non-interpretable MIC results due to presence of skip wells ➔ heteroresistance



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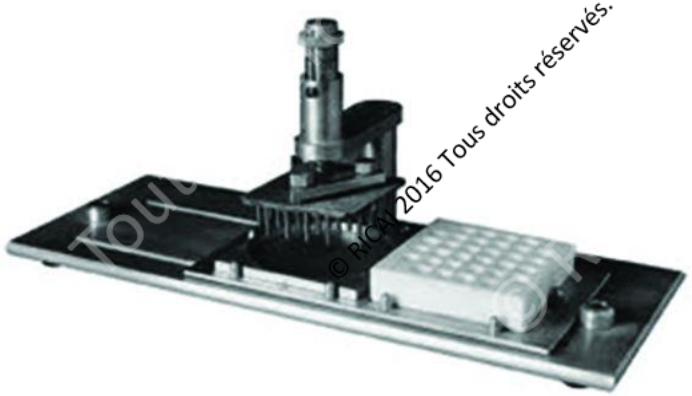
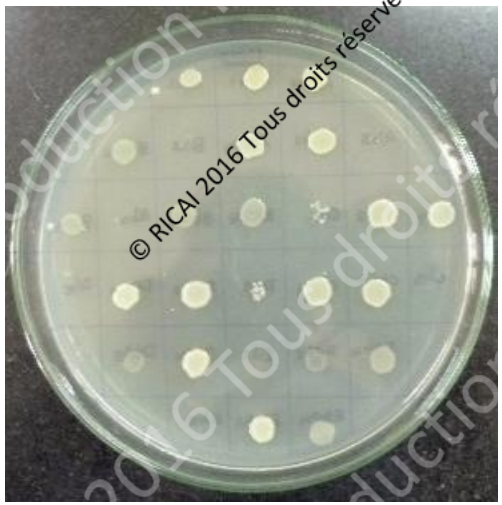
Irreproducible and Uninterpretable Polymyxin B MICs for *Enterobacter cloacae* and *Enterobacter aerogenes*

David Landman, Julius Salamera,* John Quale

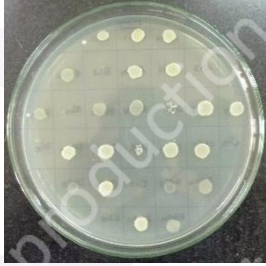
Agar dilution method

- Avoid the adsorption of the colistin to the plates ?
- Strong correlation between agar dilution and BMD
- Exception for *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* from cystic fibrosis patients





Agar dilution method (2)



Journal of Antimicrobial Chemotherapy (2004) **54**, 1057–1061
DOI: 10.1093/jac/dkh470
Advance Access publication 27 October 2004

JAC

Pitfalls of polymyxin antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients

Michael Högardt*, Sabine Schmoldt, Monika Götzfried, Kristin Adler and Jürgen Heesemann

➔ Better performance of the BMD after prolonged incubation (48h)

Colistin susceptibility testing: evaluation of reliability for cystic fibrosis isolates of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*

Samuel M. Moskowitz[1]*, Elizabeth Garber[2], Yunhua Chen[2], Sarah A. Clock[2], Setareh Tabibi[2], Amanda K. Miller[1]†, Michael Doctor[2] and Lisa Saiman[2]

J Antimicrob Chemother 2010; **65**: 1416–1423
doi:10.1093/jac/dkq131 Advance Access publication 29 April 2010

➔ Discrepancies between the 2 methods but who is right ?

- **Dilution methods**
  - Broth microdilution method
  - Agar dilution method
- **Routine test susceptibility methods**
  - **Non automatic systems**
    - Disk diffusion test
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- Qualitative detection techniques
  - Rapid Polymyxin NP test
  - SuperPolymyxin medium

Disk diffusion test

- Easy, cheap, and does not require specific equipment
- Very high and unacceptable rate of false susceptibility (up to 35 %) compared with BMD

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2007, p. 3726–3730 Vol. 51, No. 10
0066-4804/07/$08.00+0 doi:10.1128/AAC.01406-06

Comparative Evaluation of the VITEK 2, Disk Diffusion, Etest, Broth Microdilution, and Agar Dilution Susceptibility Testing Methods for Colistin in Clinical Isolates, Including Heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* Strains▽

Jerome R. Lo-Ten-Foe,[1] Anne Marie G. A. de Smet,[2] Bram M. W. Diederen,[1]†
Jan A. J. W. Kluytmans,[2,3] and Peter H. J. van Keulen[1]*

Letters in Applied Microbiology ISSN 0266-8254

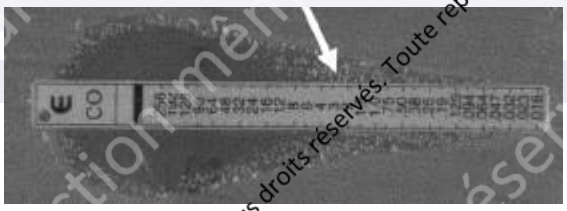
ORIGINAL ARTICLE

Comparison of disc diffusion, Etest and agar dilution for susceptibility testing of colistin against *Enterobacteriaceae*

S.M. Maalej, M.R. Meziou, F.M. Rhimi and A. Hammami

E-test strips

- High rates of false susceptibility (up to 32%) compared to dilution methods
- Failures to detect resistance even when isolates exhibit high MICs with dilution methods
- Underestimates the level of resistance of polymyxin-resistant strains (MIC ≥4 µg/ml)



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Colistin MIC Variability by Method for Contemporary Clinical Isolates of Multidrug-Resistant Gram-Negative Bacilli

Janet A. Hindler, Romney M. Humphries

Recommendations for MIC determination of colistin
As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group

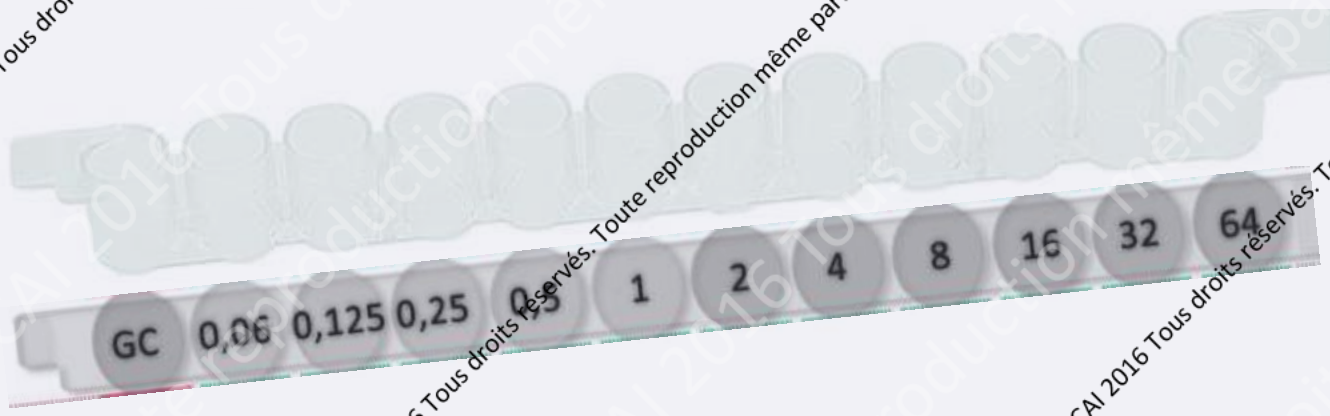
1. Reference testing of Gram negative rods is the broth microdilution method.

2. Susceptibility testing by other methods, including agar dilution, disk diffusion and gradient diffusion, cannot be recommended until historical data have been reviewed or new study data have been generated.

Published on www.eucast.org 22 March 2016

UMIC system (Biocentric)

- Broth microdilution method
- 11 colistin dilutions
- Optical or automatic reading after 18 to 24 hours of incubation



- No paper on the performances of this method

- **Dilution methods**
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- Qualitative detection techniques
  - Rapid Polymyxin NP test
  - SuperPolymyxin medium

Vitek-2 system (bioMérieux)

- Sensitivity = 82 % for detection of colistin-resistant Gram-negative isolates
- Not reliable to detect heteroresistant subpopulations

Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin

Y. Y. Tan and S. Y. Ng

Clin Microbiol Infect 2007; 13: 541–544
10.1111/j.1469-0691.2007.01708.x

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2007, p. 3726–3730 Vol. 51, No. 10
0066-4804/07/$08.00+0 doi:10.1128/AAC.01406-06

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Jerome R. Lo-Ten-Foe,[1] Anne Marie G. A. de Smet,[2] Bram M. W. Diederen,[1]†
Jan A. J. W. Kluytmans,[1,3] and Peter H. J. van Keulen[1]*

Phoenix system (Becton Dickinson)

- Evaluation of this system :
  - 100 enterobacterial isolates
  - 60 colistin-resistant and 40 colistin-susceptible isolates
- High rate (15%) of false-susceptible results
- Low sensitivity for colistin heteroresistance in *K. pneumoniae* and *E. cloacae* isolates
- Good sensitivity to detect plasmid-mediated *mcr-1* gene resistance

Microscan WalkAway system (Beckman Coulter)

- Sensitivity = 88% for *Acinetobacter* and *K. pneumoniae* isolates

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Comparison of the Vitek 2, MicroScan, and Etest Methods with the Agar Dilution Method in Assessing Colistin Susceptibility of Bloodstream Isolates of *Acinetobacter* Species from a Korean University Hospital

Seung Yeob Lee,[a] Jong Hee Shin,[a] Kyungwon Lee,[b] Min Young Joo,[a] Kyung Hwa Park,[c] Myung Geun Shin,[a] Soon Pal Suh,[a] Dong Wook Ryang,[a] Soo Hyun Kim[a]

Evaluation of polymyxin susceptibility profile among KPC-producing *Klebsiella pneumoniae* using Etest and MicroScan WalkAway automated system

LEANDRO REUS RODRIGUES PEREZ[1,2]

Sensititre system (TREK Diagnostic)

- Sensitivity = 96% compared to BMD
- Research use only

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Colistin MIC Variability by Method for Contemporary Clinical Isolates of Multidrug-Resistant Gram-Negative Bacilli

Janet A. Hindler, Romney M. Humphries

Automatic systems

- Sensitivity between 82 and 96%
- Detection of plasmid mediated colistin resistance ?
- Low range of colistin concentrations
- Results between 16 and 20 hours
- Easy use in routine labs

- Dilution methods
  - Broth microdilution method
  - Agar dilution method
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    - Phoenix system
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    - TREK Sensititre
- **Qualitative detection techniques**
  - Rapid Polymyxin NP test
  - SuperPolymyxin medium

Rapid Polymyxin NP test

Test principle :

- Detection of the glucose metabolism related to bacterial growth in presence of a defined concentration of colistin
- Formation of acid metabolites evidenced by a color change (orange to yellow) of a pH indicator (red phenol)

Rapid Detection of Polymyxin Resistance in *Enterobacteriaceae*

Patrice Nordmann, Aurélie Jayol, Laurent Poirel

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 22, No. 6, June 2016

Rapid Polymyxin NP test

- **Rapid Polymyxin NP solution :**

Medium composition
Mueller Hinton Broth Cation Adjusted
Phenol red
Glucose

CS

Ø CS

- **Bacterial inoculum :**

Bacterial colonies into NaCl 0.9 %
(density of 3 Mac Farland)

Rapid Polymyxin NP test

- Results at 2 hours

➔ The strain tested is resistant to colistin

Rapid Polymyxin NP test directly from blood cultures

Positive blood culture
(aerobic or anaerobic vial)

Gram staining: Gram negative rods

50 µl of blood culture

+

450 µl of NaCl 0.9 %

- Ten-fold dilution of the positive blood culture in NaCl 0.9%
- Rapid Polymyxin NP test

50 µl

in 150 µl of Polymyxin NP solution without colistin

in 150 µl of Polymyxin NP solution with colistin 5 µg/ml

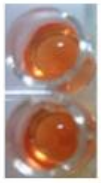
NEGATIVE	POSITIVE	NON-INTERPRETABLE
T = 4h	T = 2-4h	T = 4h
Colistin-susceptible strain	Colistin-resistant strain	Non enterobacterial strain

without colistin

with colistin

without colistin

with colistin



without colistin

with colistin

Jayol et al. JCM 2016

Rapid Polymyxin NP test

Advantages of the test :

- Sensitivity = 99.3% and specificity = 95.4% compared to BMD method
- Reliable for any enterobacterial species and regardless of the molecular mechanisms
- Easy to perform
- Rapid (2 hours from bacterial colonies and 4 hours from blood cultures)
- Cheap

Rapid Polymyxin NP test

Limits of the test :

- Visual reading of the color change
- Not adapted for *Pseudomonas aeruginosa* or *Acinetobacter* spp.
- Performances to test heteroresistant isolates ?

Rapid Polymyxin NP test

- Rapid Polymyxin NP test commercialisé depuis début novembre par ELITech

Rapid Polymyxin NP test

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Large Nosocomial Outbreak of Colistin-Resistant, Carbapenemase-Producing *Klebsiella pneumoniae* Traced to Clonal Expansion of an *mgrB* Deletion Mutant

Tommaso Giani,[a] Fabio Arena,[a] Guendalina Vaggelli,[b] Viola Conte,[a] Adriana Chiarelli,[a] Lucia Henrici De Angelis,[a] Rossella Fornaini,[c] Maddalena Grazzini,[d] Fabrizio Niccolini,[d] Patrizia Pecile,[b] Gian Maria Rossolini[a,b,e,f]

October 2015 Volume 53 Number 10 Journal of Clinical Microbiology

We describe a large hospital outbreak (93 bloodstream infections) of colistin-resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* isolates which was mirrored by increased colistin consumption. The outbreak was mostly traced to the clonal expansion of an *mgrB* deletion mutant of an ST512 strain that produced KPC-3.

SuperPolymyxin medium

Screening culture medium containing :

- EMB agar powder
- Colistin
- Daptomycin ➔ inhibition of Gram +
- Amphotericin B ➔ inhibition of fungi

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

CrossMark click for updates

A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates

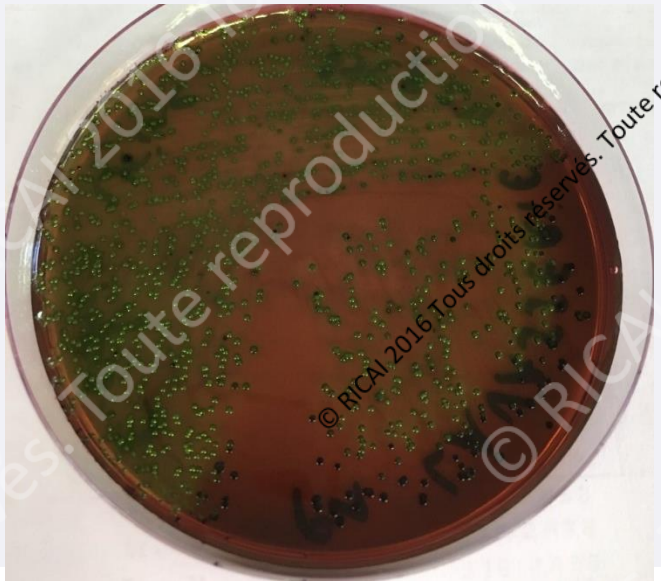
Patrice Nordmann,[a,b] Aurélie Jayol,[a] Laurent Poirel[a]

Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg,[a] and HFR-Hôpital Cantonal,[b] Fribourg, Switzerland

SuperPolymyxin medium

Bacterial colonies on the SuperPolymyxin medium :

- Distinguish lactose fermenters (colored colonies) of lactose non-fermenters (colorless or light lavender colonies)
- Lactose positive *E. coli* : characteristic metallic green sheen



E. coli

P. aeruginosa

SuperPolymyxin medium

- Sensitivity = 100% and specificity = 98%, regardless of the nature of the polymyxin resistance mechanisms and of the level of resistance
- Growth of colistin-resistant strains in 24h, except some isolates of *P. aeruginosa*, *S. maltophilia* and *Burkolderia* spp. that grow in 24 to 48h

En cours de développement industriel par la société ELITech

ELITechGroup MICROBIOLOGY

SuperPolymyxin medium

National survey of colistin resistance among carbapenemase-producing *Enterobacteriaceae* and outbreak caused by colistin-resistant OXA-48-producing *Klebsiella pneumoniae*, France, 2014

A Jayol [1], L Poirel [1], L Dortet [2 3 4], P Nordmann [1 2 5]

www.eurosurveillance.org

Que fait-on en pratique au CHU de Bordeaux ?

En pratique au CHU de Bordeaux

Liberté • Égalité • Fraternité
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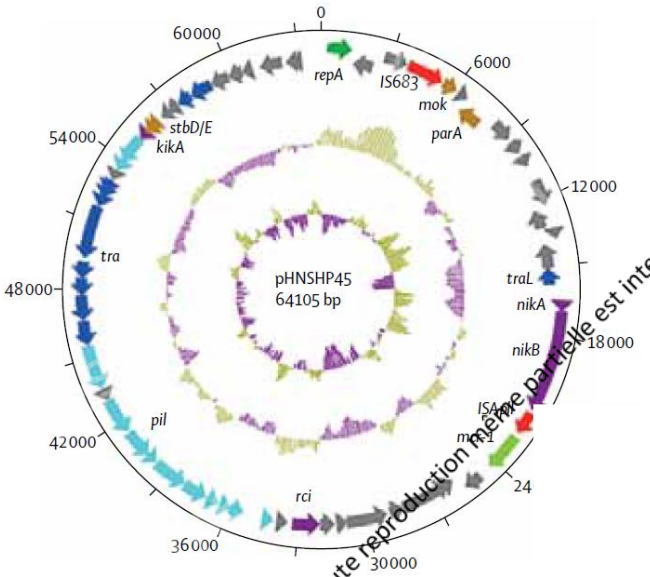
MARS

Message d'Alerte Rapide Sanitaire

MINISTERE DES AFFAIRES SOCIALES DE LA SANTE ET DES DROITS DES FEMMES
DIRECTION GENERALE DE LA SANTE
SOUS-DIRECTION VEILLE ET SECURITE SANITAIRE

DATE : 02/09/2016 **REFERENCE :** MARS N°2016_12

OBJET : ENTEROBACTERIES PORTEUSES DU GENE MCR-1 DE RESISTANCE PLASMIDIQUE A LA COLISTINE



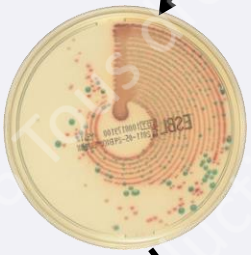
Liu et al, 2015

Dans ce contexte et dans l'attente de recommandations spécifiques à venir du Haut conseil de la santé publique, nous vous demandons :

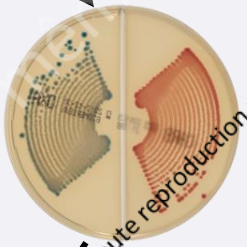
- de rechercher une résistance phénotypique à la colistine pour toute entérobactérie :
  1. Résistante aux céphalosporines de troisième génération ou aux carbapénèmes, à l'exception des entérobactéries naturellement résistantes à la colistine comme les souches appartenant aux genres *Morganella, Proteus, Serratia et Providencia* ;
  2. Et isolée chez un patient rapatrié ou ayant des antécédents récents d'hospitalisation à l'étranger ou dans les départements et territoires d'Outre-Mer ;

En pratique au CHU de Bordeaux

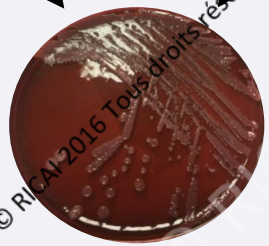
Patient rapatrié ou
hospitalisé à l'étranger



Gélose
BLSE



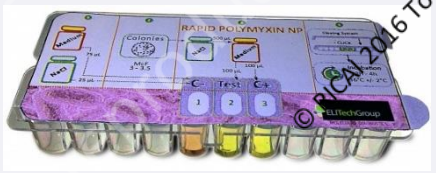
Gélose
CARBA SMART



Gélose
SuperPolymyxin

16h

Rapid Polymyxin NP test



2h

PCR *mcr-1* et *mcr-2*

En pratique au CHU de Bordeaux

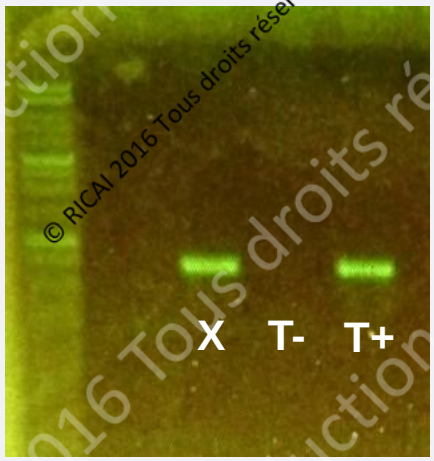
Mr X, patient rapatrié du Cambodge

Ecouvillon rectal

K. pneumoniae sur la gélose BLSE

Croissance sur gélose SuperPolymyxin

mcr-1 positif



En pratique au CHU de Bordeaux

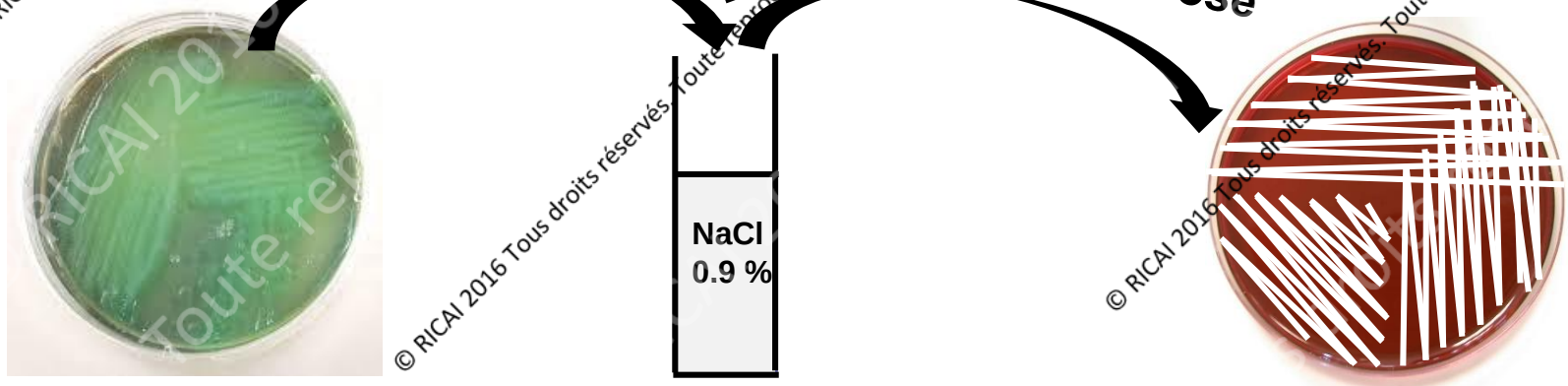
- Résistance à la colistine chez *P. aeruginosa*
  - Retrait des bandelettes E-test du marché
  - Système Phoenix inutilisable pour tester certains *P. aeruginosa*

➔ Réisolement sur gélose SuperPolymyxin

Ensemencement de la gélose SuperPolymyxin

- Ne pas réisoler directement les colonies sur la gélose !!!

➔ Risque de faux positifs (effet inoculum)



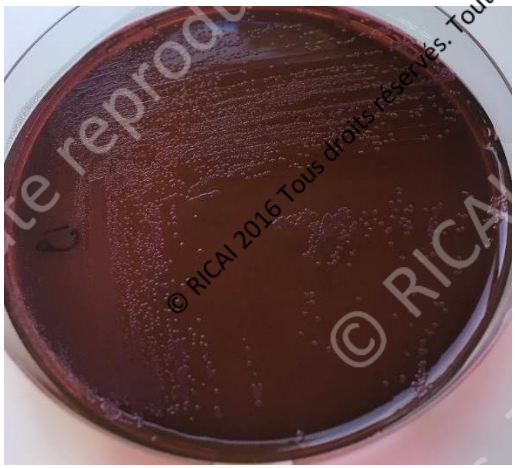
Lecture à 24h et 48h

Effet inoculum

Négatif

➔ **Souche sensible à la colistine**

Positif



➔ **Suspicion de résistance à la colistine**
En cas d'utilisation clinique, contacter le laboratoire pour la détermination des CMI en milieu liquide

Conclusion

- Méthode des disques, bandelettes E-test : à bannir
- Systèmes automatisés et système UMIC : performances à préciser
- Deux nouveaux outils pour le dépistage rapide et le screening :
  - Rapid Polymyxin NP test
  - Gélose SuperPolymyxin
- Méthode de référence : CMI en milieu liquide

Merci pour votre attention

aurelie.jayol@hotmail.fr