Journée Régionale d'Infectiologie
Nancy
12 octobre 2013

Nouvelles méthodes en bactériologie médicale

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Service de Bactériologie

CHU NANCY
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Bactériologie médicale : défis actuels

- Nécessité de résultats fiables et rapidement disponibles
- Qualité – accréditation
- Evolutivité bactérienne ++ (résistance, espèces …)
- Réduction des moyens

Défis en bactériologie médicale : les solutions ?

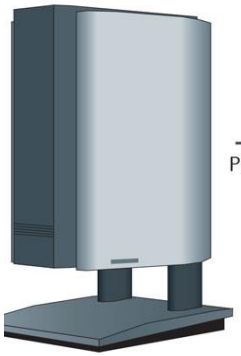
- Organisations nouvelles
- Personnel formé et habilité
- Méthodes nouvelles : rapides, fiables, évolutives et … à un coût raisonnable

Nouvelles méthodes utilisables en routine ?

« PCR tout en un »

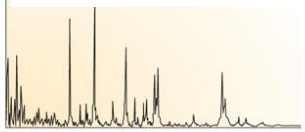
Spectrométrie de masse

Séquençage à haut débit
(génome entier)

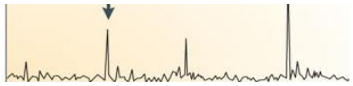


MALDI–TOF MS
PCR–ESI–QTOF MS

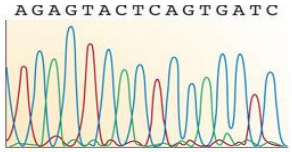
Profil protéique



Dégradation des antibiotiques

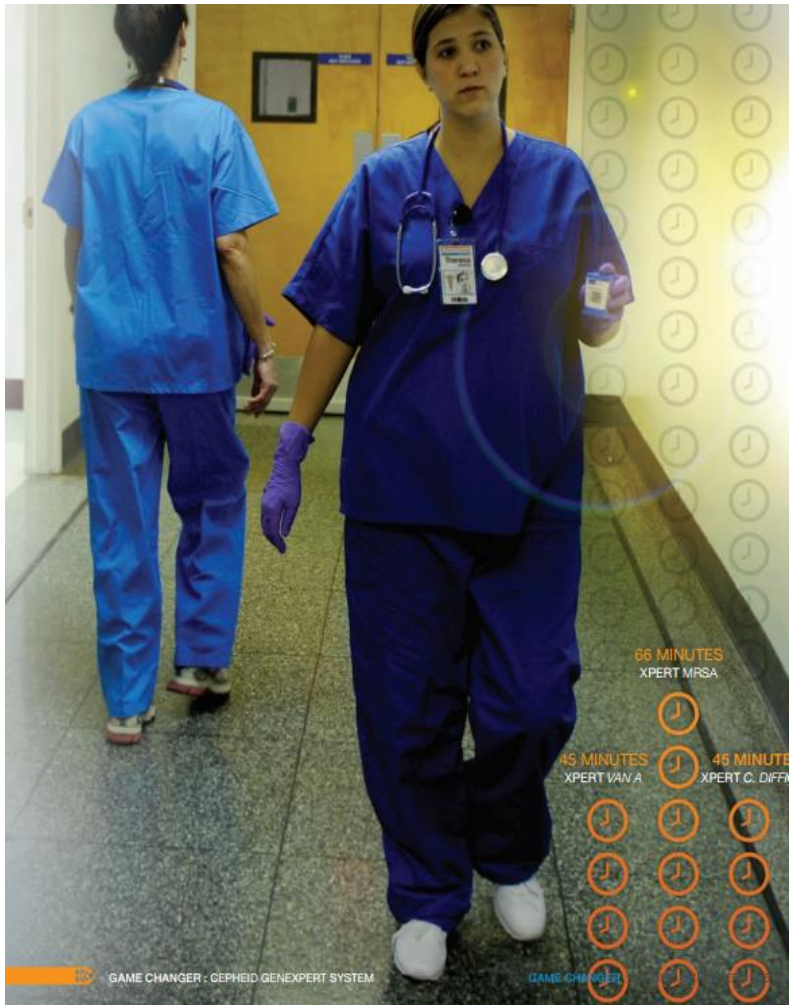


Amplicons



PCR « tout en un »

- Réalisation rapide et facile
- Personnel non spécialisé
- Nombre de cibles limité
- Coût ++



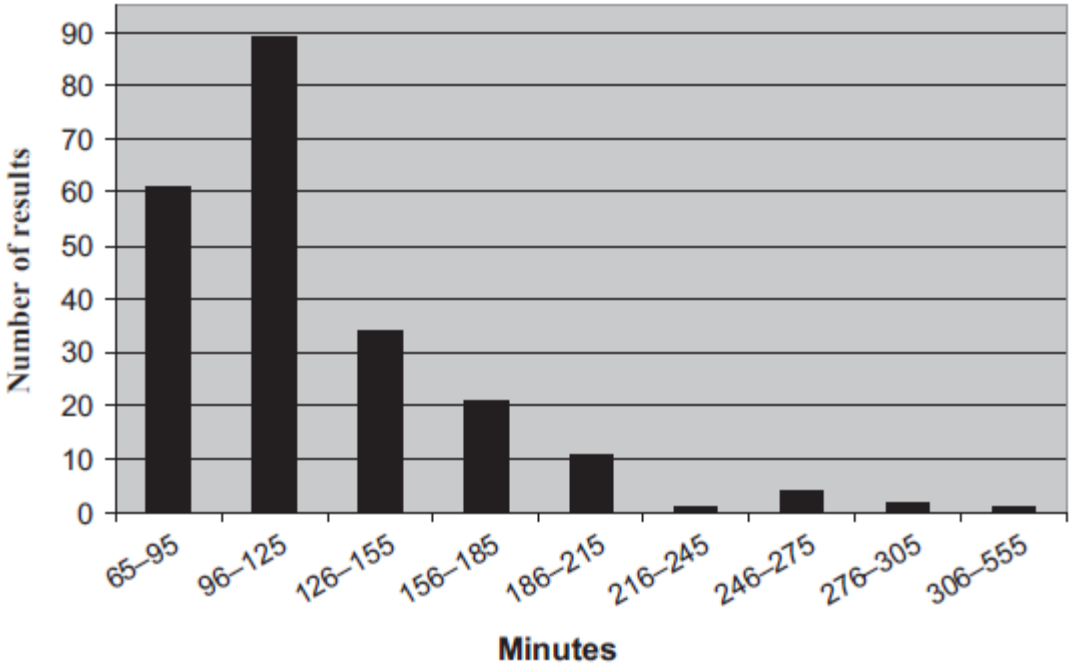
Indication and performances of commonly used point-of-care (POC) tests

Test/pathogen	Type of test	Sample	Indication	Performances
Group A streptococcal rapid test	EIA	Pharyngeal swab	Sore throat	Sensitivity: 53–99% Specificity: 62–100%
Pneumococcal antigen	ICT	Urine (pleural fluid, CSF)	Severe pneumonia (empyema, meningitis)	Sensitivity: 66–70% Specificity: 90–100%
Legionella antigen	ICT	Urine	Severe pneumonia/risk factors for legionellosis	Sensitivity: 76% Specificity: 99%
Group B streptococci	POC test–PCR	Vaginal swab	Peripartum detection of colonization	Sensitivity: 94–97% Specificity: 96–100%
MRSA carriage detection	POC test–PCR	Nasal swab	Risk factors, screening	Sensitivity: 86–94% Specificity: 93–95%
Clostridium difficile toxin detection	ICT	Stool	Antibiotic-associated diarrhoea	Sensitivity: 49–80% Specificity: 95–96%
Chlamydia antigen	ICT	Vaginal swab, urine	Screening, suspicion of PID	Sensitivity: 83% Specificity: 99%
Rapid malaria test	ICT	Blood	Fever in returning traveller	Sensitivity: 87–100% Specificity: 52–100%
Giardia lamblia rapid diagnosis	EIA	Stool	Diarrhoea, especially for returning travellers	Sensitivity: 58–98% Specificity: 97–98%
RSV antigen	ICT	Nasopharyngeal swab	Viral symptoms, especially during the winter season	Sensitivity: 59–97% Specificity: 75–100%
Influenza rapid test	ICT	Nasopharyngeal swab	Flu-like symptoms	Sensitivity: 20–55% Specificity: 99%
Rotavirus antigen	ICT	Stool	Diarrhoea (children)	Sensitivity: 75–99% Specificity: 95%
Adenovirus antigen	ICT	Stool	Diarrhoea	Sensitivity: 22% Specificity: 84%
HIV rapid test	ICT	Blood (oral fluid)	Screening, prevention of vertical transmission	Sensitivity: 99–100% Specificity: 99–100%
Enterovirus	POC test–PCR	CSF	Meningitis	Sensitivity: 97% Specificity: 100%

Clerc et Greub Clin Microbiol Infect 2010

Détection de *Streptococcus agalactiae*

PCR temps réel : Xpert GBS (Cepheid)



Poncelet-Jasserand et al. *Int J Obst Gynaecol* 2013

Reduction of the use of antimicrobial drugs following the rapid detection of *Streptococcus agalactiae* in the vagina at delivery by real-time PCR assay

E Poncelet-Jasserand,[a,*] F Forges,[b,*] M-N Varlet,[a] C Chauleur,[a] P Seffert,[a] C Siani,[d] B Pozzetto,[c,e] A Ros[e]

	Culture at 34–38 weeks of gestation	PCR at delivery	Statistical analysis*
Diagnostic performance (%):			
Sensitivity (95% CI)	55.6 (35.3–74.5)	66.7 (46.0–83.5)	
Specificity (95% CI)	84.5 (78.7–89.3)	94.9 (90.9–97.5)	
Positive predictive value (95% CI)	33.3 (20.0–48.9)	64.3 (44.1–81.4)	
Negative predictive value (95% CI)	93.2 (88.4–96.4)	95.4 (91.5–97.9)	
Women inadequately treated with prophylactic antimicrobial treatment (%)	13.6	4.5	$P < 0.001$
Women adequately treated with prophylactic antimicrobial treatment (%)	6.8	8.0	NS
Women inadequately not treated with prophylactic antimicrobial treatment (%)	5.4	4.0	NS
Women adequately not treated with prophylactic antimicrobial treatment (%)	74.2	83.5	$P < 0.05$
Technical performances (%):			
Sensitivity (95% CI)	58.3 (36.6–77.9)	76.9 (56.3–91.0)	
Specificity (95% CI)	92.7 (87.8–96.0)	95.4 (91.4–97.9)	
Positive predictive value (95% CI)	51.9 (31.9–71.3)	69.0 (49.2–84.7)	
Negative predictive value (95% CI)	94.3 (89.7–97.2)	96.9 (93.3–98.8)	

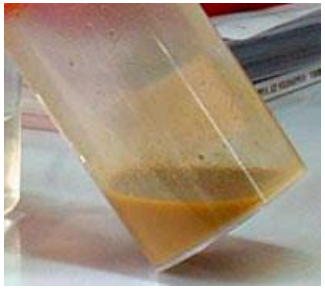
*McNemar test.

Poncelet-Jasserand et al. *Int J Obst Gynaecol* 2013

CME

Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections

Christina M. Surawicz, MD[1], Lawrence J. Brandt, MD[2], David G. Binion, MD[3], Ashwin N. Ananthakrishnan, MD, MPH[4], Scott R. Curry, MD[5], Peter H. Gilligan, PhD[6], Lynne V. McFarland, PhD[7,8], Mark Mellow, MD[9] and Brian S. Zuckerbraun, MD[10]



« Nucleic acid amplifications tests (NAAT) for *C. difficile* toxin genes such as PCR are superior to toxins A + B EIA testing as a standard diagnostic test for CDI »

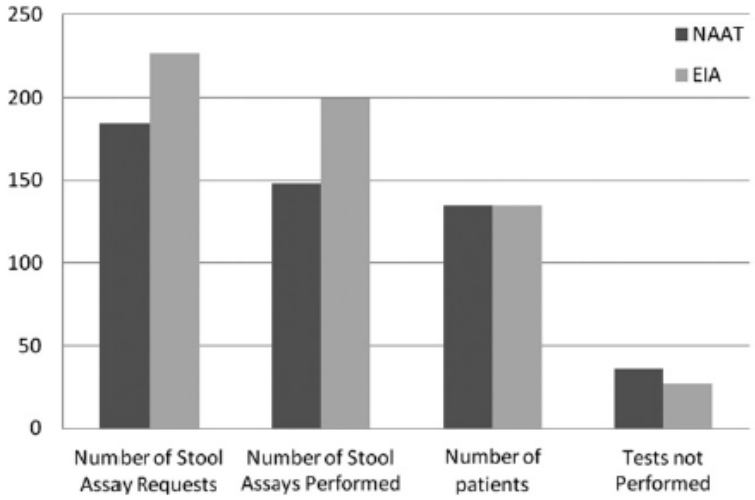
45 min

(strong recommendation, moderate-quality evidence)

Am J Gastroenterol April 2013

Clinical impact of switching conventional enzyme immunoassay with nucleic acid amplification test for suspected *Clostridium difficile*-associated diarrhea

Steven W. Johnson PharmD [a,b,*], Meganne Kanatani PharmD [c], Romney M. Humphries PhD [d], Daniel Z. Uslan MD [e]



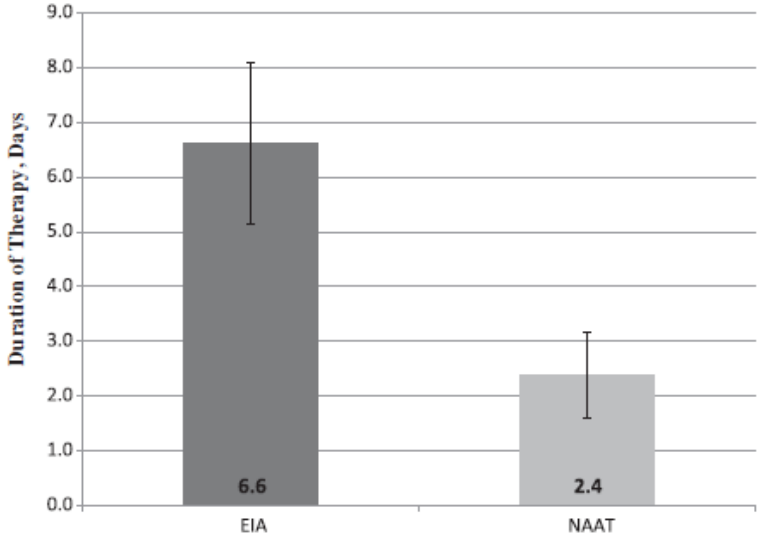


Fig 2. Days of empiric therapy in patients who had negative stool assays.

American Journal of Infection Control 41 (2013) 373-5

Patient avec diarrhée PCR (+) Tox (-)
uniquement excréteur ?

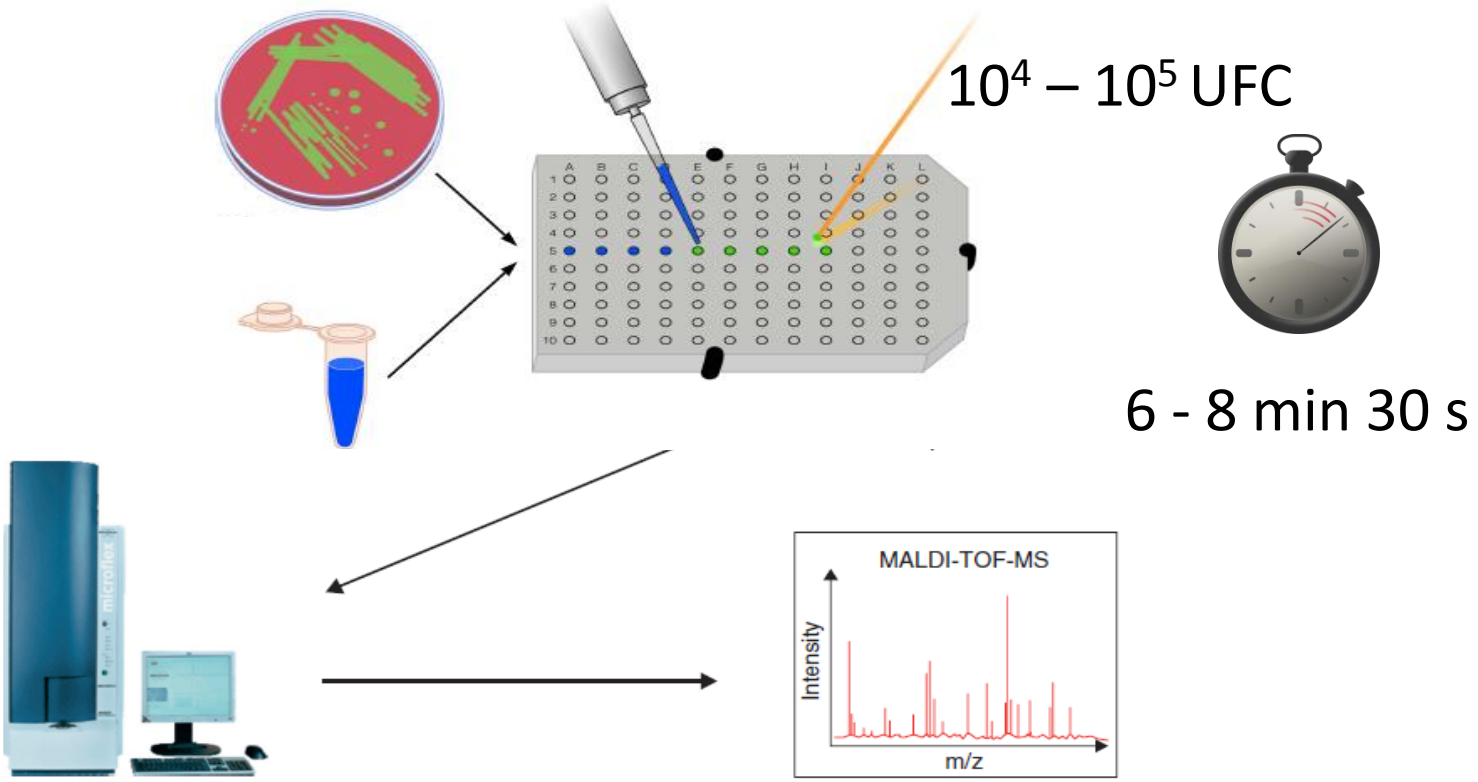
⇩

GDH ou PCR & si + : EIA Tox
ou …

Baker et al. J Hosp Infect - July 2013
Planche et al. Lancet Infect Dis – September 2013

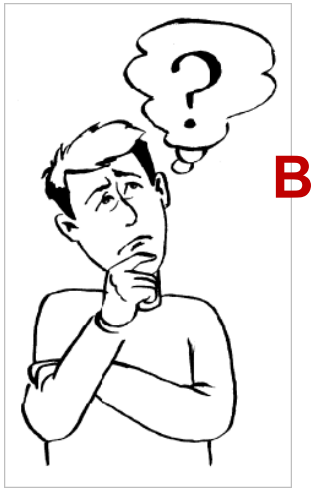
Identification des bactéries par spectrométrie de masse MALDI-TOF

Matrix **A**ssisted **L**aser **D**esorption/**I**onization – **T**ime **O**f **F**light

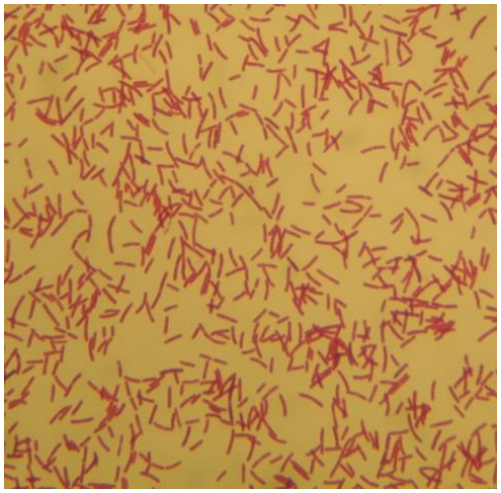


D'après Clark et al. CMR 2013 - Seng et al. JCM 2013 & Kliem et al. Curr Opin Microbiol 2012

Cardiac Device-Related Endocarditis Caused by *Paenibacillus glucanolyticus*



Bacille à Gram négatif …
ou non ??



→ **Identification phénotypique standard**

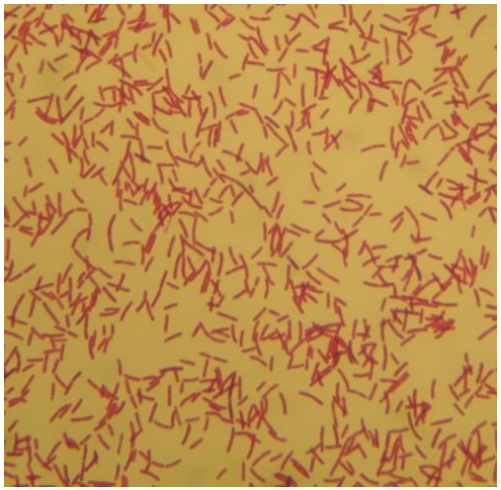
A partir des colonies (**24-48 h**) → délai : **+ 24-48 h**

- identification Gram négatif : *Cronobacter* ou *Aeromonas ?*
- identification Gram positif : *Paenibacillus glucanolyticus*

Ferrand et al. J Clin Microbiol 2013

Cardiac Device-Related Endocarditis Caused by *Paenibacillus glucanolyticus*

Bacille à Gram positif !



→ **Spectrométrie de masse :** *Paenibacillus glucanolyticus*
A partir des colonies (**24-48 h**) → délai : **+ 20 minutes**

→ **PCR universelle (ADNr 16S) :** *Paenibacillus glucanolyticus*
A partir prélèvements : **48 h**
A partir colonies (**24-48 h**) → délai : **+ 24-48 h**

Ferrand et al. J Clin Microbiol 2013

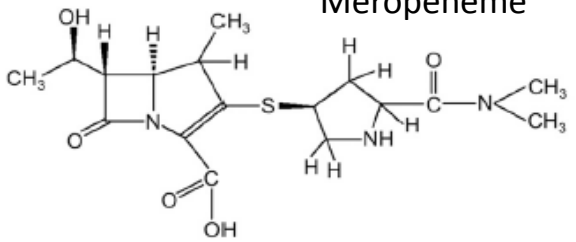
Impact of MALDI-TOF MS on the clinical management of patients with **Gram-negative bacteremia** : a prospective observational study

- Centre hospitalier et universitaire de Lausanne (2010)
- 202 épisodes de bactériémies
- Identification correcte / genre : 86,7% (hémocultures monomicrobiennes)
- **Impact de l'examen direct** (Gram) : **20,8%**
- **Impact SM-Maldi-TOF** : **35,1%** (modification antibiothérapie)

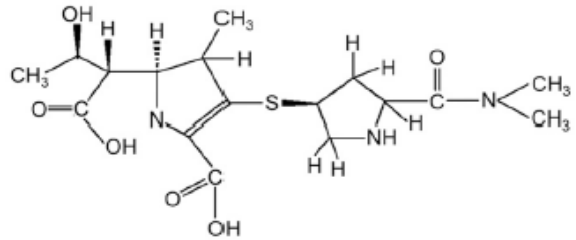
Clerc et al. Clin Infect Dis 2013

Spectrométrie de masse MALDI-TOF et détection de β-lactamases

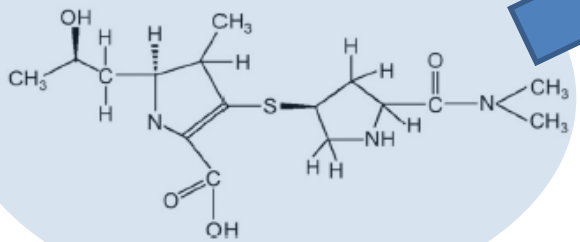
Méropénème



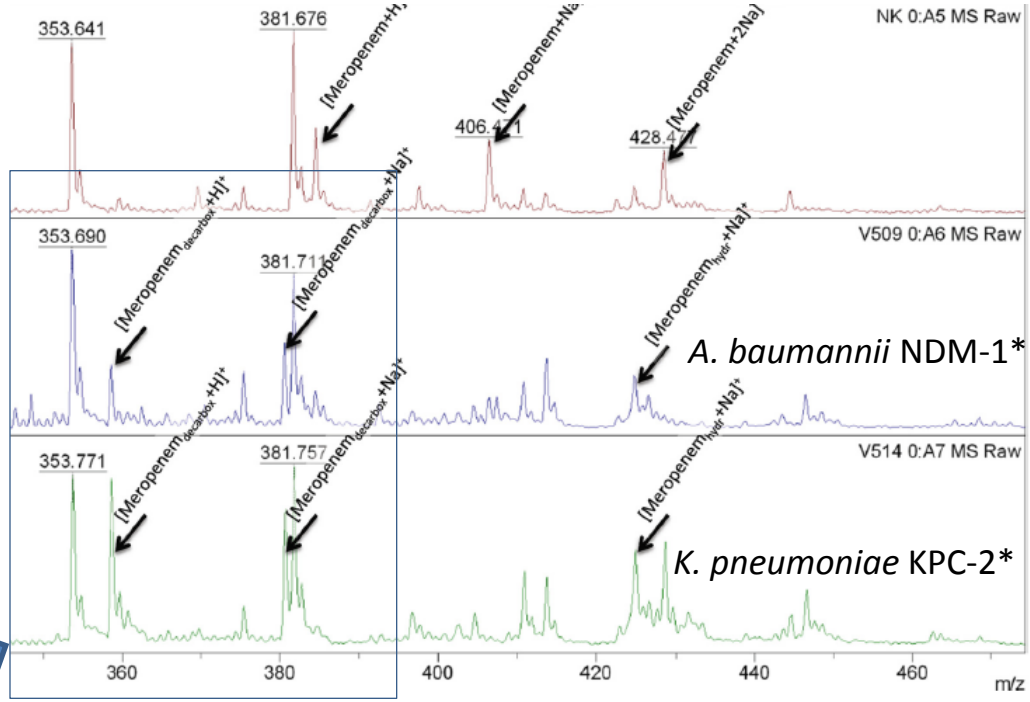
Hydrolyse



Décarboxylation



Ex : carbapénémases



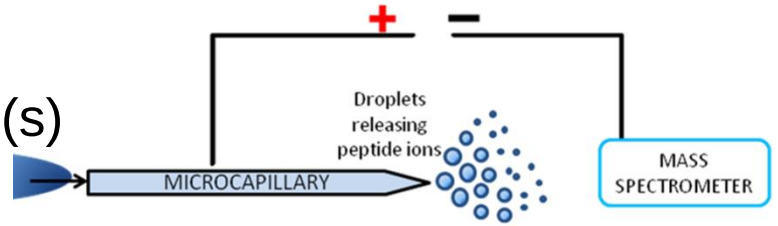
* Après 2 h d'incubation (35° C) avec méropénème

Hrabak et al. Clin Microbiol Rev 2013

Détection des bactéries par PCR – ESI-TOF

Electro**s**pray **i**onization – **T**ime **O**f **F**light

Seuil détection = 1-5 génome(s)
dans prise d'essai



Extraction → PCR → SM ESI-TOF → Analyse des spectres → Résultats

6 h

PCR « à large spectre »
16 rDNA, 23S rDNA, *rpo*C, *val*S, *rpo*B, *tuf*B, *rpl*B, *inf*B, *ssp*E
vanA, *vanB*, bla_{KPC}, *mecA*

PCR/ESI-MS CHARACTERIZATION OF BLOODSTREAM INFECTIONS

Sample no.	Organisms contained in mixture	False negative[a]	Gram stain result
BCB028	*Staphylococcus capitis*		Positive
	Staphylococcus epidermidis		Positive
	Staphylococcus warneri		Positive
BCB089	Coagulase-negative staphylococcus sp.	FN	Positive
	Beta-hemolytic streptococci		Positive
BCB095	*Staphylococcus hominis*		Positive
	Pseudomonas stutzeri		* Negative
BCB110	*Streptococcus dysgalactiae*		Positive
	Streptococcus pneumoniae		Positive
BCB115	*Bacteroides thetaiotaomicron*		* Negative
	Staphylococcus aureus		Positive
BCB124	*Staphylococcus aureus*	FN	Positive
	Streptococcus oralis (viridans group)		Positive
	Streptococcus sanguinis (viridans group)		Positive
BCB135	Coagulase-negative staphylococcus sp.	FN	Positive
	Streptococcus oralis (viridans group)		Positive
	Streptococcus sanguinis (viridans group)		Positive
BCB138	Viridans group streptococci		Positive
	Streptococcus pneumoniae		Positive
BCB144	*Staphylococcus hominis*		Positive
	Staphylococcus carnosus		Positive
	Staphylococcus auricularis		Positive
	Staphylococcus piscifermentans		Positive
	Staphylococcus aureus		Positive
BCB152	*Staphylococcus hominis*		Positive
	Acinetobacter baumannii		* Negative
BCB178	Coagulase-negative staphylococcus sp.		Positive
	Staphylococcus aureus		Positive
BCB200	Coagulase-negative staphylococcus sp.	FN	Positive
	Beta-hemolytic streptococci		Positive
	Enterococcus sp.	FN	Positive

Sample no.	Organisms contained in mixture	False negative[a]	Gram stain result
BCB206	*Klebsiella oxytoca*		* Negative
	Staphylococcus aureus		Positive
BCB217	*Klebsiella oxytoca*		* Negative
	Staphylococcus aureus		Positive
BCB218	*Streptococcus oralis* (viridans group)		Positive
	Streptococcus sanguinis (viridans group)		Positive
BCB222	*Candida albicans*		
	Acinetobacter baumannii		* Negative
BCB228	*Staphylococcus epidermidis*		Positive
	Enterococcus faecalis	FN	Positive
BCB268	*Streptococcus pyogenes*		Positive
	Staphylococcus aureus		Positive
BCB271	Coagulase-negative staphylococcus sp.	FN	Positive
	Streptococcus agalactiae		Positive
BCB284	*Enterobacter hormaechei*		* Negative
	Enterobacter hormaechei (second strain)		* Negative
BCB293	*Acinetobacter lwoffii*		* Negative
	Acinetobacter baumannii		* Negative
BCB296	*Streptococcus cristatus* (viridans group)		Positive
	Streptococcus pneumoniae		Positive
BCB309	Coagulase-negative staphylococcus sp.		Positive
	Enterococcus faecium	FN	Positive
BCB338	*Acinetobacter baumannii*		* Negative
	Acinetobacter calcoaceticus		‡ Negative
BCB347	*Stomatococcus mucilaginosus*		Positive
	Streptococcus salivarius (viridans group)		Positive
BCB365	*Streptococcus sanguinis* (viridans group)		Positive
	Streptococcus pneumoniae		Positive
BCB373	*Staphylococcus epidermidis*		Positive
	Enterococcus faecalis		Positive
BCB374	*Staphylococcus epidermidis*		Positive
	Candida albicans		
BCB440	*Streptococcus oralis* (viridans group)		Positive
	Streptococcus sanguinis (viridans group)		Positive

Kaleta et al. J Clin Microbiol 2011

Spectrométrie et hémocultures

	PCR-ESI-TOF	MALDI-TOF
Seuil de détection	1-5 génome(s)	10^4 UFC
Hémocultures mono-microbiennes Identifications (espèce) correctes	Flacon positif : 95%	Flacon positif : 80% Subculture : 94.9%
Hémocultures pluri-microbiennes Erreurs	24%	76 – 100 %
Détection directe à partir du sang	Sensibilité : 50% Spécificité : 94%	-
Coût	+++++	+++

D'après : Jordana-Lluch et al. PLOS ONE 2013 - Chen et al. J Clin Microbiol 2013
Kaleta et al J Clin Microbiol - 2011 Kaleta et al Clin Chem 2011

Successful identification of pathogens by PCR ESI-TOF-MS in culture negative **peripostheticjoint infection**
Jacovides et al. J Bone Joint Surg 2012

PCR-ESI-TOF MS : a new tool for the diagnosis of infective **endocarditis** from **heart valves**
Wallet et al. Diagn Microbiol Infect Dis 2013

PCR-ESI MS for direct detection of pathogens and antimicrobial resistance from **heart valves** in patients with infective **endocarditis**
Brinkman et al. J Clin Microbiol 2013

PCR and ESI MS for detection of persistent *Enterococcus faecalis* in cerebrospinal fluid following treatment of **postoperative ventriculitis**
Farrell et al. J Clin Microbiol 2013

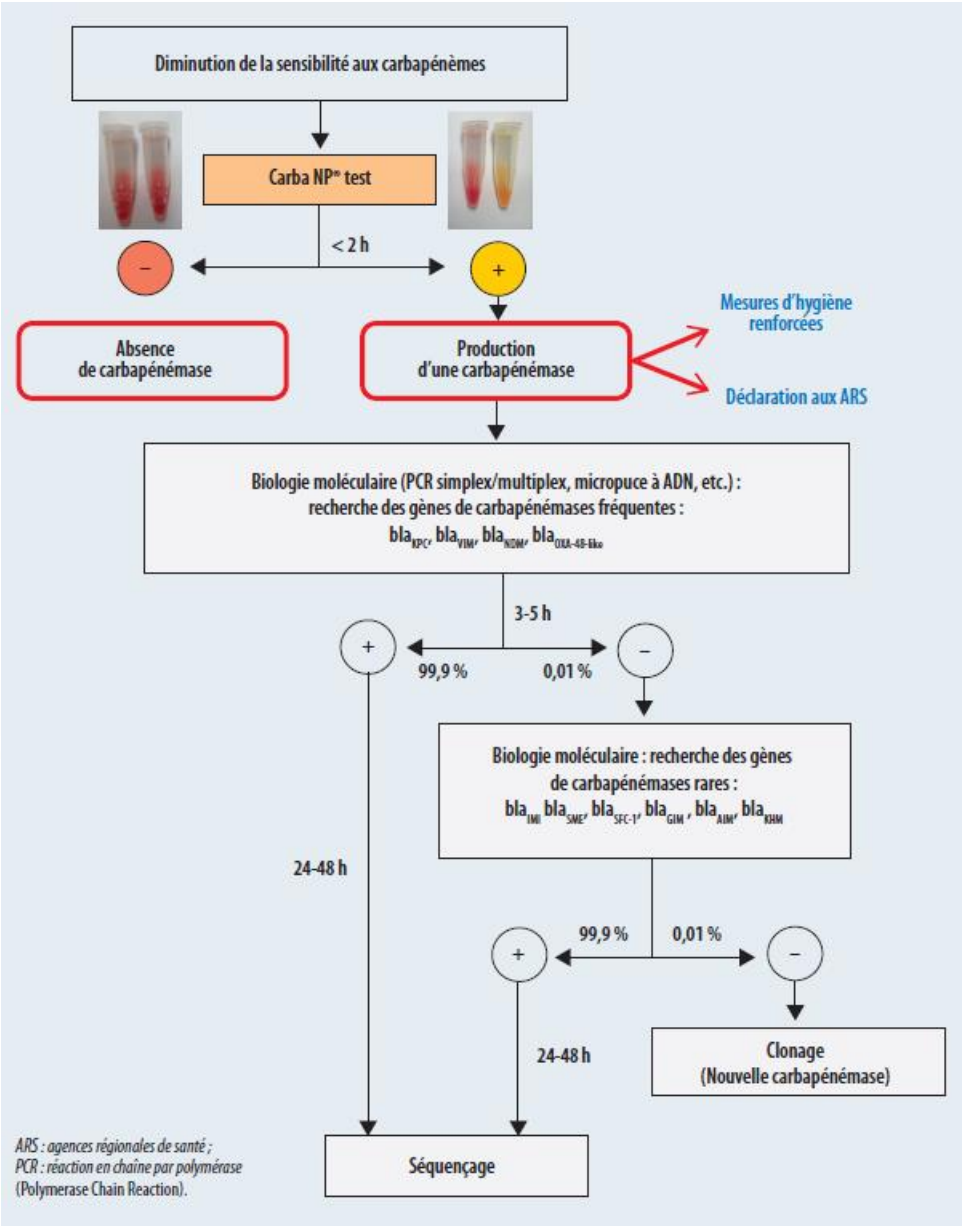
Les nouvelles « anciennes méthodes »

Le Carba NP test

Ambler class, carbapenemase type	Species	β-Lactamase	No.	MIC range, mg/L			Carba NP test result
				IMP	ERT	MER	
Class A							
KPC-type	*Klebsiella pneumoniae*	KPC-2	27	0.5–>32	4–>32	1–>32	+
		KPC-3	3	0.5–8	4–>32	1–8	+
	Klebsiella ozaenae	KPC-3	1	>32	>32	2	+
	Escherichia coli	KPC-2	5	0.5–4	0.5>32	0.5–2	+
	Enterobacter cloacae	KPC-2	7	1–24	1.5–32	0.75–16	+
	Enterobacter aerogenes	KPC-2	1	8	>32	8	+
	Citrobacter freundii	KPC-2	2	8–>32	1.5–>32	1.5–3	+
	Serratia marcescens	KPC-2	2	>32	>32	>32	+
	Salmonella spp.	KPC-2	1	4	1	0.25	+
NMC-A	*E. cloacae*	NMC-A	1	16	>32	16	+
SME-type	*S. marcescens*	SME-1	1	32	4	12	+
		SME-2	1	32	4	12	+
GES-type	*E. cloacae*	GES-5	1	>32	>32	>32	+
IMI-type	*Enterobacter asburiae*	IMI-2	1	>32	>32	>32	+
Class B							
NDM-type	*K. pneumoniae*	NDM-1	16	0.5–>32	2–>32	1–>32	+
		NDM-4	1	>32	>32	>32	+
	E. coli	NDM-1	7	1–16	3–>32	1–16	+
	E. cloacae	NDM-1	1	2	16	2	+
	C. freundii	NDM-1	1	>32	>32	>32	+
	Providencia stuartii	NDM-1	1	12	0.38	1.5	+
	Proteus rettgeri	NDM-1	1	3	0.5	1.5	+
VIM-type	*K. pneumoniae*	VIM-1	15	0.5–>32	0.5–>32	0.38–>32	+
		VIM-19	1	8	16	4	+
	E. coli	VIM-1	2	1.5–3	0.38–1.5	0.5–1	+
		VIM-2	2	2–4	0.5–1.5	0.38–0.5	+
		VIM-19	1	8	16	4	+
	E. cloacae	VIM-1	4	1–>32	0.38 to >32	0.5–>32	+
	S. marcescens	VIM-2	1	>32	>32	>32	+
IMP-type	*K. pneumoniae*	IMP-1	5	0.5–8	2–4	1–8	+
		IMP-8	2	0.5–1	0.5–1	0.5	+
	E. coli	IMP-1	2	0.5	3–4	0.5–1	+
		IMP-8	1	6	8	3	+
	E. cloacae	IMP-1	12	8–>32	>32	2–>32	+
		IMP-8	2	0.75–1.5	0.5–1	0.5–1	+
	S. marcescens	IMP-1	2	8–>32	>32	2–>32	+
		IMP-11	1	8	>32	2	+
Class D							
OXA-48 type	*K. pneumoniae*	OXA-48	15	0.38–>32	0.38–>32	0.38–>32	+
		OXA-181	2	0.5-1	2–4	0.5–1	+
	E. coli	OXA-48	6	0.38–3	0.5–16	0.12–1	+
	E. cloacae	OXA-48	3	0.5–1	0.5–16	0.5–1.5	+
	P. rettgeri	OXA-181	1	8	1	2	+

Nordmann et al Emerging Infectious Diseases September 2012

La réconciliation



Poirel et al. La Lettre de l'Infectiologue juillet-août 2013

En conclusion

•Nouvelles méthodes en bactériologie = nécessaires mais non suffisantes

•Les industriels ne sont pas philanthropes : coût !

•A l'ère des nouvelles méthodes

- L'examen microscopique reste nécessaire (*Ex : hémocultures plurimicrobiennes)*
- La culture des bactéries (antibiogramme …) : aussi !
- La « culture » des biologistes et des technicien(ne)s : aussi !