Apport des nouvelles techniques microbiologiques pour le bon usage des antibiotiques

Jean-Luc Mainardi

Service de Microbiologie, HEGP-Université Paris Descartes

Plan

- Tests rapides par immunochromatographie
- Apport des techniques moléculaires
- Identification bactérienne par spectrométrie de masse
- Détection rapide de la résistance aux antibiotiques: exemple des bêta-lactamases à spectre étendu

Avantages de ces nouvelles techniques

- Faciles à utiliser pour la plupart

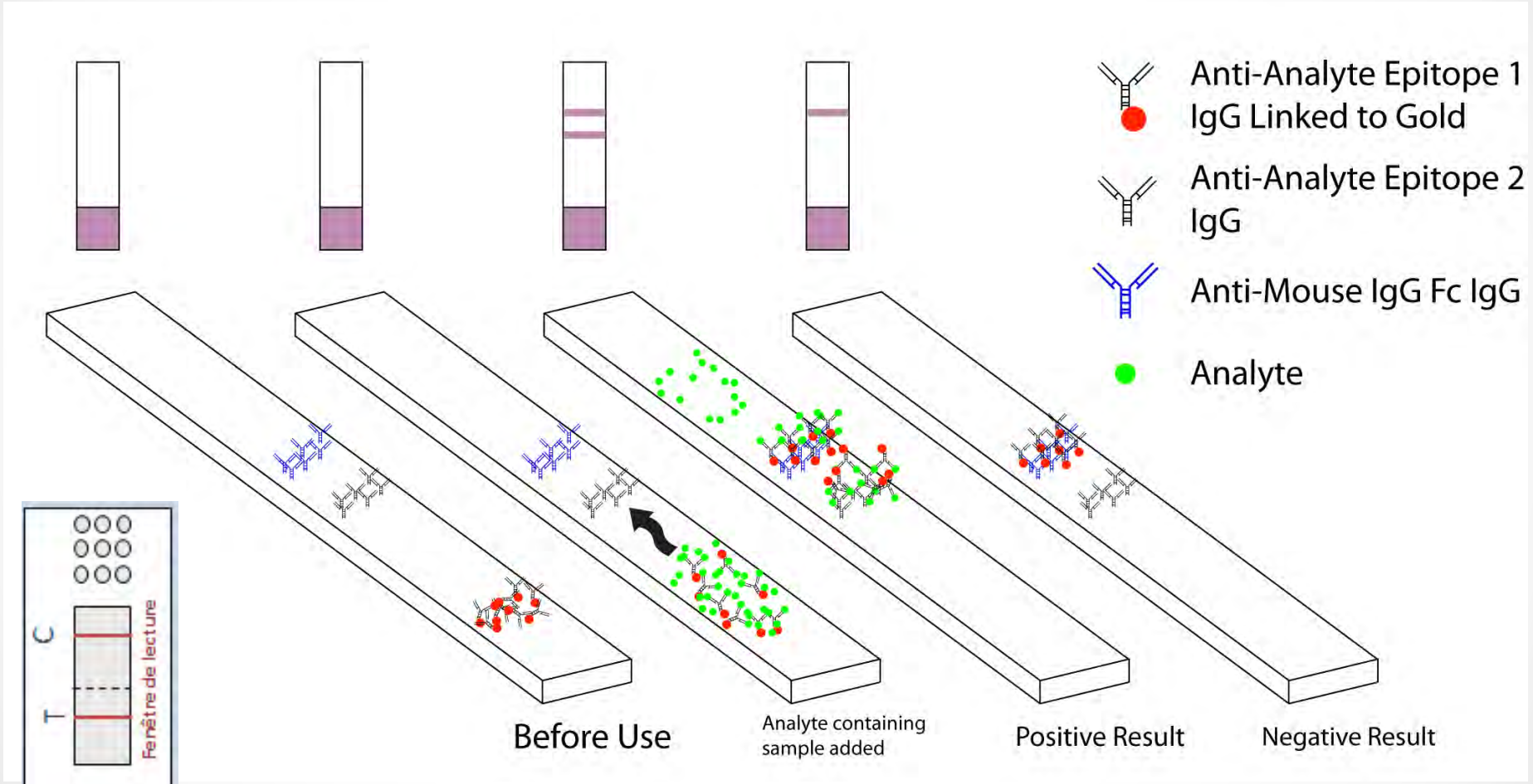
- Résultats rapides (30 min à 3h)
  - Identification des microorganismes
  - Résistance aux antibiotiques
  - Virulence (Toxine)

⇒ Meilleure prise en charge ?
- Antibiothérapie ciblée et adaptée
- Isolement (BMR, IST, …)

⇒ Mais connaître les limites des tests +++

Tests rapides

Immunochromatography tests (ICT)



Immunochromatography tests (ICT):
Exemple du test BinaxNOW® *S. pneumoniae*

- Cible=polyoside C de la paroi

- Sensibilité 0,74 (0,72-0,77)
- Spécificité 0,94 (0,93-0,95)

Boulware *et al.* J Infection 2007

- 1ère lecture à 15 minutes : validation d'un résultat positif
- 2ème lecture à 30 minutes pour valider un résultat négatif

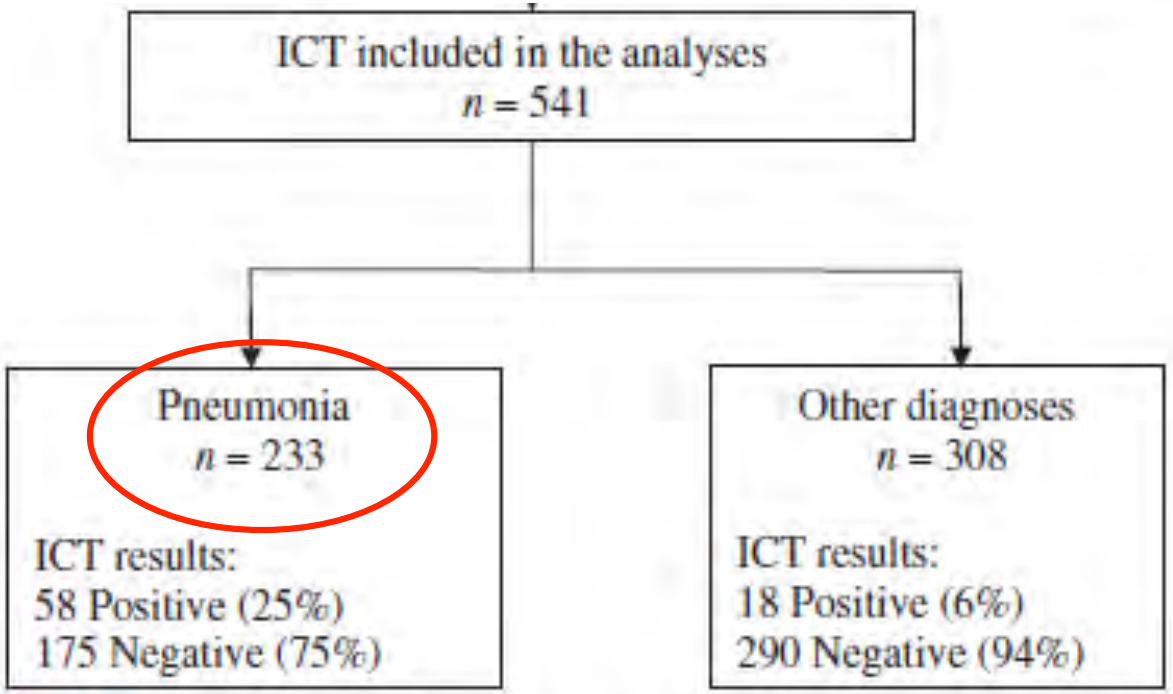
- Faux positifs :
- Porteur sain (enfants +++), autres Streptocoques non-*pneumoniae*
- Excrétion des Ag dans l'urine jusqu'à 6 mois après la pneumonie

Andreo et al, Eur J Clin Microbiol Infect Dis. 2009

Do clinicians consider the results of the BinaxNOW *Streptococcus pneumoniae* urinary antigen test when adapting antibiotic regimens for pneumonia patients?

M. Matta[1,2], S. Kernéis[2,3,4], N. Day[1], M. Lescat[1,2], A. Buu Hoi[1,2], E. Varon[1,5], L. Gutmann[1,2,5,6] and J.-L. Mainardi[1,2,6]

Clin Mic Infect 2010



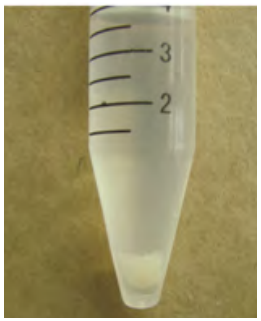
Impact of immunochromatographic test (ICT) results on the antibiotic regimen in the pneumonia group (*n* = 233, percentage in parentheses)

Impact on the antibiotic regimen	Number (%) of patients
ICT positive (*N* = 58)	
Change adapted[a]	22 (9)
Change not adapted	14 (6)
No change	20 (9)
ICT negative (*N* = 175)[b]	
Initiation of therapy	11 (5)
Step-down[c]	8 (3)
Broader-range therapy[d]	6 (3)
Other change	9 (4)
No change	141 (61)

Groupe pneumonie (58 patients):

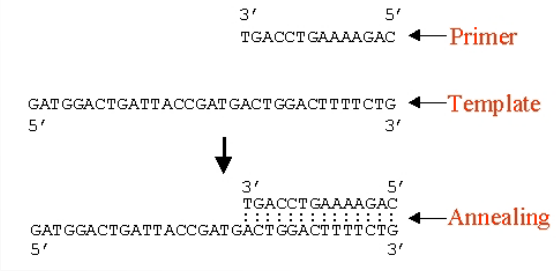
ATB	Avant ICT	Après ICT	p
Amoxicilline	7%	45%	<0,01

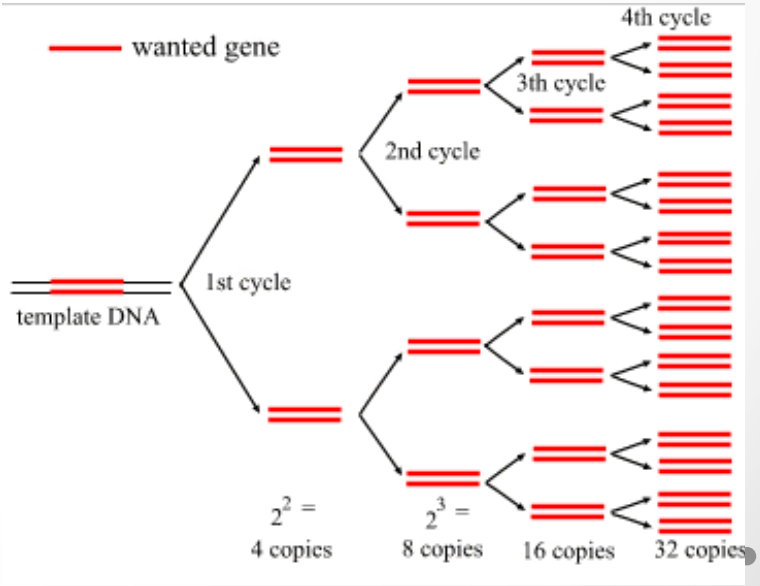
Diagnostic moléculaire : Principe



Extraction ADN

ADN purifié

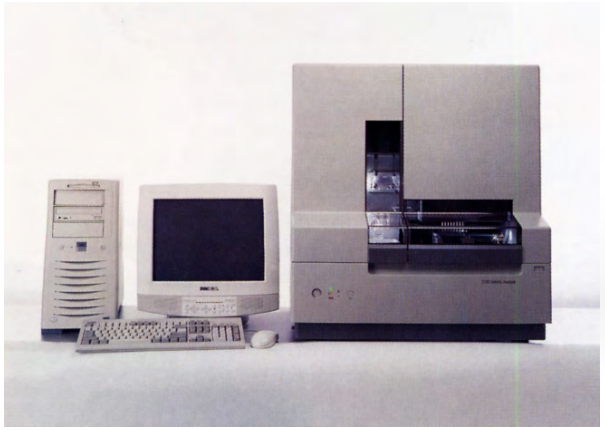


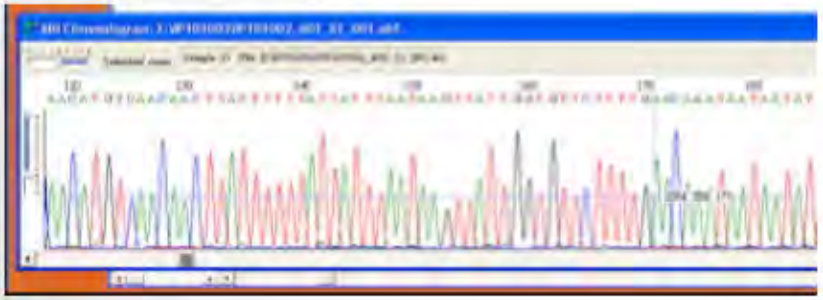


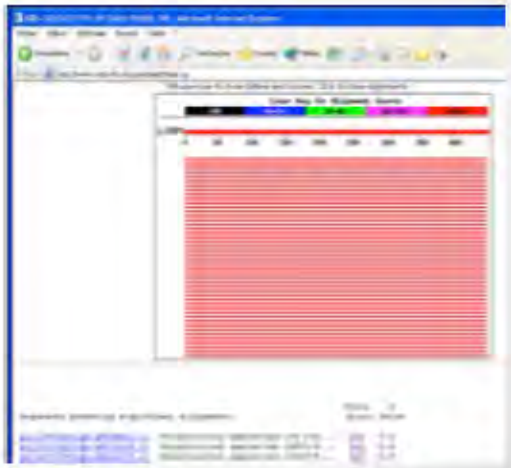
- **PCR=>amplification du gène**

Séquençage

ADN amplifié

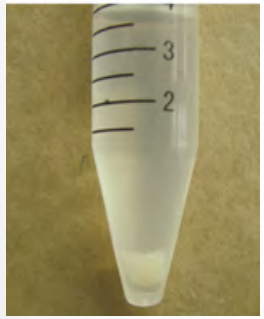




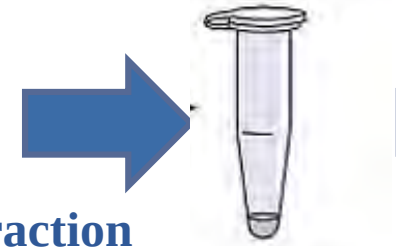


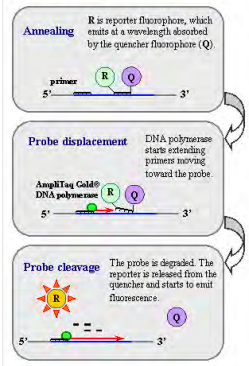
Détermination de la séquence du fragment d'ADN → Comparaison avec des banques de données informatiques → Identification de la bactérie

PCR temps réel

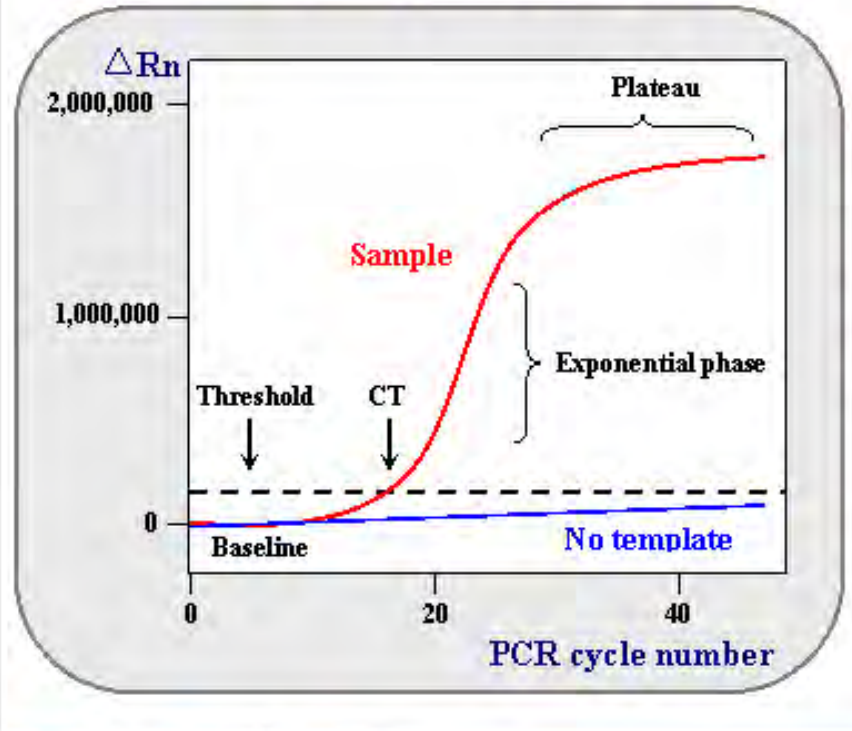


Extraction ADN





PCR + détection



PCR en milieu fermé

- PCR en milieu fermé : extraction ADN+PCR temps réel
  - Facilité d'utilisation (Préparation < 5min)
  - Rapide (45 min-2h)
  - Biologie délocalisée

- Exemple du Xpert Cepheid®

⇒ Identification
⇒ Détection multi-résistance
⇒ Détection facteurs de virulence

Clinical IVD Tests

Healthcare Associated Infections

- Xpert MRSA
- Xpert SA Nasal Complete
- Xpert MRSA/SA SSTI
- Xpert MRSA/SA BC
- Xpert C. difficile
- Xpert C. difficile/Epi
- Xpert vanA

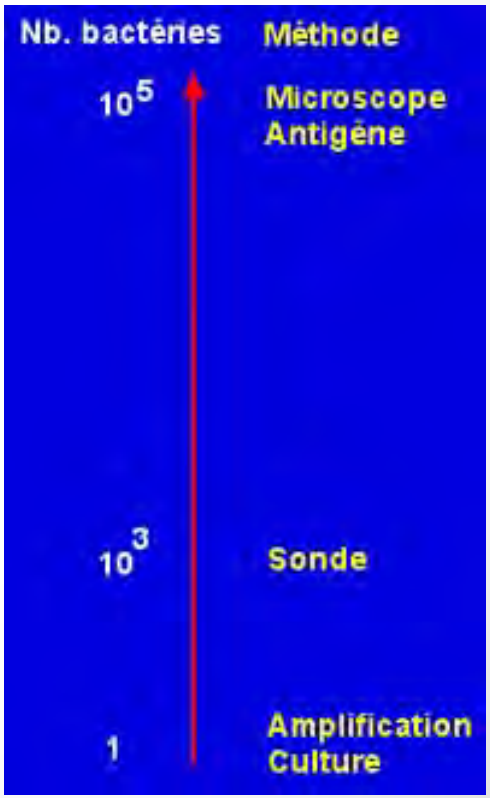
Critical Infectious Diseases

- Xpert MTB/RIF

Sexual Health

- Xpert CT/NG
- Xpert GBS
- Xpert GBS LB

Sensibilité des différentes techniques



www.microbes-edu.org/etudiant/diag1.html

Difficultés

- Choix de la cible +++ (nbre de copies du gène, présence ou non du gène, spécificité…)
- Spécificité des primers=> amplification du mauvais fragment=> faux +
- Mutations dans le gène => les primers ne s'hybrident plus=> faux négatif

Patient rapatrié de Singapour ;
Test colorimétrique positif et Xpert Carba-R négatif

P. aeruginosa

Diapositive I. Podglajen HEGP

Patient rapatrié de Centre Afrique :
Xpert Carba-R « nouvelle version » positif

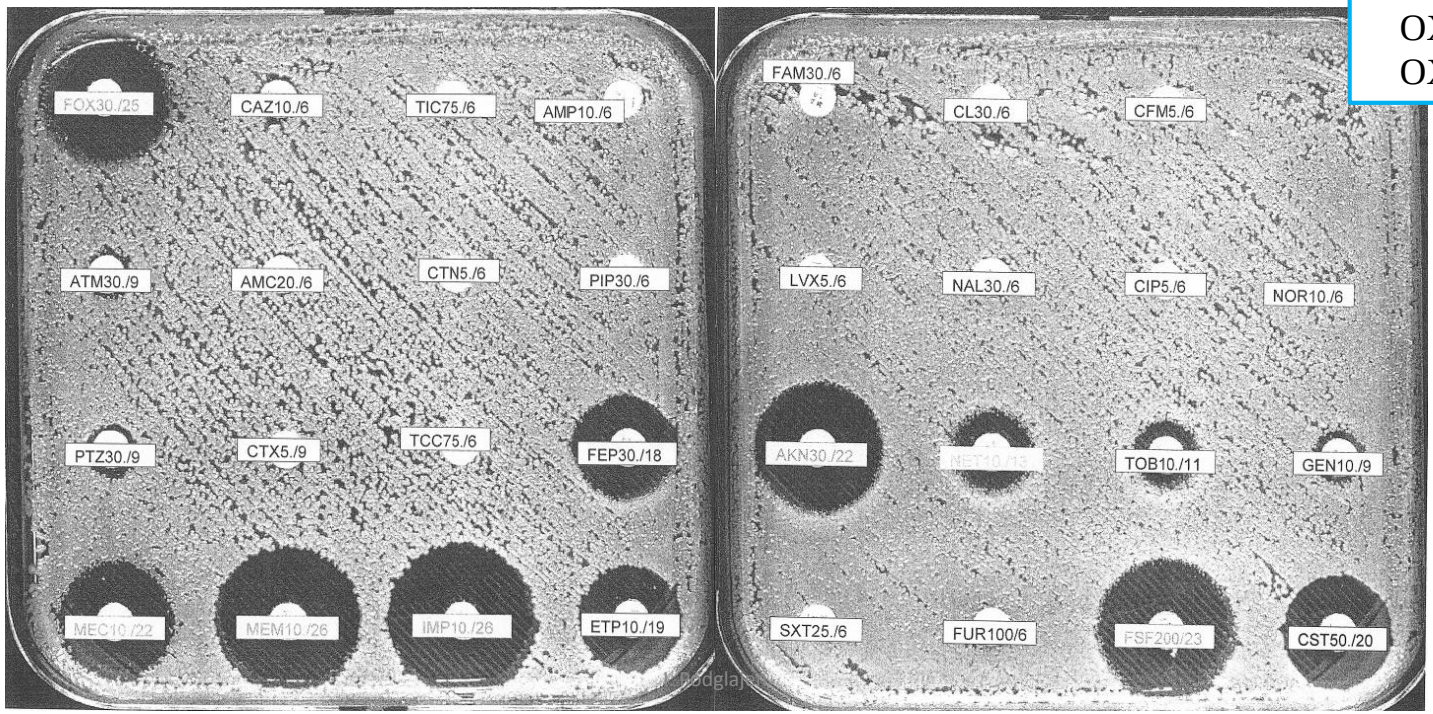
K. Pneumoniae : BLSE + OXA-181

- KPC
- NDM
- VIM
- IMP-1
- OXA-48 T

avec

OXA-181

OXA- 232



Diapositive I. Podglajen HEGP

PCR directe sur le sang

Multiplex PCR Allows Rapid and Accurate Diagnosis of Bloodstream Infections in Newborns and Children with Suspected Sepsis[▽†§]

Barbara Lucignano,[1‡] Stefania Ranno,[1‡] Oliver Liesenfeld,[2] Beatrice Pizzorno,[3] Lorenza Putignani,[1*] Paola Bernaschi,[1*] and Donato Menichella[1]

- 16S-23S rRNA
- 5-18S rRNA

3 réactions PCR temps réel

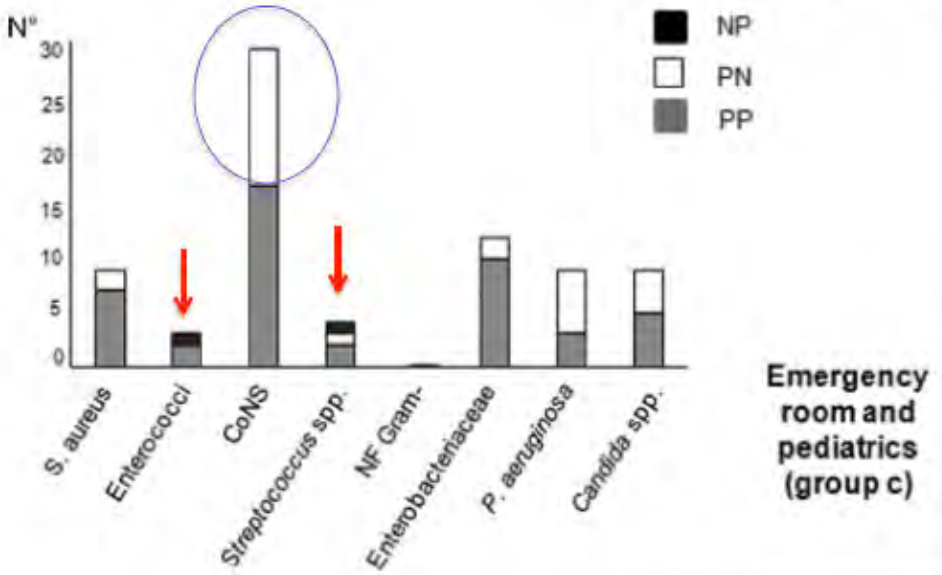
TABLE 1. SeptiFast master list

Gram-negative organisms	Gram-positive organisms	Fungi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella pneumoniae/ K. oxytoca	CoNS[a]	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter cloacae/ E. aerogenes	*Streptococcus* spp.[b]	*Candida glabrata*
Proteus mirabilis	*Enterococcus faecium*	*Candida krusei*
Pseudomonas aeruginosa	*Enterococcus faecalis*	*Aspergillus fumigatus*
Acinetobacter baumannii		
Stenotrophomonas maltophilia		

25 micro-organismes
(90% des espèces isolées d'hémocultures)

803 enfants, 1673 échantillons

Septifast®/Hémoc



Lucignano et al. J Clin Microbiol 2011

Sensitivity 85.0% (95% CI 78.7 to 89.7%)
Specificity 93.5% (95% CI 92.1 to 94.7%)
compared to blood culture.

System	Method	Time to result (hours)	Blood volume (mL)	Microorganism coverage	Resistance and virulence markers	Sensitivity, specificity, and correlation with conventional methods (%)	Comments
SepsiTest Molzym, Bremen, Germany	Broad-range PCR + sequencing	6	1–10[a]	>345 bacteria (Gram positive and Gram negative) and fungi	0	21–87, 85–96, NR	Pros: can be used in other sterile samples; Cons: variable sensitivity and specificity
SeptiFast Roche Molecular System, Basel, Switzerland	Multiple broad-range real-time PCR	3.5–5	1.5	6 Gram positive, 8 Gram negative, 5 fungi	*mecA*[b]	43–95, 60–100, 43–83	Pros: time to result; Cons: variable sensitivity and specificity, no quantification
MagicPlex Seegene, Seoul, Korea	Multiple PCR + multiplex real-time PCR	3–5	1	21 bacteria (Gram positive and Gram negative) at species level (90 at genus level), 6 fungi	*mecA*, *vanA/B*	37–65, 77–92, 73	Pros: fast; Cons: limited number of studies, succession of reaction and device, no quantification
VYOO SIRS-Lab, Jena, Germany	Multiplex PCR + electrophoresis	8	5	14 Gram positive, 18 Gram negative, 7 fungi	0	NR, NR, 70	Pros: highly sensitive; Cons: limited number of studies, several manual steps
PLEX-ID, Abbott Molecular, Carlsbad, CA, USA	Multiplex broad-range PCR/ESI-MS	6	1.25–5[c]	Up to 800 (Gram positive, Gram negative, fungi)	*mecA*, bla_{KPC}, *vanA/B*	50–91[d], 98–99, 79–97	Pros: universal, detection of mixed bacterial populations, semiquantitative; Cons: no interventional studies

Clinical Microbiology and Infection, 2015

Review of Rapid Diagnostic Tests Used by Antimicrobial Stewardship Programs

CID 2014

Karri A. Bauer,[1] Katherine K. Perez,[2,3,4] Graeme N. Forrest,[5] and Debra A. Goff[1]

[1]Department of Pharmacy, The Ohio State University Wexner Medical Center, Columbus; Departments of [2]Pharmacy, and [3]Pathology and Genomic Medicine, Houston Methodist Hospital, and [4]Center for Outcomes Research, Houston Methodist Research Institute, Texas; and [5]Division of Infectious Diseases, Portland Veterans Affairs Medical Center, Oregon

Xpert MRSA/SA—blood culture	Parta et al [14]	*Staphylococcus* spp	212 patients with GPCC (89 in group 1, whose physicians were notified of results by use of Xpert MRSA/SA BC, 123 patients in group 2, with delayed reporting after traditional microbiological studies)	Patients in rapid diagnostic and result notification protocol group who did not have *S. aureus* bacteremia had a significant decrease in treatment for *S. aureus* infection (76% vs 55%; $P < .01$). Patients with MSSA had significantly reduced mean time to initiation of β-lactam therapy (44.6-h reduction).
	Bauer et al [15]	*Staphylococcus* spp	156 patients with *Staphylococcus aureus* (74 pre-rPCR, 82 post-rPCR)	Mean time to switch from empiric to targeted antimicrobial therapy in patients with MSSA was 1.7 d shorter after rPCR ($P = .002$). In the post-rPCR MSSA, and MRSA groups, mean LOS was reduced by 6.2 d ($P = .07$). Mean hospital costs were reduced by $21 387 ($P = .02$) for the post-rPCR group.
	Wong et al [16]	CoNS	53 patients (31 preintervention, 22 intervention)	In postintervention group: antistaphylococcal antibiotics were discontinued 32.0 h sooner from time of rPCR result (median, 57.7 vs 25.7 h; $P = .005$), total antibiotic exposure was decreased by 43.5 h (97.6 vs 54.1 h; $P = .011$), infection-related LOS was decreased by 4.5 d (10 vs 5.5 d; $P = .018$), infection-related costs were decreased by $8338 ($28 973 vs $20 635; $P = .144$). Vancomycin was initiated in 7 (21.9%) patients with CoNS bacteremia.
Xpert MRSA/SA SSTI—PCR assay	Terp et al [17]	MRSA	165 patients with purulent SSTI	No significant reduction in excessive empiric prescription of MRSA-active antibiotics in the absence of an effective stewardship implementation strategy.
PNA FISH	Forrest et al 2006 [18]	CoNS	87 patients (53 with CoNS, 34 with positive blood cultures with GPCC not tested in same time period in control group)	Case patients: significant reduction in median LOS from 6 to 4 d in PNA FISH group ($P < .05$; CI, .95–1.87); decrease in costs of approximately $4000 per patient.
	Schweizer et al [19]	*S. aureus*	814 patients with bacteremia admitted between 2001 and 2007	Of 774 patients who received appropriate antimicrobial therapy, the time to appropriate therapy was shorter among patients who were admitted after the PNA FISH assay was instituted compared to pre–PNA FISH implementation (0.34 d vs 0.56 d; $P = .06$).
	Holtzman et al [20]	*S. aureus*, CoNS	199 patients (100 pre–PNA FISH, 99 post–PNA FISH)	No reduction in LOS or vancomycin use. Study did not include active notification or antimicrobial stewardship intervention.
	Forrest et al [21]	*Enterococcus* spp	224 patients with hospital-acquired enterococcal bacteremia (129 preintervention period, 95 PNA FISH period)	PNA FISH identified *E. faecalis* a median of 3 d earlier and OE 2.3 d earlier compared with standard microbiology ($P < .001$). The OE had significantly shorter time to initiation of effective therapy (1.3 d vs 3.1 d; $P < .001$) and decreased 30-day mortality (26% vs 45%; $P = .04$).
	Ly et al 2008 [22]	*S. aureus*	202 patients with gram-positive cocci in clusters and blood cultures	Significant reduction in mortality in the intervention group compared with the standard management group (7.9% vs 16.8%; $P = .05$); hospitalization charges were less by approximately $20 000 in the intervention group.

Apport pour le bon usage des antibiotiques

- Diminution de la durée de la mise en route d'une antibiothérapie adaptée sur SAMS
- Diminution de la durée d'hospitalisation
- Diminution du coût
-Diminution de la prescription d'antibiotiques en cas de contamination à staphylocoque à coagulase negative

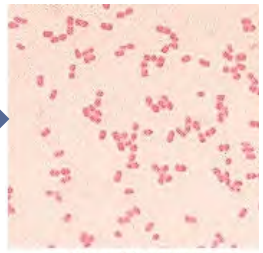
Mais

- Pas de différence si les référents en antibiothérapie ne sont pas partie prenante (messager et éducateur)
- Peu de valeurs si résultats pas donnés en temps rééls
- Peu d'étude de l'impact sur la mortalité

Identification bactérienne par spectrométrie de masse

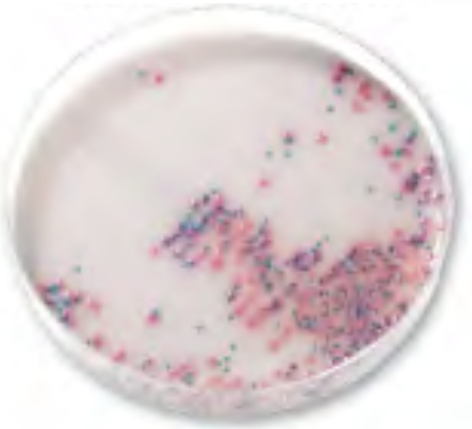
Révolution de l'identification bactérienne : MALDI-TOF MS

Qques heures

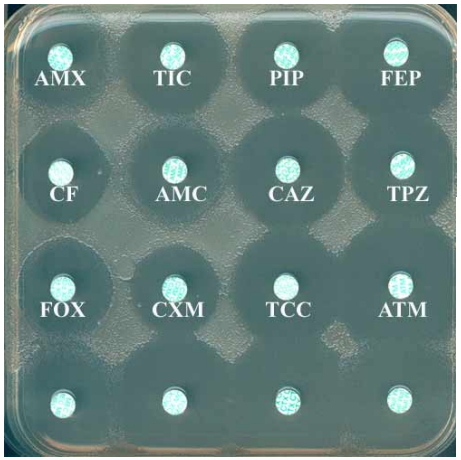


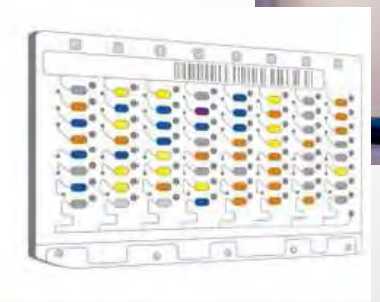
Examen direct

24 h



48 h





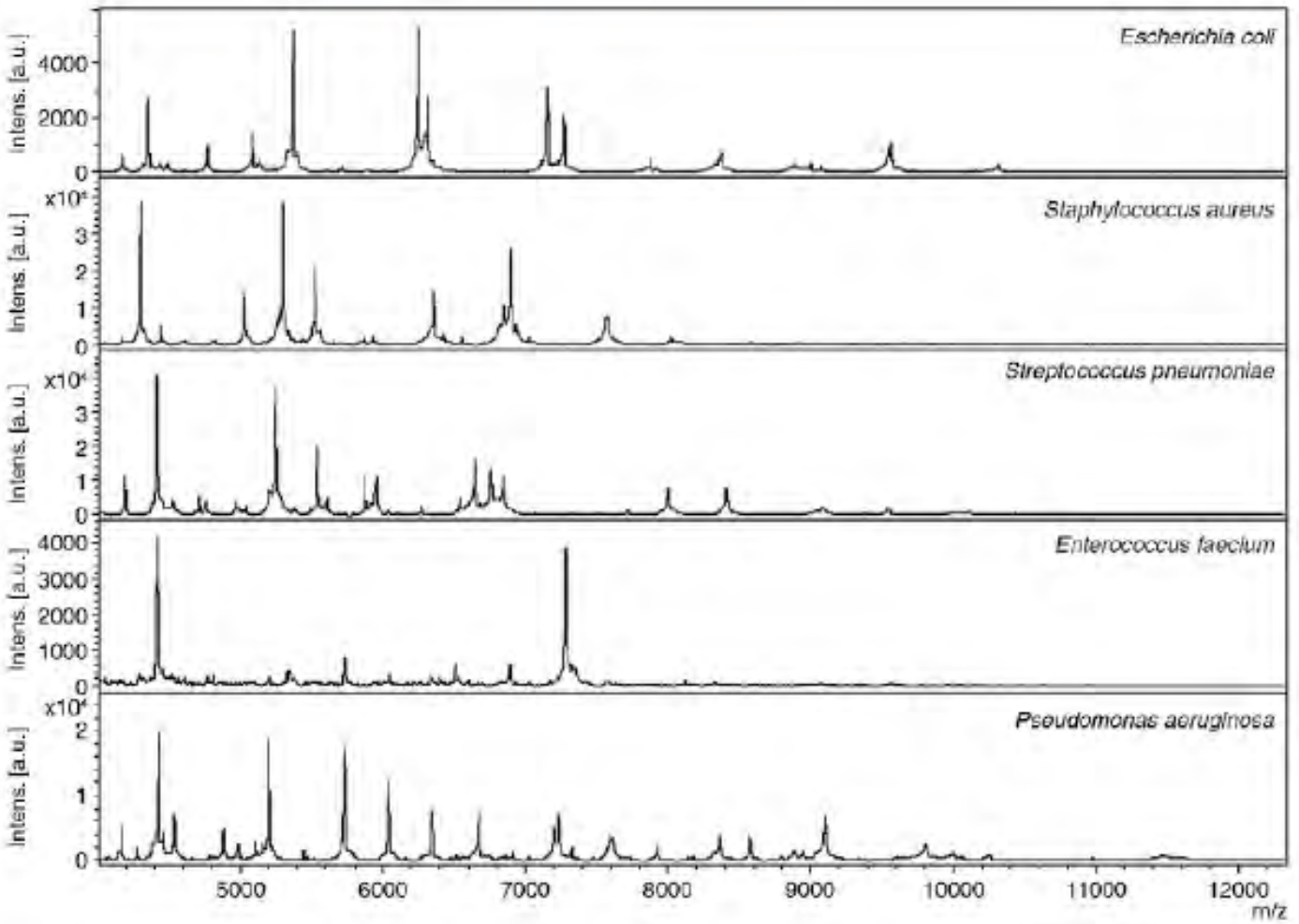
Spectrométrie de masse MALDI-TOF

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

- **Matrice + échantillon => Cible (plaque métallique)**
- **Laser => désorption /ionisation**
- **Analyseur du temps de vol**

MALDI-TOF MS

- Pics spécifiques
  - Genre
  - Espèce
  - (Sous-espèce)
- Reproductibles SI conditions de croissance identiques



Profil de pics (protéines bactériennes) :
- **ratio m/z**
- **intensité relative**

Carbonnelle *et al.* Clin Biochem, 2011

=> Identification à J1 (le jour de la culture)

JOURNAL OF CLINICAL MICROBIOLOGY, May 2010, p. 1549–1554 Vol. 48, No. 5
0095-1137/10/$12.00 doi:10.1128/JCM.01794-09

Performance of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of Bacterial Strains Routinely Isolated in a Clinical Microbiology Laboratory▽

A. Bizzini, C. Durussel, J. Bille, G. Greub,†* and G. Prod'hom†*

Identification à l'espèce : 93,2%
Identification au genre : 5,3%

Review

Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture

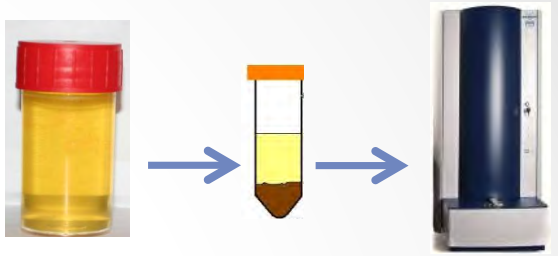
Silpak Biswas, Jean-Marc Rolain *

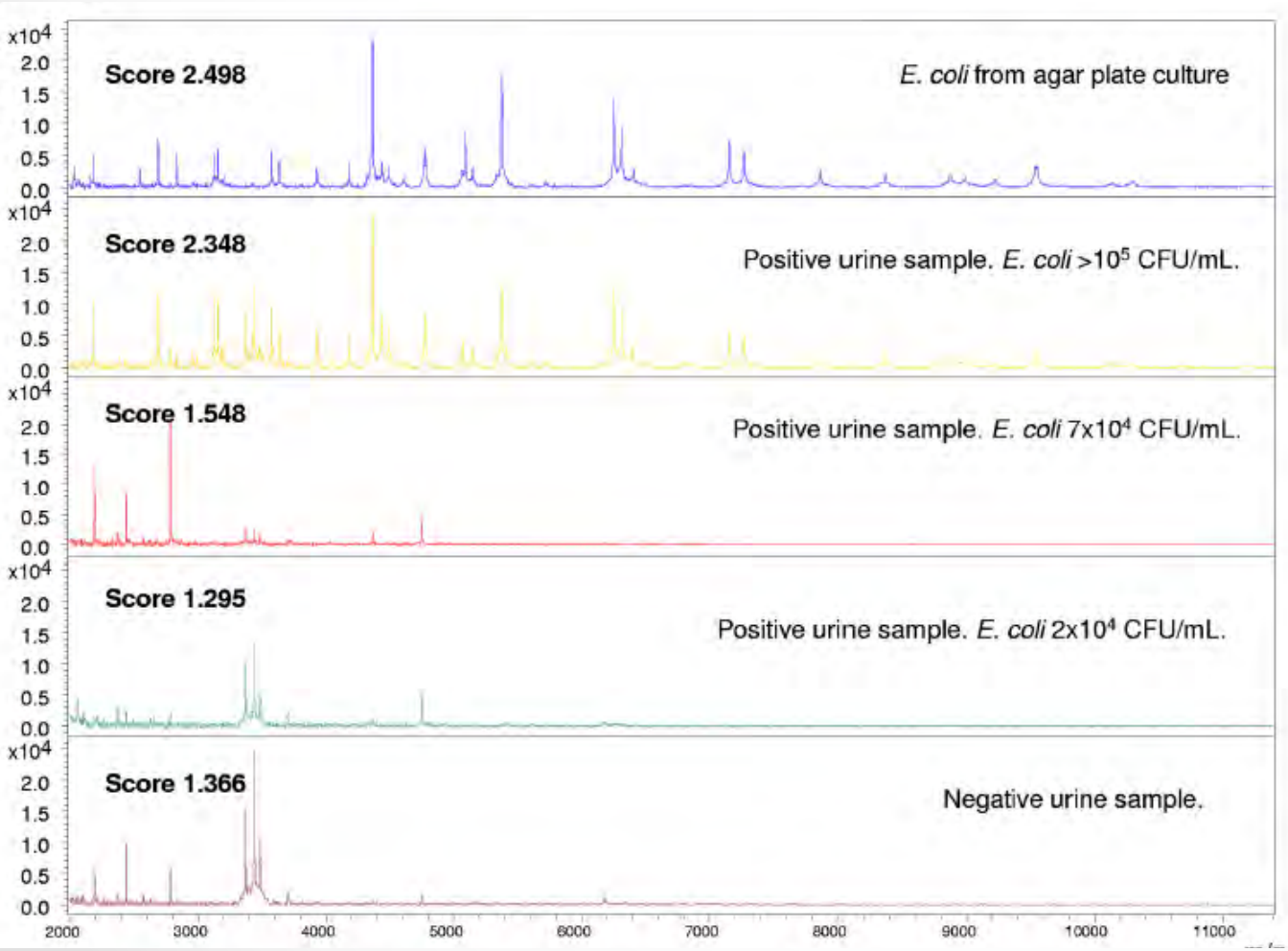
CNRS-IRD, UMR 6236, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), IHU Méditerranée Infection, Faculté de Médecine et de Pharmacie, Aix-Marseille Université, 27, boulevard Jean-Moulin, 13385 Marseille cedex 05, France

=> identification of anaerobes, fastidious bacteria and slow growing bacteria has been improved by the arrival of MALDI-TOF-MS in clinical laboratories

MALDI-TOF MS et urine

⇒ Identification directement sur le culot de centrifugation





If >10^5 UFC/mL of a single species
=> 92% identification

Ferreira et al, JCM 2010

MALDI-TOF et urines

- **Identification à J0 !**
- **Excellentes identifications sur BGN+++** Ferreira et al, JCM 2010
- **Limites :**

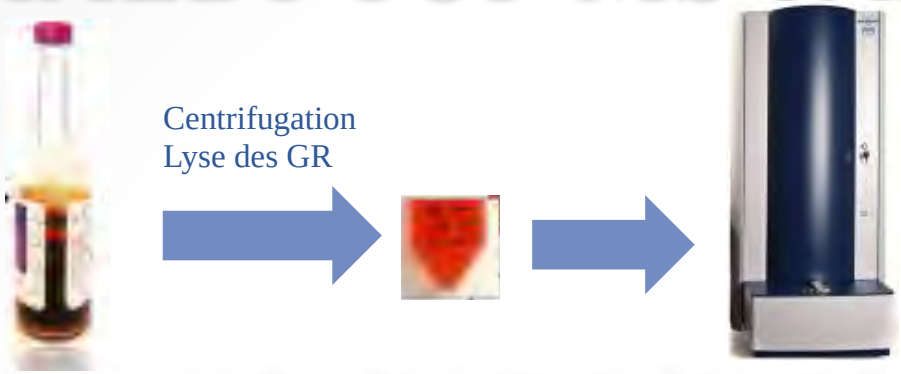
- Seuil de détection : ≈10^5 UFC/mL

=> pb si < 10^5 UFC/mL

- Prélèvements plurimicrobiens

Clin. Microbiol. Rev. 2014 vol. 27 no. 4 783-822

MALDI-TOF MS et hémoculture



Direct Bacterial Identification in Positive Blood Cultures by Use of Two Commercial Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems

Jonathan H. K. Chen,[a] Pak-Leung Ho,[a,c] Grace S. W. Kwan,[a] Kevin K. K. She,[a] Gilman K. H. Siu,[a] Vincent C. C. Cheng,[b,c] Kwok-Yung Yuen,[a,c] Wing-Cheong Yam[a,c]

JCM 2013

- Identification espèce >90% des cas
- En <3h (extraction)
- HC plurimicrobienne: identification de l'espèce majoritaire+++

OPEN ACCESS Freely available online PLoS one 2012

Direct Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry Improves Appropriateness of Antibiotic Treatment of Bacteremia

Anne L. M. Vlek*, Marc J. M. Bonten, C. H. Edwin Boel

		Direct MALDI-TOF MS (n = 89)	Standard care (n = 164)	p-value
Median identification time in hours (IQR)		16.4 (10.3–42.9)	45.2 (35.5–55.9)	<0.001
Episodes with ID time	<10 h	23.6%	0.6%	<0.001
	10–35 h	44.9%	23.2%	0.001
	35–50 h	16.9%	36.6%	0.001
	>50 h	14.6%	39.6%	<0.001
Median time until first switch in antibiotic therapy in hours (IQR)		17.5 (9.8–38.8)	24.0 (9.5–47.0)	0.30
Number of switches	0	55.0%	58.8%	0.59
	1	41.6%	34.8%	0.28
	2	3.4%	6.7%	0.27
1st switch same day BC[#] positive		40.0%	29.2%	0.20
1st switch 1 day after BC[#] positive		30.0%	38.5%	0.47
1st switch >1 day after BC[#] positive		30.0%	32.3%	0.92

Effect of direct MALDI-TOF MS on proportion of appropriate treatment.

	Direct MALDI-TOF MS	Standard care
% (n) of episodes with appropriate therapy<24 h after positive BC[a]	75.3% (67)*	64.0% (105)*
% (n) of episodes with inappropriate therapy<24 h after positive BC[a]	4.5% (4)*	14.6% (24)*
% (n) of episodes without antibiotic therapy<24 h after positive BC[a]	20.2% (18) (6.7% (6) other interventions[b], 13.5% (12) contaminated BC)	21.4% (35) (4.3% (7) other interventions[b], 11.0% (18) contaminated BC, 6.1% (10) not applicable[c])

[a]blood culture, [b]removal of intravenous catheters, [c]palliative care or patient died shortly after blood culture was positive.
*p value 0.01.
doi:10.1371/journal.pone.0032589.t004

Vlek *et al*. Plos 2012

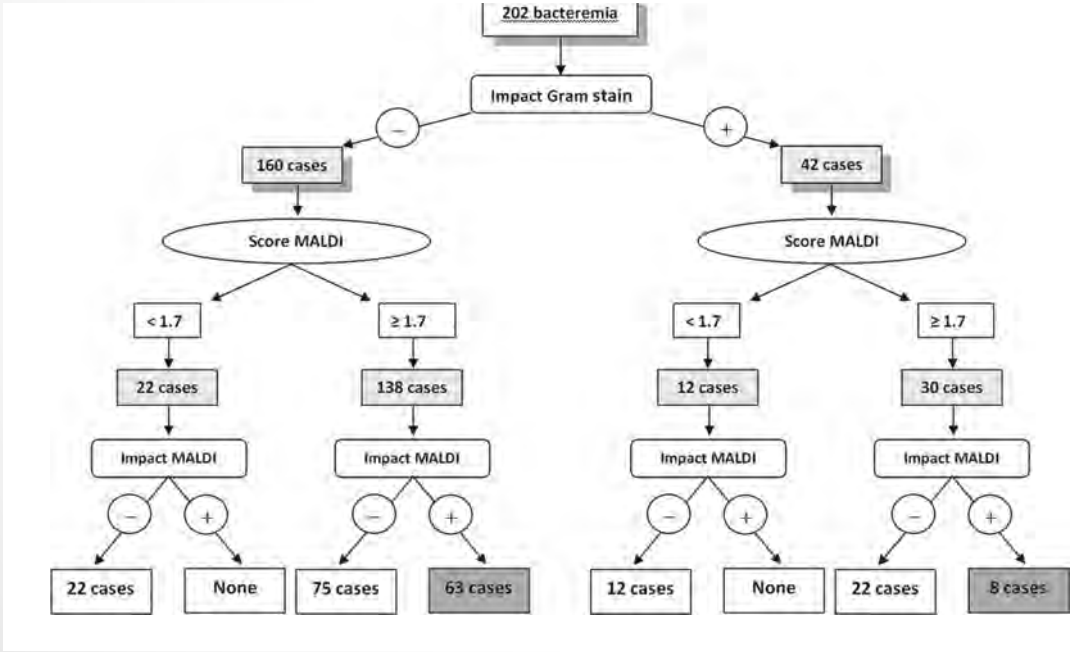
- Gain sur le rapidité de l'identification bactérienne
- Pas d'évaluation de la mortalité
- Durée d'hospitalisation pas étudiée
- Pas d'évaluation du devenir des patients

Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospective Observational Study

Olivier Clerc,[1] Guy Prod'hom,[2] Christelle Vogne,[2] Alain Bizzini,[2] Thierry Calandra,[1] and Gilbert Greub[1,2]

[1]Infectious Diseases Service and [2]Institute of Microbiology, Lausanne University Hospital Center and University of Lausanne, Switzerland

CID 2013



Impact of Sequential Gram Stain and MALDI-TOF Reporting

Impact of the Sequential Reporting	N = 202
Gram stain	42 (20.8)
Streamlining	16 (7.9)
Spectrum broadening	16 (7.9)
Introduction of empirical antibiotic therapy	10 (5.0)
MALDI-TOF MS	71 (35.1)
Streamlining	22 (10.9)
Spectrum broadening	31 (15.3)
Introduction of focused empirical antibiotic therapy	18 (8.9)

-Pas de groupe controle

- Pas de devenir des patients.

Impact of Rapid Organism Identification via Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Combined With Antimicrobial Stewardship Team Intervention in Adult Patients With Bacteremia and Candidemia

Angela M. Huang,[1,2] Duane Newton,[5,6] Anjly Kunapuli,[1,2] Tejal N. Gandhi,[3] Laraine L. Washer,[3,4] Jacqueline Isip,[1,2] Curtis D. Collins,[1,2] and Jerod L. Nagel[1,2]

CID, 2013

Clinical and Treatment-Related Outcomes

Outcome	Total		
	Preintervention (n = 256)	Intervention (n = 245)	*P* Value
Clinical outcomes			
30-day all-cause mortality	52 (20.3)	31 (12.7)	.021
Time to microbiological clearance, d	3.3 ± 4.8	3.3 ± 5.7	.928
Length of hospitalization, d[a]	14.2 ± 20.6	11.4 ± 12.9	.066
Length of ICU stay, d[a]	14.9 ± 24.2	8.3 ± 9.0	.014
Recurrence of same BSI	15 (5.9)	5 (2.0)	.038
30-day readmission with same BSI	9 (3.5)	4 (1.6)	.262
Treatment-related outcomes			
Time to effective therapy, h	30.1 ± 67.7	20.4 ± 20.7	.021
Time to optimal therapy, h	90.3 ± 75.4	47.3 ± 121.5	<.001

Impact of Antimicrobial Stewardship Intervention on Coagulase-Negative *Staphylococcus* Blood Cultures in Conjunction with Rapid Diagnostic Testing

JCM 2014

MALDI_TOF

Jerod L. Nagel,[a] Angela M. Huang,[a,c] Anjly Kunapuli,[a] Tejal N. Gandhi,[b] Laraine L. Washer,[b,c] Jessica Lassiter,[a] Twisha Patel,[a] Duane W. Newton[a]

Departments of Pharmacy Services and Clinical Sciences, University of Michigan Health System and College of Pharmacy, Ann Arbor, Michigan, USA[a]; Department of Internal Medicine, Division of Infectious Diseases,[b] Department of Infection Control and Epidemiology,[c] and Clinical Microbiology Laboratories and Department of Pathology,[d] University of Michigan Health System and Medical School, Ann Arbor, Michigan, USA; Froedtert Hospital and The Medical College of Wisconsin, Milwaukee, W[illegible]

Outcomes for patients with CoNS bacteremia

Characteristic	Preintervention group (n = 46)	AST intervention group (n = 32)	P value
Time to organism identification[a] (h)	83.4 ± 29.5	57.0 ± 32.3	<0.001
Time to effective therapy[a] (h)	37.7 ± 40.1	23.0 ± 10.7	0.064
Time to optimal therapy[a] (h)	58.7 ± 56.4	34.4 ± 29.9	0.030
No. (%) of patients with 30-day all-cause mortality	10 (21.7)	1 (3.1)	0.023
Length of hospitalization[a,b] (days)	14 ± 22	15 ± 14	0.954
Length of ICU stay[a,b] (days)	28 ± 33	11 ± 11	0.188
No. (%) of patients with recurrent bacteremia	6 (13.0)	0 (0.0)	0.076
No. (%) of patients with 30-day readmission with CoNS bacteremia	2 (4.3)	0 (0.0)	0.51

Antimicrobial use and outcomes for patients with CoNS contamination

Characteristic	Preintervention group (n = 83)	AST intervention group (n = 85)	P value
Duration of CoNS antibiotic therapy[a] (days)	4.4 ± 4.2	3.0 ± 1.6	0.015
Vancomycin utilization[a] (g)	4.8 ± 6.3	3.0 ± 3.9	0.038
Daptomycin utilization[a] (g)	2.88	0	0.243
No. of vancomycin serum assays obtained[a]	2.0 ± 2.2	0.9 ± 1.4	<0.001
No. (%) of patients with 30-day all-cause mortality	9 (10.8)	10 (11.8)	>0.99
Length of hospitalization[a] (days)	14.6 ± 22.9	15.8 ± 18.6	0.7
No. (%) of patients with recurrent bacteremia	3 (3.6)	2 (2.4)	0.68
No. (%) of patients with 30-day readmission with CoNS bacteremia	2 (2.4)	1 (1.2)	0.618
No. (%) of patients *Clostridium difficile* colitis	7 (8.4)	4 (4.7)	0.367

- Spectrométrie de masse pas réalisée directement sur les hémocultures

Integrating Rapid Pathogen Identification and Antimicrobial Stewardship Significantly Decreases Hospital Costs

Arch Pathol Lab Med—Vol 137, September 2013

Katherine K. Perez, PharmD; Randall J. Olsen, MD, PhD; William L. Musick, PharmD; Patricia L. Cernoch, BS; James R. Davis, PhD; Geoffrey A. Land, PhD; Leif E. Peterson, PhD; James M. Musser, MD, PhD

MALDI-TOF

Bactériémie à Gram négatif

Table 2. Length of Stay and Cost Outcomes in Survivors[a]

Outcome	Preintervention Cohort (n = 100)	Intervention Cohort (n = 101)	*P*
Hospital length of stay	11.9 ± 9.3	9.3 ± 7.6	.01
Hospital length of stay after BSI onset	9.9 ± 7.1	8.1 ± 6.4	.01
ICU length of stay	7.3 ± 8.5	6.3 ± 8.7	.05
ICU length of stay after BSI onset	6.1 ± 6	4.9 ± 6.7	.09
Total hospital costs	$45 709 ± $61 806	$26 162 ± $28 996	.009
MS DRG weight	2.7 ± 2.4	±1.9	54

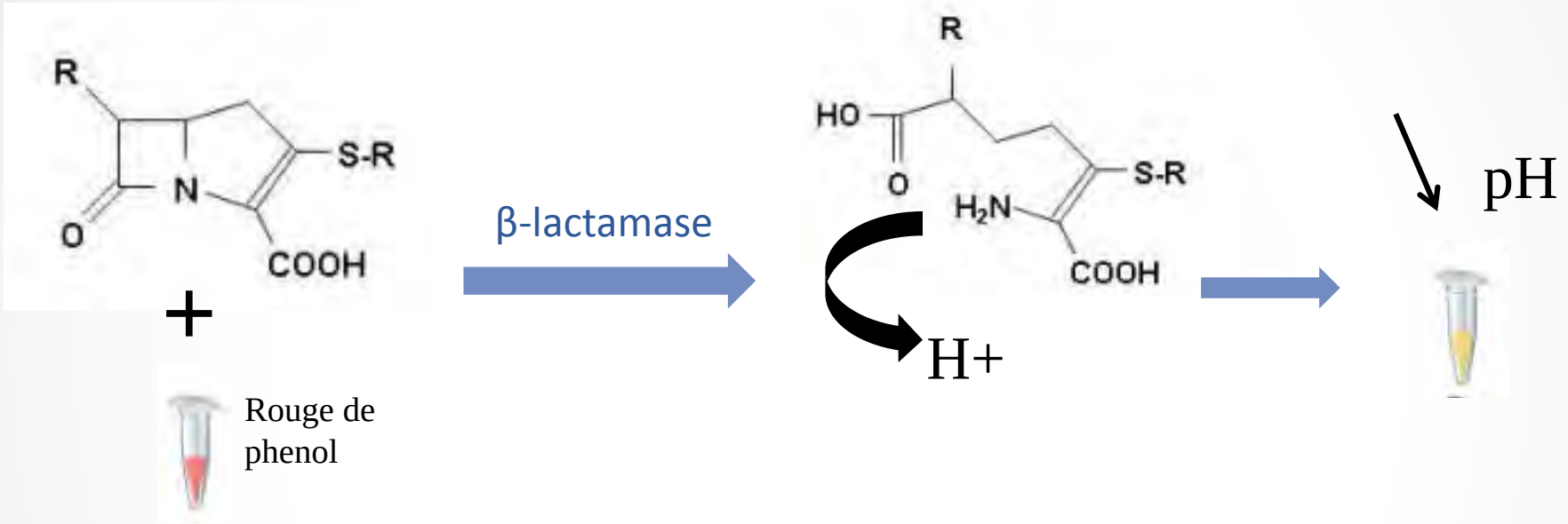
Table 3. Independent Factors Associated with Length of Stay[a]

Factor	Univariate			Multivariate[b]		
	HR	95% CI	*P*	HR	95% CI	*P*
Active antibiotic therapy at 48 h	2.24	1.23–4.08	.009	2.90	1.15–7.33	.02
MALDI-TOF MS antimicrobial stewardship intervention	1.40	1.06–1.85	.02	1.38	1.01–1.88	.04
APACHE II	0.96	0.93–0.99	.003	0.97	0.93–0.999	.05
Preinfection LOS	0.87	0.83–0.91	<.001	0.86	0.83–0.91	<.001
Preexisting lung disease	0.62	0.40–0.94	.02	0.54	0.35–0.84	.006

Détection rapide de la résistance aux antibiotiques: les tests chromogéniques

Tests chromogéniques: BGN et BLSE/Carbapénémase

- Principe :



- Détection rapide (< 2 heures) de la résistance aux *β*-lactamines
  - **BLSE :** βLacta® test (Biorad), ESBL NDP test
  - **Carbapénémases** : Rapidec® Carba NP test (Biomerieux)

⇒**Adaptation de l'antibiothérapie +++**

Tests chromogéniques:
à partir de cultures de BGN

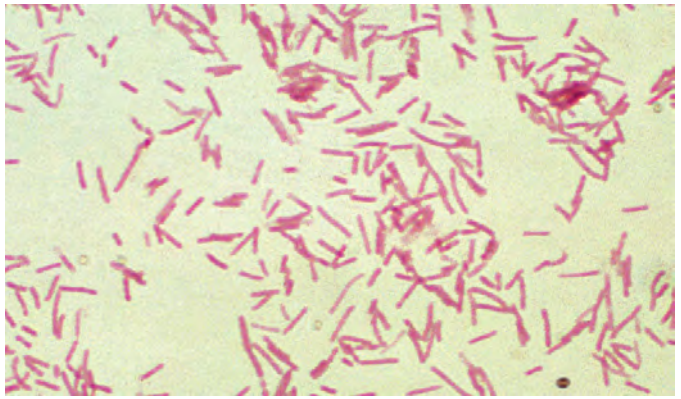
Test	Cible	Sensibilité	Spécificité	Référence
βLacta test (Biorad)	R aux C3G (BLSE+ ++, HCASE, Carbapénémases)	87,7%	99,6%	Renvoisé et al, JCM 2014
ESBL NDP test	BLSE	92,6%	100%	Nordmann et al, JCM 2012
Rapidec Carba NP test (Biomérieux)	Carbépénémases	96%	96%	Poirel et al, JCM 2015

Détection des carbapénèmases

		Carba NP test on positive blood culture					
		Positive		Negative			
Carbapenemase types	Tested isolates (*n*)	*n*	%	*n*	%	Sensitivity (%)	Specificity (%)
KPC	50	50	100	0	0	100	100
IMP	27	27	100	0	0	100	100
VIM	37	37	100	0	0	100	100
NDM	33	33	100	0	0	100	100
OXA-48-like	46	42	91.3	4	8.7	91.3	100
No carbapenemase	74	0	0	74	100	–	–
Total results						97.9	100

Dortet et al, Clin Micr Infection 2014

Tests chromogéniques:
directement sur ECBU positif

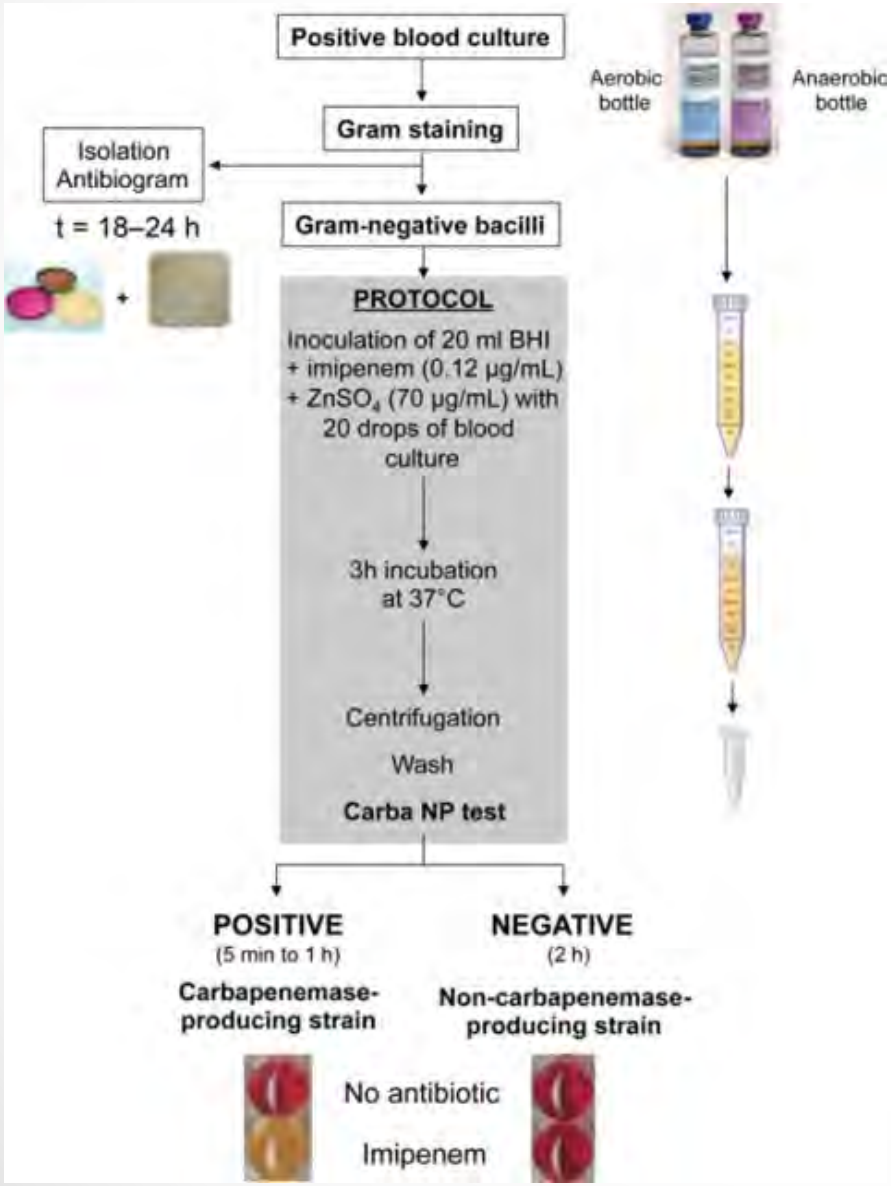


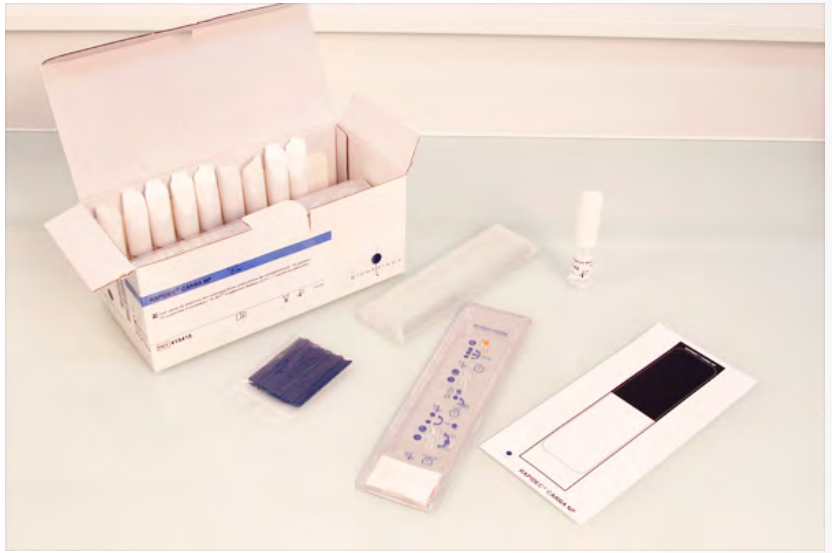
- 200 ECBU avec BGN à l'examen direct (culture =>10^4 à 10^5 UFC/mL)
  - o Sensibilité : 94%
  - o Spécificité : 100%
  - Comparé à culture + antibiogramme
- Résultats en <30 min

Gallah et al, JCM 2014

Tests chromogéniques:

directement sur flacon d'hémoculture positif





Dortet et al, Clin Micr Infection 2014

The β-Lacta test for rapid detection of *Enterobacteriaceae* resistant to third-generat cephalosporins from positive blood cultures using briefly incubated solid medium cultures

Fabrice Compain[1,2*], Hayat Bensekhri[1], Hidayeth Rostane[1], Jean-Luc Mainardi[1,2], Marie Lavollay[1,2]

- Identification par spectrométrie de masse après 3 heures d'incubation d'une hémoculture positive
- 108 hémocultures positives à entérobactéries étudiées
- Détection de la résistance aux C3G: sensibilité de 84.8%, spécificité de 100%, une valeur prédictive positive de 100% et négative de 94.%
- Pour détecter les BLSE: sensibilité de 100% et une spécificité de 96.3%, valeur prédictive positive de 90.3% et négative de 100%
- Impact sur la prise en charge des patients ?

J Med Microbiol. 2015

Antimicrobial Stewardship Combined With Maldi-tof and β-Lacta Test Performed on Gram-Negative Bacilli Blood Culture is Effective for Sparing the use of Carbapenems

A. Aubry, A. Fournier, H. Pereira, S. Katsahian, H. Bensekhri, J-L. Mainardi, M-P. Fernandez-Gerlinger[1.] **ICAAC 2015**

● Prospective observational study (168 days- 24 weeks):
-All patients with GNB positive blood cultures
-Analyzed was performed from Monday to Friday morning (120 days).

● MT and BLT were performed simultaneously on 3h incubated solid medium subcultures (Compain *et al*., J Med Microbiol. 2015)

● Three strategies were compared:
(A) empiric antibiotic therapy initiated by the physician in charge of the patient knowing GNB bacteremia without MT and BLT results : preintervention group
(B) empiric antibiotic therapy recommended by AMS without MT and BLT results
(C) AMS advice with MT and BLT results.

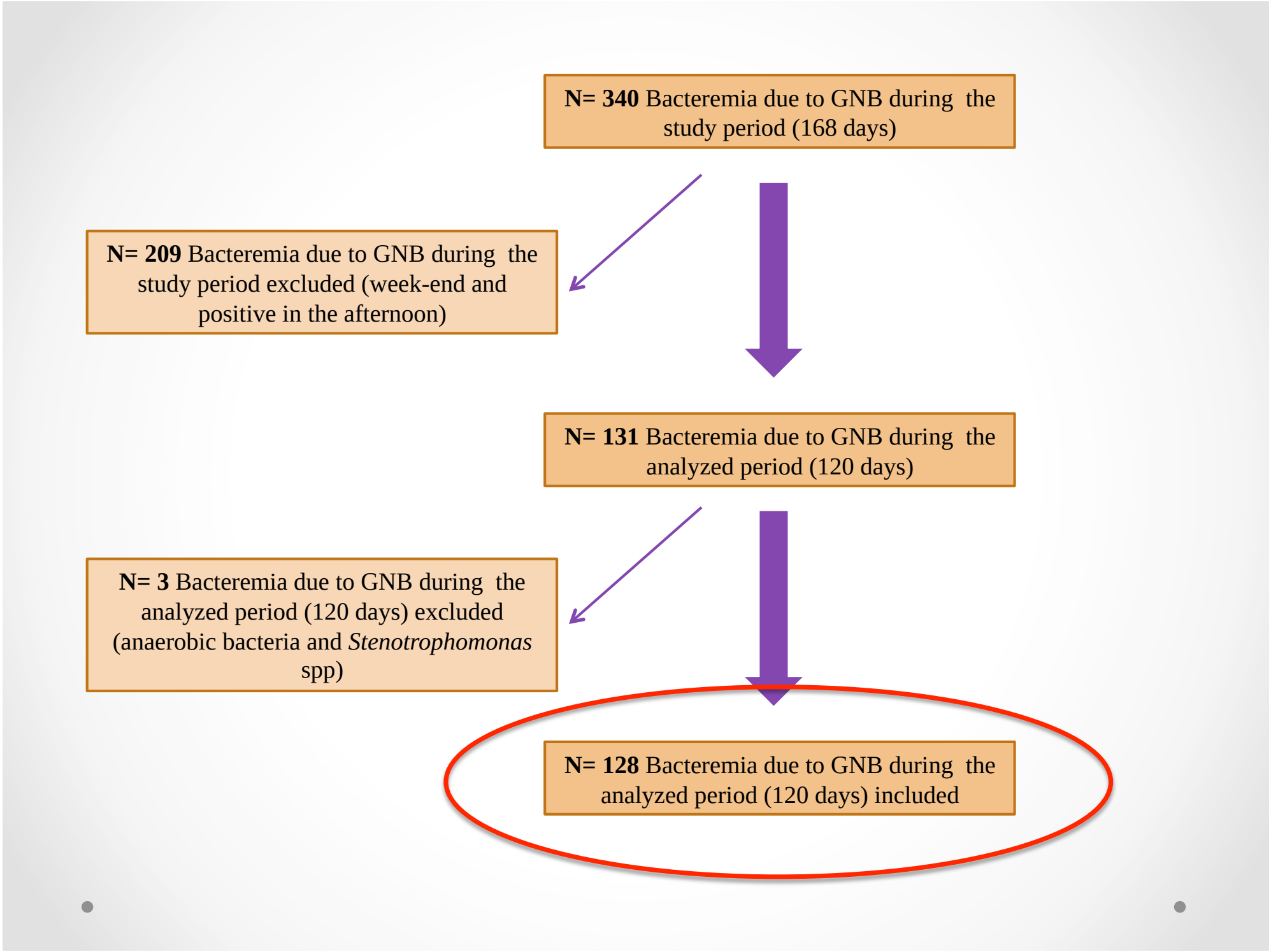

N= 340 Bacteremia due to GNB during the study period (168 days)
N= 209 Bacteremia due to GNB during the study period excluded (week-end and positive in the afternoon)
N= 131 Bacteremia due to GNB during the analyzed period (120 days)
N= 3 Bacteremia due to GNB during the analyzed period (120 days) excluded (anaerobic bacteria and Stenotrophomonas spp)
N= 128 Bacteremia due to GNB during the analyzed period (120 days) included

Table 2: Organism distribution

Pathogens	n (%)
Enterobacteriaceae	100 (84)
Escherichia coli	62 (50)
Proteus mirabilis	1
Klebsiella pneumoniae	17 (14)
Klebsiella oxytoca	3 (2)
Enterobacter cloacae	8 (7)
Enterobacter aerogenes	2 (1)
Enterobacter sakasakii	1
Serratia marcescens	3 (2)
Pantoea agglomerans	1
Morganella morganii	1
Citrobacter freundii	1
Nonfermentative	17 (12)
Pseudomonas aeruginosa	15 (12)
Acinetobacter pittii	1
Chryseobacterium spp.	1
Other aerobic	2 (1)
Salmonella spp.	2 (1)
Polymicrobial	9 (7)

Table 1: Characteristics of the 128 cases of Gram-negative bacteremia analyzed

Female n (%)	60 (47)
Age mean years	69
Nosocomial* n (%)	77 (60)
Previous ESBL carriage n (%)	14 (10)
Recent antimicrobial therapy n (%)	69 (54)
Severe sepsis/septic shock n (%)	29 (23)
ICU admission n (%)	19 (15%)
Polymicrobial bacteremia n (%)	9 (7)
Source of infection	
Urinary tract n (%)	50 (39)
Catheter infection n (%)	45 (35)
- Catheter	22
- PAC	17
- lymphangitis	6
Digestive tract n (%)	11 (9)
- Peritonitis	3
- Translocation	8
- Angiocholitis	9
- Cholecystitis	1
- Liver abscess	1
- Other	1
Pneumonia n (%)	6 (5)
Others n (%)	4 (3)
- OSI	2
- Unknown	2

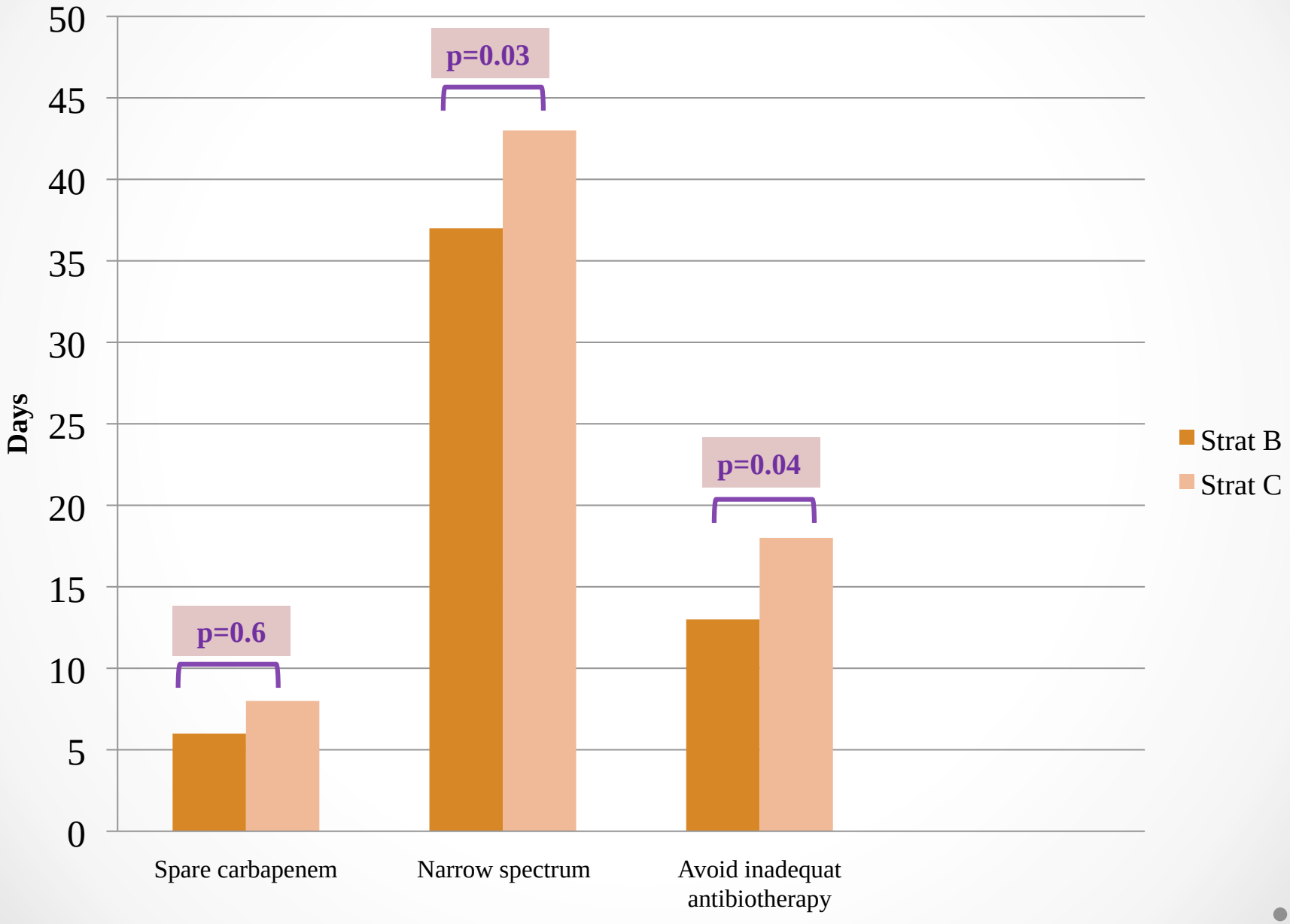
*Nosocomial: hospital acquired and healthcare associated; ICU: intensive care unit; PAC : Port-a-cath implantable central venous access device ;OSI operative site infection.

ESBL carriage and bacteremia

	All Bacteremia N (%)	ESBL bacteremia N (%)	Non ESBL bacteremia N (%)
ESBL known carriage N (%)	14 (10)	6 (43)	8 (57)
ESBL not known carriage N (%)	114 (90)	8 (7)	106 (93)

p = 0.001
OR = 9.65

Antimicrobial Stewardship with and without Maldi-tof and β-Lacta Test: Strat. B compared to Strat. C



Agreement Strat.B and β-Lacta Test or Maldi-tof MS estimated through Cohen's kappa

	B-Lacta Test	Maldi-tof MS	
Spare carbapenem Strat. B	K = -0,0465 p = 2 .10^{-13}	K = -0,0483 p = 0	not agreement
Avoid inadequate antibiotherapy Strat.B	K = 0,85 p = 0	K = 0,82 p = 0	agreement
Narrow spectrum Strat. B	K = 0,95 p = 0	K = 0,885 p = 0	

Conclusion:

1) Rapid identification with Maldi-tof MS and EBLSE β-Lacta Test associated with antimicrobial stewardship (Strat C) seems to be more efficient than AMS alone to narrow spectrum and avoid inadequate antibiotherapy.

En conclusion…

- Multiples tests (ICT, PCR, MS, tests chromogéniques, …)
- De plus en plus d'informations… => faire le tri
- Toujours garder un œil critique:
  - En général bonne spécificité des tests,sSensibilité des examens ?
  - Interprétation ? (clearance de l'ADN, antigènuries…)

⇒ Apport pour l'antibiothérapie si dialogue clinico-biologique et/ou intervention des équipes mobiles d'infectiologie+++

⇒ Nécéssite d'avoir des évaluations clinico/économiques