SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale della Romagna

Metodologie molecolari per la identificazione dei batteri multiresistenti

Vittorio Sambri, MD, PhD
Unit of Microbiology
the Greater Romagna Area Hub Laboratory
DIMES – University of Bologna
Pievesestina, Cesena (Italy)
Vittorio.sambri@auslromagna.it – vittorio.sambri@unibo.it

U.O. MICROBIOLOGIA
Pievesestina

A “syndromic” approach

- Classic Microbiology
  - Culture based
  - Phenotypic ID
  - Phenotypic AST
  - Immunocomplex ID
  - Immune response detection
  - **Time is an issue**
  - First come First got

- Molecular Microbiology
  - Specific gene(s) ID
  - Growth is not necessary (***sometime!***)
  - Multiple techniques
  - Very low LOD
  - **Fast and quick**
  - More germs “who is the bad guy”

Antibiogramma Molecolare

- Determina la presenza di geni di resistenza
  - Non serve il batterio vitale
    - Bassi LOD (dipendente da numero di target e da reazione)
    - Non influenzato da on going therapy
    - TAT molto rapido
  - Determina ciò che “noi vogliamo, non quello che c’è”
    - Singolo target
    - Pannelli (quanto completi)
    - Sensibilità della reazione
    - Mutazioni

Point-of-care multiplex PCR promises short turnaround times for microbial testing in hospital-acquired pneumonia – an observational pilot study in critical ill patients

Table 1 Pathogens detected by the mPCR device (according to the manufacturer)

Gram-positive	Gram-negative	Fungal pathogens
Streptoccocus pneumoniae	*Pseudomonas aeruginosa*	*Pneumocystis jirovecii*
Staphylococcus aureus	*Acinetobacter baumanii*	
	Legionella pneumophilia	
	Moraxella catarrhalis	
	Stenotrophomonas maltophilia	
	Enterobacter species	
	Escherichia coli	
	Klebsiella pneumoniae	
	Klebsiella oxytoca	
	Proteus species	
	Serratia marcescens	
	Morganella morganii	
	Haemophilus influenzae	
	Chlamydophila pneumoniae	

Table 2 Resistance markers detected by the mPCR device (according to the manufacturer)

Resistance marker	Resistance against	Relevant pathogen group[a]
mecA	Oxacillin, Methicillin	*Staphylococcus* species
msrA	Macrolides	*Staphylococcus* species
mefA/E	Macrolides	*Streptococcus* species
ermA	Macrolides / Lincosamides	*Staphylococcus* species
ermB	Macrolides / Lincosamides	*Streptococcus* species
ermC	Macrolide / Lincosamides	*Staphylococcus* species
tem	Penicillins, 3rd Gen. Cephalosporins	Enterobacteriaceae, Non-fermenting bacteria, *Haemophilus influenzae*
shv	Penicillins, 3rd Gen. Cephalosporin	Enterobacteriaceae, Non-fermenting bacteria
ctx-M	Penicillins, 3rd Gen. Cephalosporins	Enterobacteriaceae, Non-fermenting bacteria
dha	3rd Gen. Cephalosporins	Enterobacteriaceae
ebc	3rd Gen. Cephalosporins	Enterobacteriaceae
oxa51 like	Carbapenems	*Acinetobacter baumanii*
kpc	Carbapenems	Enterobacteriaceae, Non-fermenting bacteria
int1	Multidrug resistance	Enterobacteriaceae, Non-fermenting bacteria
sul1	Multidrug resistance, Sulfonamides	Enterobacteriaceae, Non-fermenting bacteria
gyrA83	Fluoroquinolones	*Escherichia coli*, *Pseudomonas aeruginosa*
gyrA87	Fluoroquinolones	*Escherichia coli*, *Pseudomonas aeruginosa*
parC	Fluoroquinolones	*Pseudomonas aeruginosa*

Kunze *et al. Annals of Clinical Microbiology and Antimicrobials* (2015) 14:33
DOI 10.1186/s12941-015-0091-3

Emerging methodologies for pathogen identification in positive blood culture testing

Grégory Dubourg & Didier Raoult

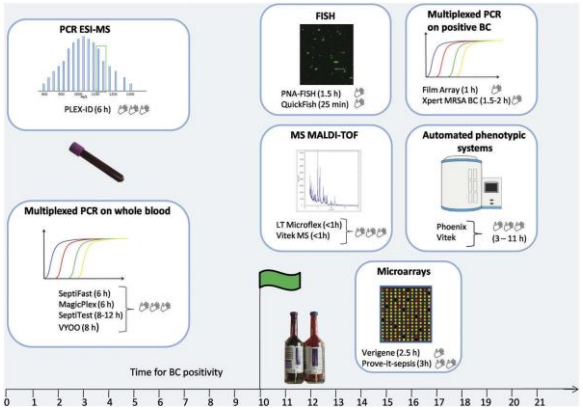
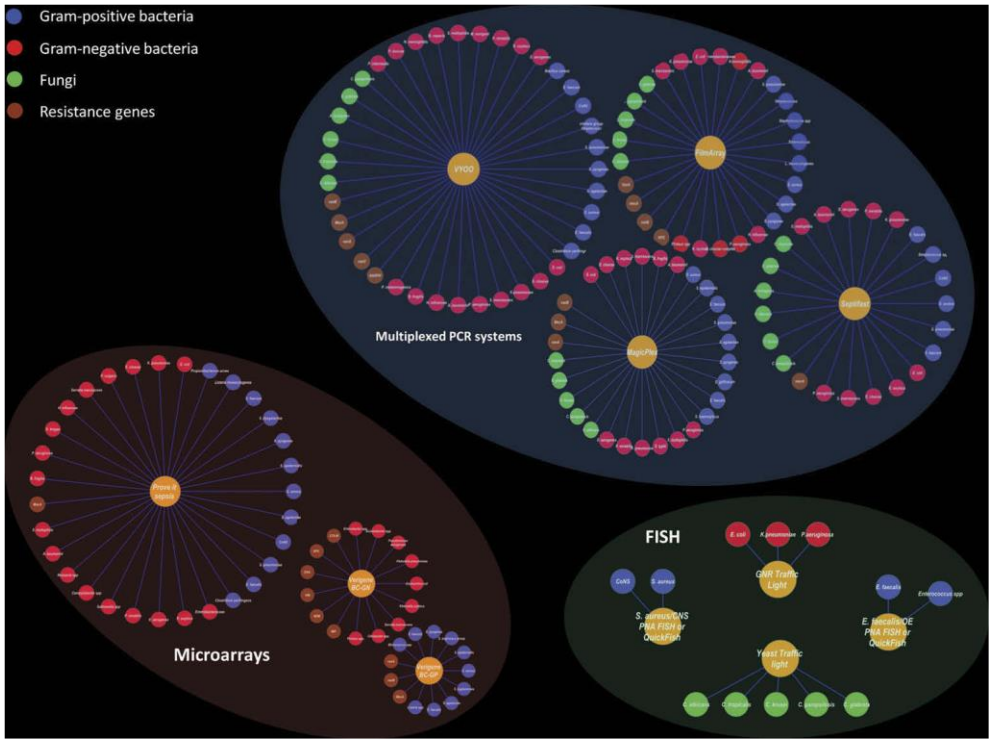


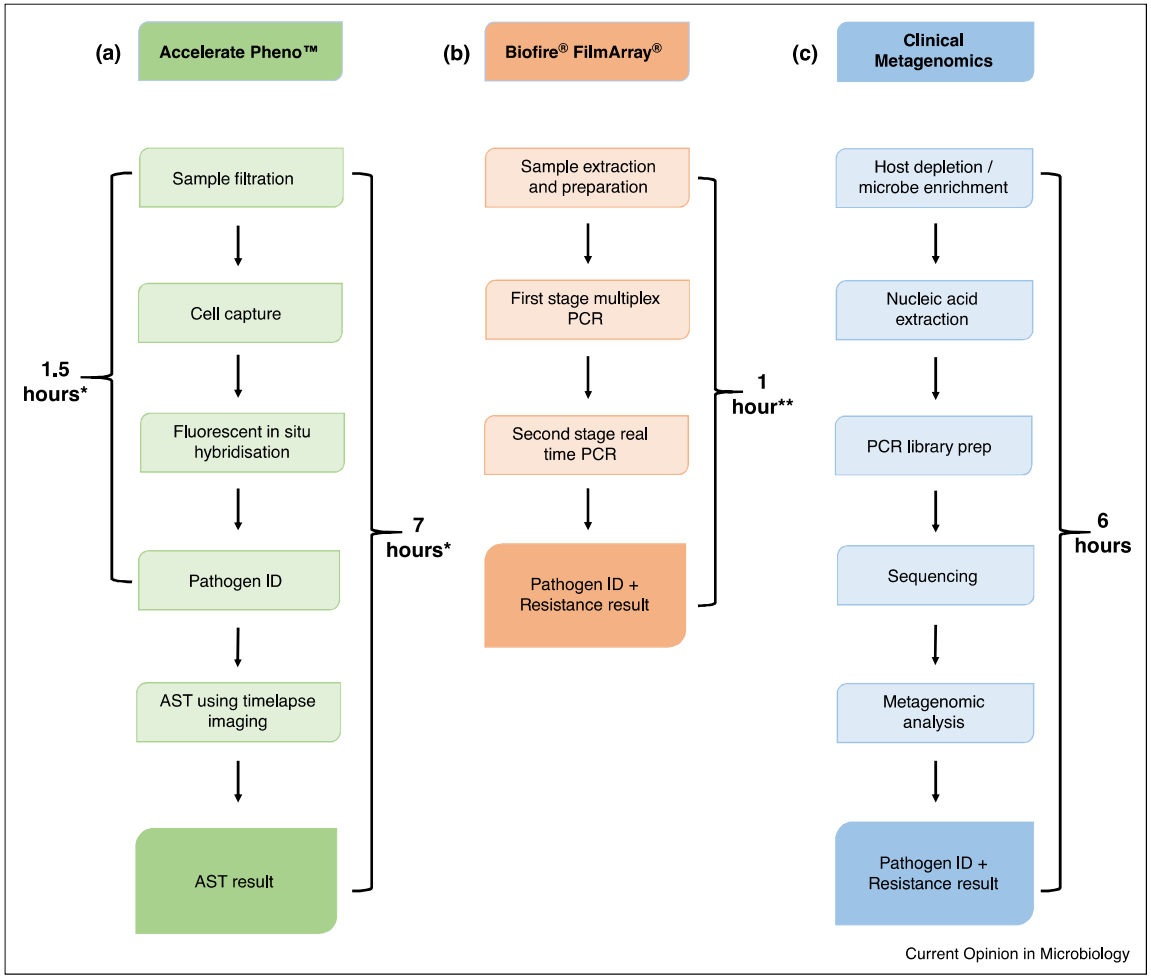
Figure 1. Detection methods for the early diagnosis of bloodstream infections, performed on whole blood or positive blood culture. Number of hands represent the hands-on time (one : <10 minutes; two: 10–30 minutes; three: >30 minutes).

Expert Review of Molecular Diagnostics



Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance

Alexander J Trotter[1,2], Alp Aydin[1,2], Michael J Strinden[1,2] and Justin O'Grady[1,2]



Current Opinion in Microbiology 2019, 51:39–45

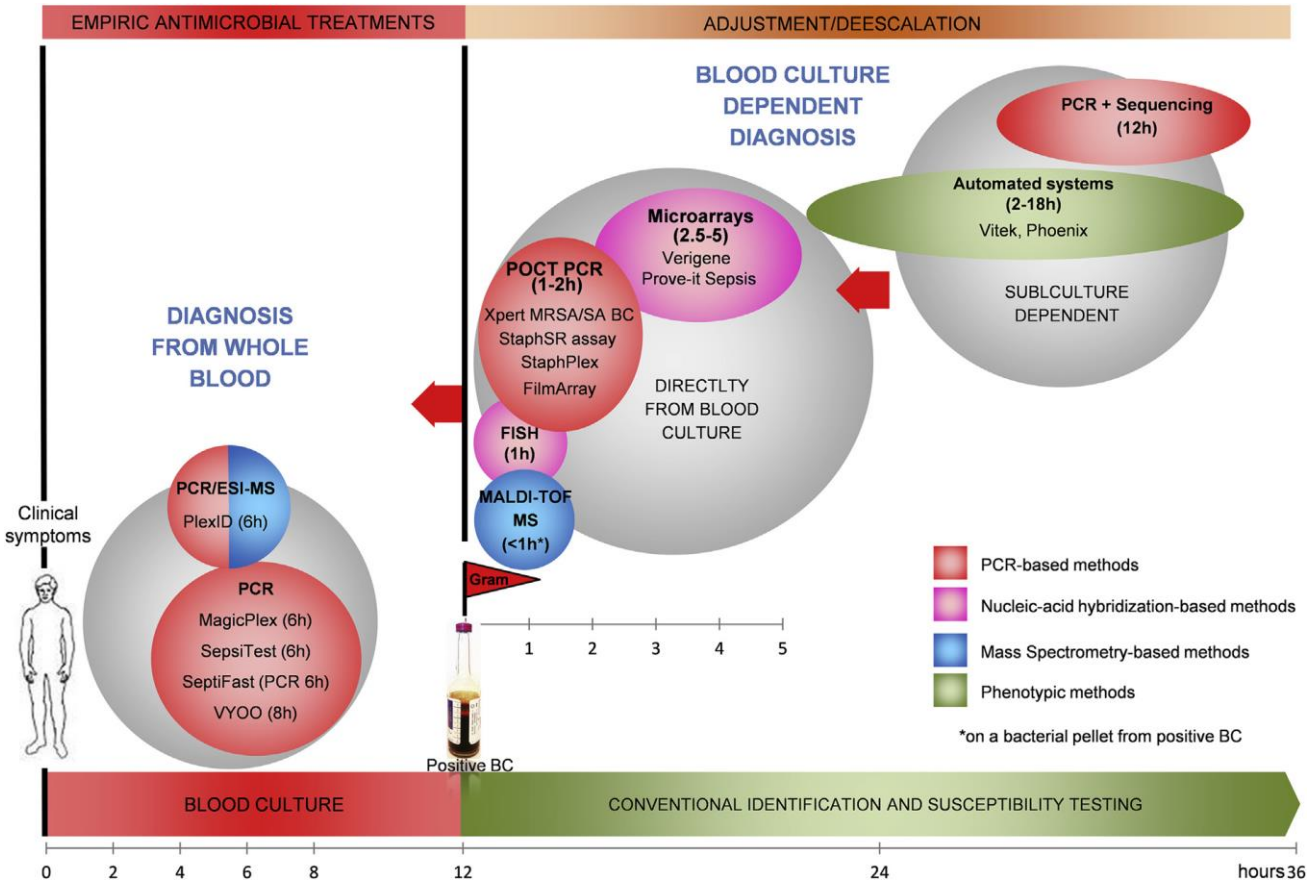


FIG. 2. Nucleic acid methods for the microbial diagnosis of BSI, BC-independent and BC-dependent methods. Nucleic acid–based methods have shortened the time to result BSI diagnosis. In the absence of microbial documentation of the etiologic agent of the BSI, anti-infectious treatments are initiated on the basis of clinical and epidemiologic information. Diagnosis directly from blood samples could shorten the length of empiric treatment.

Opota *et al.* Diagnosis of bacteremia directly from blood

Direct blood pathogen identification panel

Status: Final result **Visible to patient:** This result is not viewable by the patient. **Next appt:** None

	1d ago
KPC	Not Applicable
mecA	Not Detected
vanA/B	Not Applicable
Enterococcus genus	Not Detected
Listeria monocytogenes	Not Detected
Staphylococcus genus	DETECTED
Comments: Detection of Staphylococcus genus but not S. aureus is interpreted as detection of coagulase negative Staphylococcus sp.	
Staphylococcus aureus	Not Detected
Streptococcus genus	Not Detected
Streptococcus agalactiae	Not Detected
Streptococcus pneumoniae	Not Detected
Streptococcus pyogenes	Not Detected
Acinetobacter baumannii	Not Detected
Enterobacteriaceae family	Not Detected
Enterobacter cloacae complex	Not Detected
Escherichia coli	Not Detected
Klebsiella oxytoca	Not Detected
Klebsiella pneumoniae	Not Detected
Proteus	Not Detected
Serratia marcescens	Not Detected
Haemophilus influenzae	Not Detected
Neisseria meningitidis	Not Detected
Pseudomonas aeruginosa	Not Detected
Candida albicans	Not Detected
Candida glabrata	Not Detected
Candida krusei	Not Detected
Candida parapsilosis	Not Detected
Candida tropicalis	Not Detected

FIG 2 Example of rapid blood culture pathogen identification (BCID) results within patient's electronic health record.

Assessment of Rapid-Blood-Culture-Identification Result Interpretation and Antibiotic Prescribing Practices

Linsey M. Donner,[a] W. Scott Campbell,[b] Elizabeth Lyden,[c] Trevor C. Van Schooneveld[d]

May 2017 Volume 55 Issue 5 Journal of Clinical Microbiology

Table 3. P1 blood culture episodes where FA-BCID test results enabled a treatment modification.

	FA-BCID test result	Routine ID result	Treatment switch initiated by FA-BCID test result		
			type	antibiotic	TAT OAT
1	*mecA*-neg *S. aureus*	*S. aureus*	de-escalation	flucloxacillin	01:10
2	*S. pneumoniae*	*S. pneumoniae*	initiation	penicillin	01:44
3	bla_{KPC}-neg *E. cloacae* complex	*E. cloacae* complex	broadening	ciprofloxacin	01:53
4	*mecA*-neg *S. aureus*	*S. aureus*	initiation	flucloxacillin	02:07
5	*mecA*-pos *S. haemolyticus*	*S. haemolyticus*	initiation	vancomycin	02:23
6	*vanA/B*-neg *Enterococcus*	*E. faecalis*	initiation	ampicillin	02:26
7	bla_{KPC}-*neg S. marcescens*	*S. marcescens*	initiation	temocillin	02:34
8	*S. pneumoniae*	*S. pneumoniae*	initiation	penicillin	02:46
9	*mecA*-neg *S. aureus*	*S. aureus*	initiation	flucloxacillin	03:03
10	*mecA*-neg *S. aureus*	*S. aureus*	initiation	flucloxacillin	03:37
11	bla_{KPC}-neg *E. coli*	*E. coli*	initiation	cefuroxime	03:42
12	*mecA*-pos *S. aureus*	*S. aureus*	broadening	vancomycin	03:47
13	*C. albicans*	*C. albicans*	initiation	fluconazole	03:50
14	*mecA*-pos *S. aureus*	*S. aureus*	initiation	vancomycin	03:57
15	*Streptococcus*	*S. milleri* group	de-escalation	ampicillin	04:25
16	*S. thermophilus*	*S. viridans*	initiation	ampicillin	04:33
17	*mecA*-neg *S. aureus*	*S. aureus*	de-escalation	flucloxacillin	05:27
18	*mecA*-neg *S. aureus*	*S. aureus*	de-escalation	flucloxacillin	06:33
19	*mecA*-neg *S. aureus*	*S. aureus*	initiation	flucloxacillin	06:40
20	bla_{KPC}-neg *P. aeruginosa*	*P. aeruginosa*	broadening	ceftazidime	06:50
21	*mecA*-neg *S. aureus*	*S. aureus*	initiation	flucloxacillin	07:13
22	*C. glabrata*	*C. glabrata*	broadening	anidulafungin	08:00
23	*mecA*-neg *S. aureus*	*S. aureus*	de-escalation	flucloxacillin	11:04
24	bla_{KPC}-neg *E. coli*	*E. coli*	de-escalation	cefuroxime	11:40
25	bla_{KPC}-neg *S. marcescens*	*S. marcescens*	initiation	piperacillin + tazobactam	13:29
26	*L. monocytogenes*	*L. monocytogenes*	de-escalation	ampicillin	15:52
27	*C. glabrata* + *mecA*-neg *S. aureus*	*C. glabrata*	initiation	anidulafungin + flucloxacillin	15:53
28	bla_{KPC}-neg *E. cloacae* complex	*E. cloacae* complex	initiation	temocillin	26:17
29	bla_{KPC}-neg *E. coli*	*E. coli*	initiation	ceftriaxone	30:30
30	bla_{KPC}-neg *E. coli*	*E. coli*	initiation	cefuroxime	33:55
31	bla_{KPC}-neg *E. coli* + *vanA/B*-neg *Enterococcus*	*E. coli* + *E. faecalis*	initiation	cefuroxime + vancoymcin	34:33
32	bla_{KPC}-neg *E. coli*	*E. coli* + *C. perfringens*	initiation	cefuroxime	37:30
33	*mecA*-neg *Staphylococcus* + *vanA/B*-neg *Enterococcus*	*S. epidermidis* + *E. faecalis*	broadening	vancomycin	40:12
34	bla_{KPC}-neg *P. aeruginosa*	*P. aeruginosa*	broadening	ceftazidime	65:30
35	bla_{KPC}-neg *A. baumannii* + *mecA*-neg *Staphylococcus*	*A. baumannii* + *S. haemolyticus*	broadening	meropenem	108:48

In episode 1–26, the modified treatment upon FA-BCID result was the OAT. In episode 27–35, further tailoring was necessary following ID and AST results. The TAT to OAT is reported in hours:minutes.

Abbreviations: AST, antimicrobial susceptibility testing; FA-BCID, FilmArray blood culture identification; ID, identification; OAT, optimal antimicrobial treatment; TAT, turn-around-time; P1, intervention period.

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RESEARCH ARTICLE

The impact of a rapid molecular identification test on positive blood cultures from critically ill with bacteremia: A pre-post intervention study

Alexia Verroken[1]*, Noémie Despas[1], Hector Rodriguez-Villalobos[1], Pierre-François Laterre[2]

1 Department of Microbiology, Cliniques Universitaires Saint-Luc – Université Catholique de Louvain, Brussels, Belgium, **2** Intensive Care Department, Cliniques Universitaires Saint-Luc – Université Catholique de Louvain, Brussels, Belgium

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1 Department of Microbiology, Cliniques Universitaires Saint-Luc – Université Catholique de Louvain, Brussels, Belgium, **2** Intensive Care Department, Cliniques Universitaires Saint-Luc – Université Catholique de Louvain, Brussels, Belgium

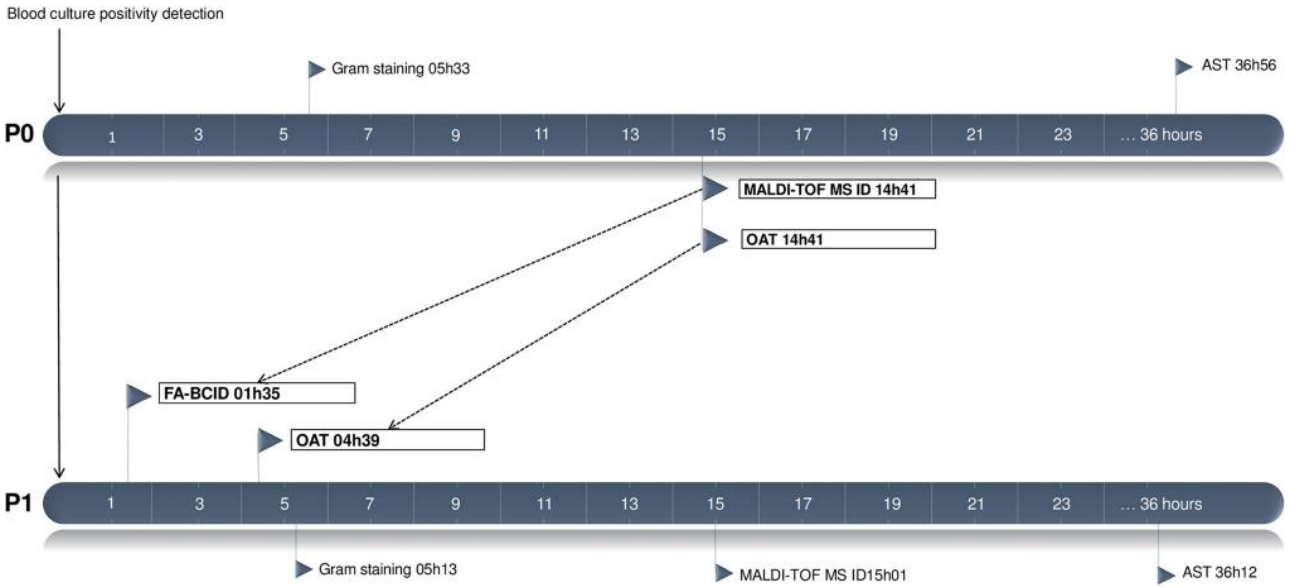


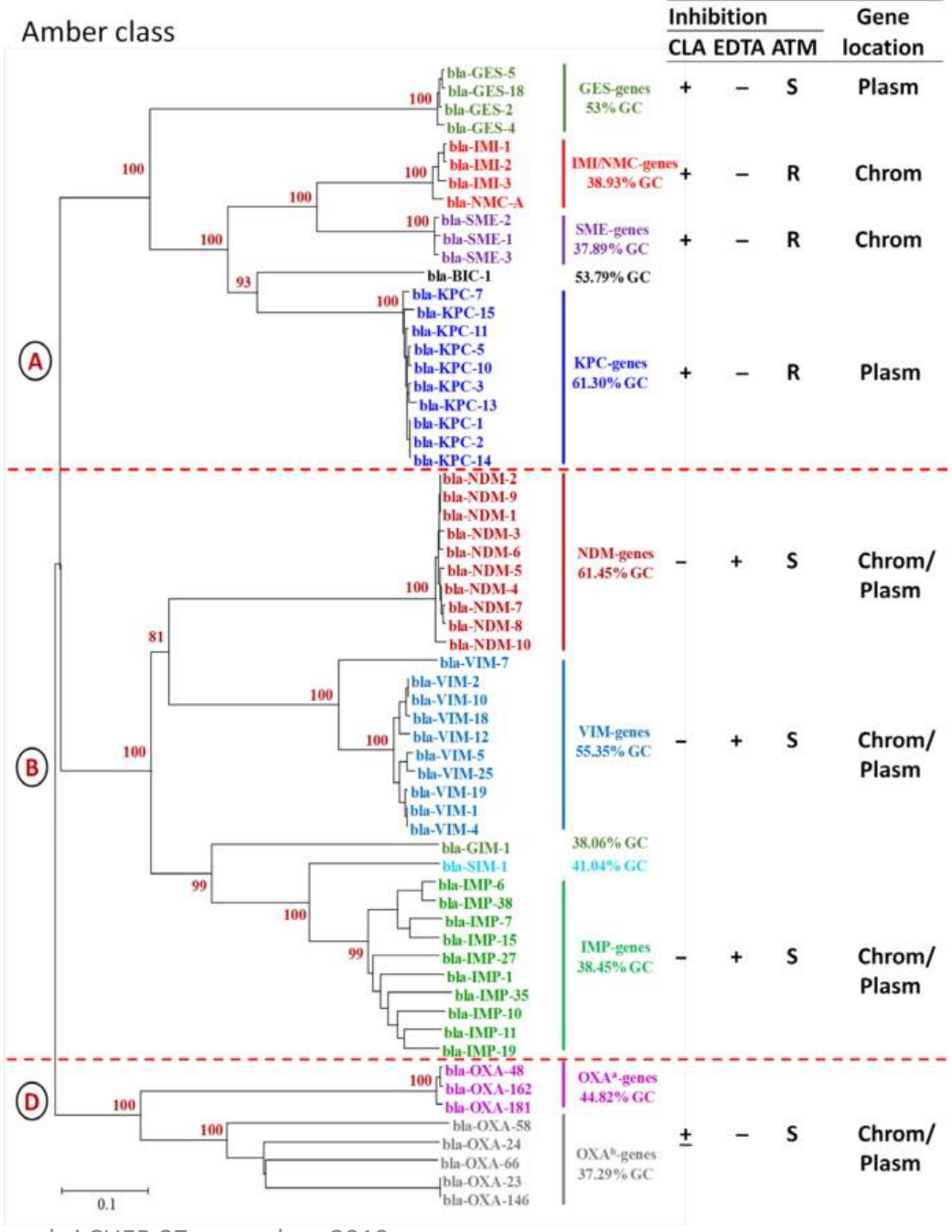
Fig 2. Comparison of median time to microbiological results and time to administration of optimal antimicrobial treatment in critically ill with bloodstream infections included in P0 and P1. Abbreviations: AST, antimicrobial susceptibility testing; FA-BCID, FilmArray blood culture identification; ID, identification; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight; OAT, administration of the optimal antimicrobial treatment; P0, pre-intervention period; P1, intervention period.

https://doi.org/10.1371/journal.pone.0223122.g002

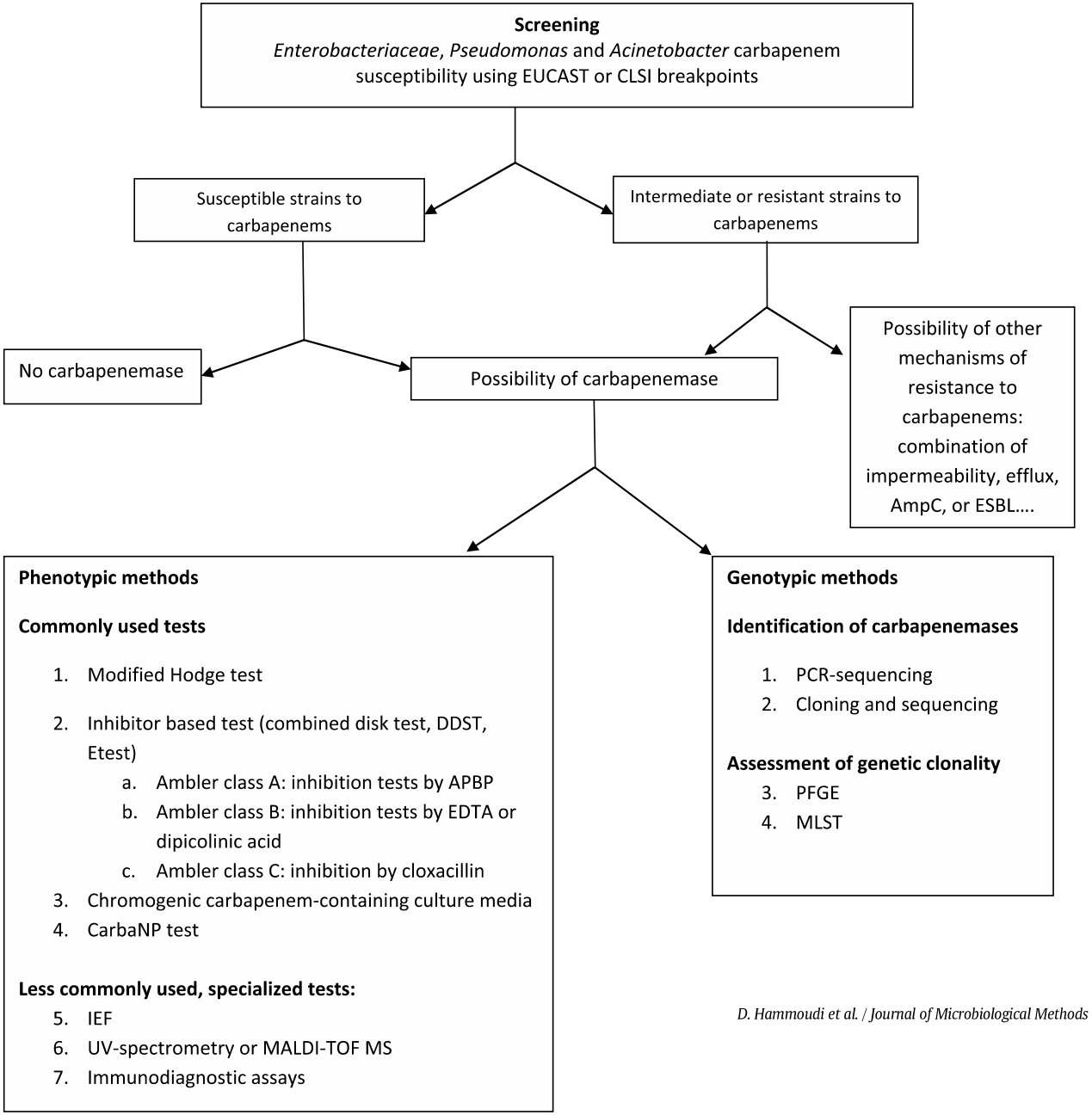
Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species

S. M. Diene and J.-M. Rolain

Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Marseille, France



Clinical Microbiology and Infection, Volume 20 Number 9, September 2014



D. Hammoudi et al. / Journal of Microbiological Methods 107 (2014) 106–118

Rapid molecular tests for detection of antimicrobial resistance determinants in Gram-negative organisms from positive blood cultures: a systematic review and meta-analysis[◊]

Clinical Microbiology and Infection

G. De Angelis [1], A. Grossi [2], G. Menchinelli [1], S. Boccia [2, 3], M. Sanguinetti [1, 4, *], B. Posteraro [5, 6]

Study eligibility criteria: Clinical studies evaluating the performance of two major commercial systems, namely the Verigeneâ and FilmArrayâ systems, for rapid testing of GNB-PBCs, in comparison with the phenotypic or genotypic methods performed on GNB-PBC isolates.

Results: Twenty studies were identified (3310 isolates) from 2006 to 2019. Nine studies were conducted in East Asia. In 15 studies using phenotypic comparators (1930 isolates), 1014 (52.5%) isolates were *Escherichia coli*, and 287 (14.9%) of all the isolates displayed AMR phenotypes. In 5 studies using genotypic comparators (1380 isolates), 585 (42.4%) were *E. coli*, and 100 (7.2%) of all the isolates displayed AMR genotypes. Pooled sensitivity and specificity estimates for detection of AMR determinants by the Verigeneâ (i.e. CTX-M, IMP, KPC, NDM, OXA and VIM) and/or FilmArrayâ (i.e. KPC) systems were 85.3% (95% CI 79.9%–89.4%) and 99.1% (95% CI 98.2%–99.5%), respectively, across the 15 studies, and 95.5% (95% CI 89.2%–98.2%) and 99.7% (95% CI 99.1%–99.9%), respectively, across the 5 studies.

https://doi.org/10.1016/j.cmi.2019.11.009

Rapid molecular tests for detection of antimicrobial resistance determinants in Gram-negative organisms from positive blood cultures: a systematic review and meta-analysis[◊]

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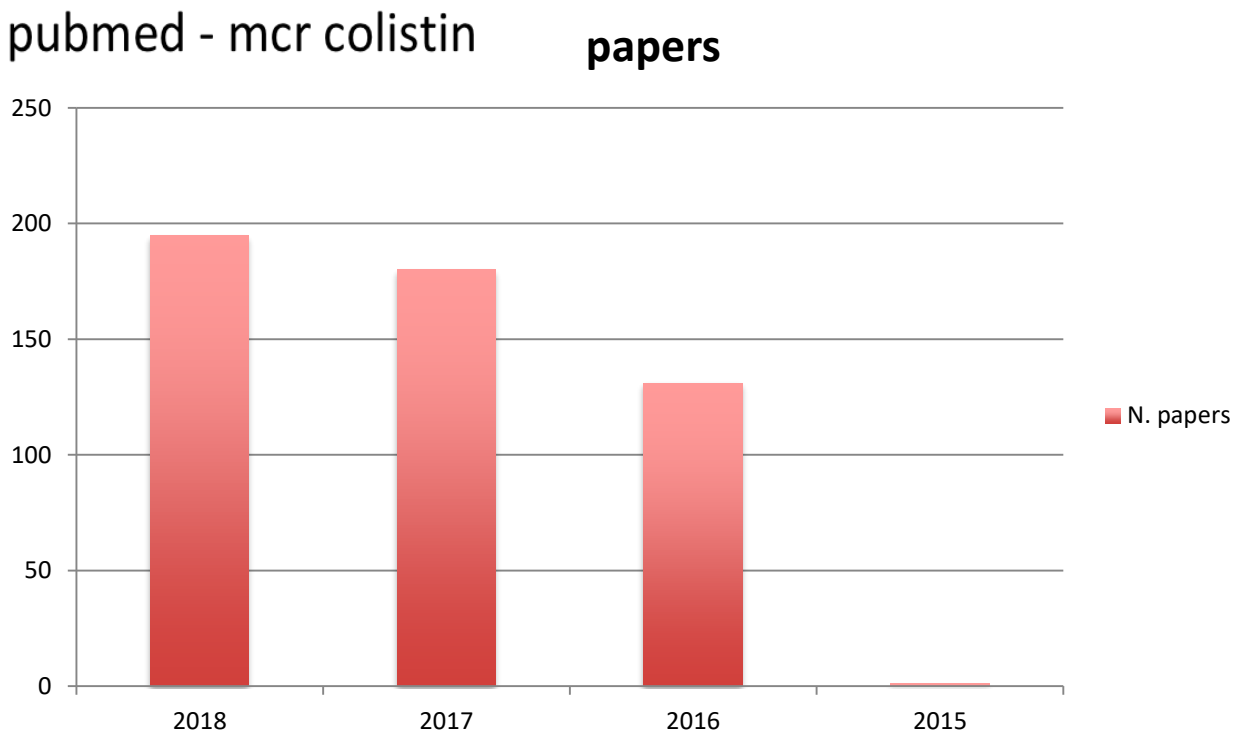
Conclusions: Our findings show that the Verigeneâ and FilmArrayâ systems may be a valid adjunct to the conventional microbiology (phenotypic or genotypic) methods used to identify AMR in GNBs. FilmArrayâ system detects only one AMR genotype, namely KPC, limiting its utilization. Both Verigeneâ and FilmArrayâ systems can miss important cephalosporin/carbapenem resistance phenotypes in a minority of cases. However, sensitivity and specificity of both systems render them valuable clinical tools in timely identification of resistant isolates. Further studies will establish the prominence of such rapid diagnostics as standard of care in patients with bloodstream infections.

https://doi.org/10.1016/j.cmi.2019.11.009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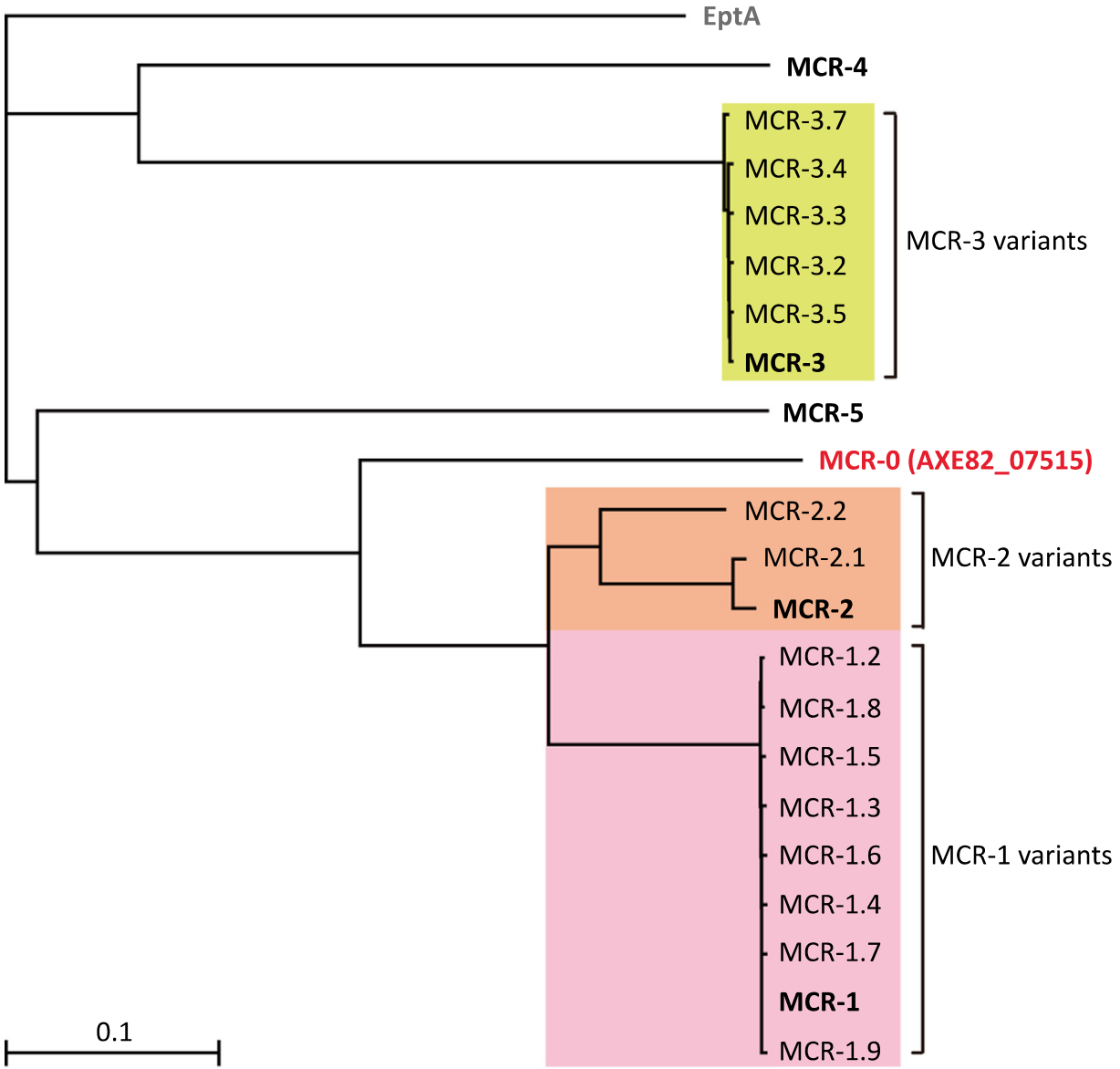


Trends in Microbiology, September 2018, Vol. 26, No. 9

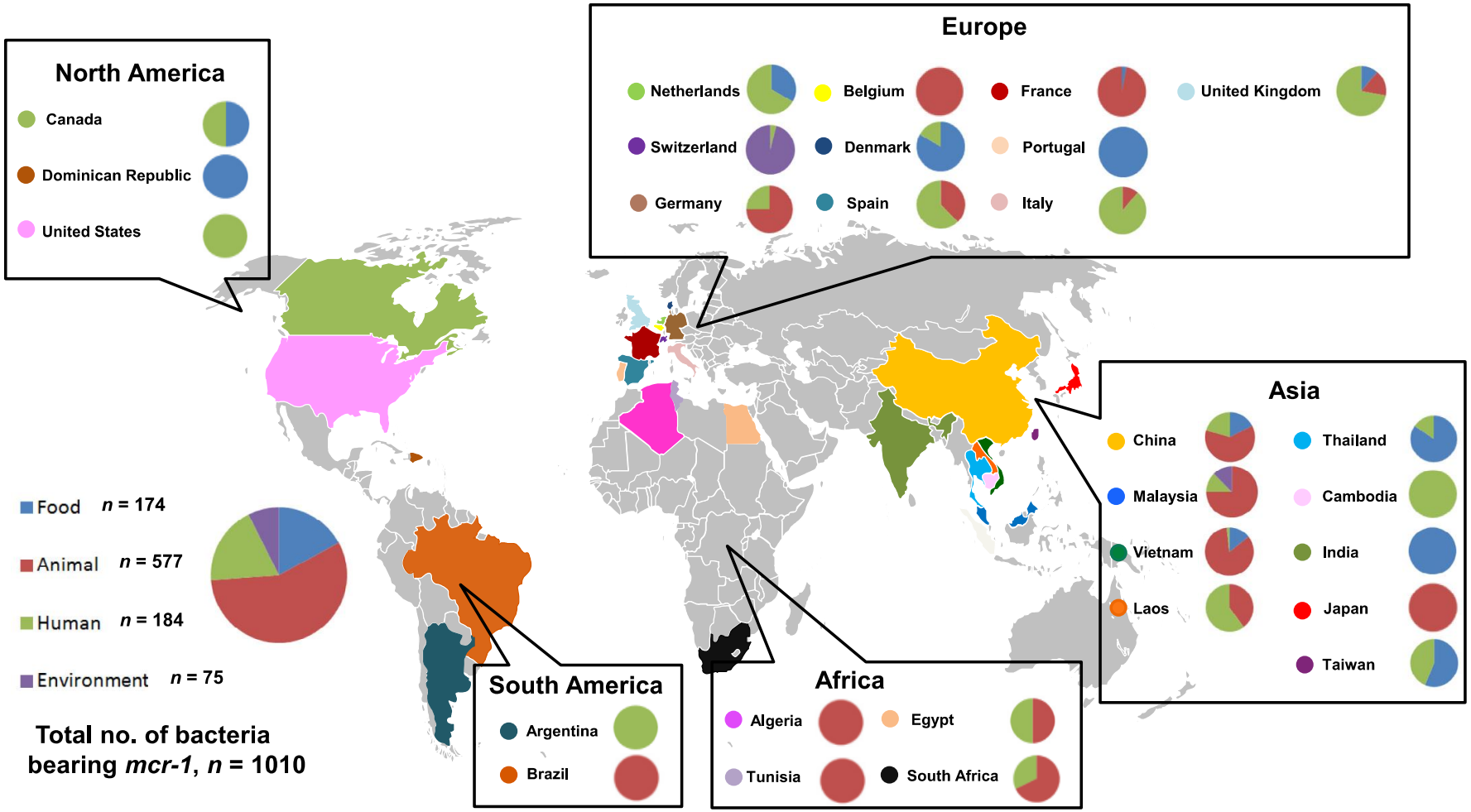


Fig. 2. Global distribution of plasmid-mediated *mcr-1* colistin-resistant strains isolated from environments, foods, animals and humans (November 2015 to April 2016).

Microbiological surveillance of plasmid mediated colistin resistance in human *Enterobacteriaceae* isolates in Romagna (Northern Italy): August 2016–July 2017

F. Del Bianco[a,*], M. Morotti[a], M.F. Pedna[a], P. Farabegoli[a], V. Sambri[a,b]

Results: Over the total of 19053 isolates belonging to *Enterobacteriaceae*, 90 were colistin resistant. The presence of *mcr-1* was detected in 26 *Escherichia coli*. The overall prevalence of *mcr-1* was 0.14%. The *mcr-1* positive *E. coli* strains were assigned to 13 distinct sequence types (STs) according to MLST.

Features of the *mcr-1*-harboring *E. coli* clinical isolates. MICs were determinated by Vitek2.

Strain	Isolation	Source	MLST[a]	MIC mg/L (S/I/R)[b]												
				AMK	AMX/CLAV	CTX	CFT	FEP	IMI	MEM	PIP/TZB	CIP	GEN	SXT	TGC	COL
1O/RA	Aug 2016	urine	ST617	≤2 S	≤2 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≤20 S	≤0.5 S	8 R
2I/RN	Aug 2016	urine	ST744	≤2 S	8 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	8 S	≥4 R	≤1 S	≥320 R	≤0.5 S	8 R
3O/RA *ESβL+*[c]	Sept 2016	urine	ST73	≤2 S	16 R	2 I	16 R	2 I	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≥16 R	≤20 S	≤0.5 S	8 R
4O/RA	Sept 2016	urine	ST410	≤2 S	≥32 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≤1 S	≥320 R	≤0.5 S	4 R
5I/RA	Sept 2016	blood	ST624	≤2 S	≥32 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	64 R	≥4 R	2 S	≥320 R	≤0.5 S	8 R
6I/RN	Sept 2016	urine	ST224	≤2 S	16 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≤1 S	≥320 R	1 S	8 R
7I/RN	Sept 2016	urine	ST69	≤2 S	≤2 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	8 R
8I/CE	Sept 2016	urine	ST69	4 S	16 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≥16 R	≥320 R	≤0.5 S	8 R
9O/RA	Sept 2016	urine	ST457	≤2 S	4 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	2 R	≤1 S	≥320 R	≤0.5 S	8 R
10O/FO	Sept 2016	urine	ST10	≤2 S	4 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≥16 R	≥320 R	≤0.5 S	8 R
11O/RA	Sept 2016	wounde	ST354	≤2 S	8 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≥16 R	≥320 R	≤0.5 S	8 R
12O/RA	Sept 2016	urine	ST10	≤2 S	4 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≥16 R	≥320 R	≤0.5 S	8 R
13I/RN	Oct 2016	urine	ST224	≤2 S	16 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	16 I	≥4 R	≤1 S	≥320 R	≤0.5 S	8 R
14O/RN	Oct 2016	urine	ST10	≤2 S	≥32 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	16 I	≥4 R	≤1 S	≥320 R	≤0.5 S	4 R
15O/FO	Nov 2016	urine	ST216	≤2 S	8 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	8 R
16O/RA	Nov 2016	urine	ST95	≤2 S	4 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	2 R	≥16 R	≥320 R	≤0.5 S	8 R
17O/RA	Oct 2016	urine	ST744	≤2 S	≤2 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	4 R
18I/RN	Dec 2016	blood	ST10	≤2 S	16 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≤1 S	≥320 R	≤0.5 S	8 R
19O/CE *ESβL+*[c,d]	Dec 2016	urine	ST131	≤2 S	4 S	8 R	≤1 S	2 I	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	8 R
20O/RA	Jan 2017	urine	ST131	≤2 S	4 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	1 I	≤1 S	≥320 R	≤0.5 S	8 R
21I/RN	Feb 2017	urine	ST10	≤2 S	≤2 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≤1 S	≤20 S	≤0.5 S	16 R
22O/FO	Apr 2017	urine	ST131	≤2 S	≤2 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≥4 R	≤1 S	≤20 S	≤0.5 S	8 R
23O/RA	Apr 2017	urine	ST224	≤2 S	16 R	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	4 R
24I/RA	May 2017	b.asp.[e]	ST10	≤2 S	8 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	4 R
25I/RA	June 2017	wounde	ST10	≤2 S	4 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	4 R
26O/RN	June 2017	urine	ST131	≤2 S	8 S	≤1 S	≤1 S	≤1 S	≤0.25 S	≤0.25 S	≤4 S	≤0.25 S	≤1 S	≥320 R	≤0.5 S	6 R

Methods: All the colistin resistant *Enterobacteriaceae*, isolated from August 1st 2016 to July 31st 2017,

International Journal of Infectious Diseases 69 (2018) 96–98

Detection of *mcr-4* positive *Salmonella enterica* serovar Typhimurium in clinical isolates of human origin, Italy, October to November 2016

Edoardo Carretto[1], Flavia Brovarone[1], Paola Nardini[1], Giuseppe Russello[1], Daniela Barbarini[2], Stefano Pongolini[3], Carlo Gagliotti[4], Alessandra Carattoli[5], Mario Sarti[6]

In this study we report the detection of the recently described *mcr-4* gene in two human isolates of *Salmonella enterica* serovar Typhimurium. The strains were isolated from faecal samples of two Italian patients with gastroenteritis, collected in 2016. The identified *mcr-4* genes (variant *mcr-4.2*) differed from the *mcr-4* gene originally described in a *Salmonella* strain of swine origin from Italy. *Salmonella* species could represent a hidden reservoir for mcr genes.

www.eurosurveillance.org

Wang et al. *Emerging Microbes & Infections* (2018)7:122
DOI 10.1038/s41426-018-0124-z

Emerging Microbes & Infections
www.nature.com/emi

ARTICLE Open Access

Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*

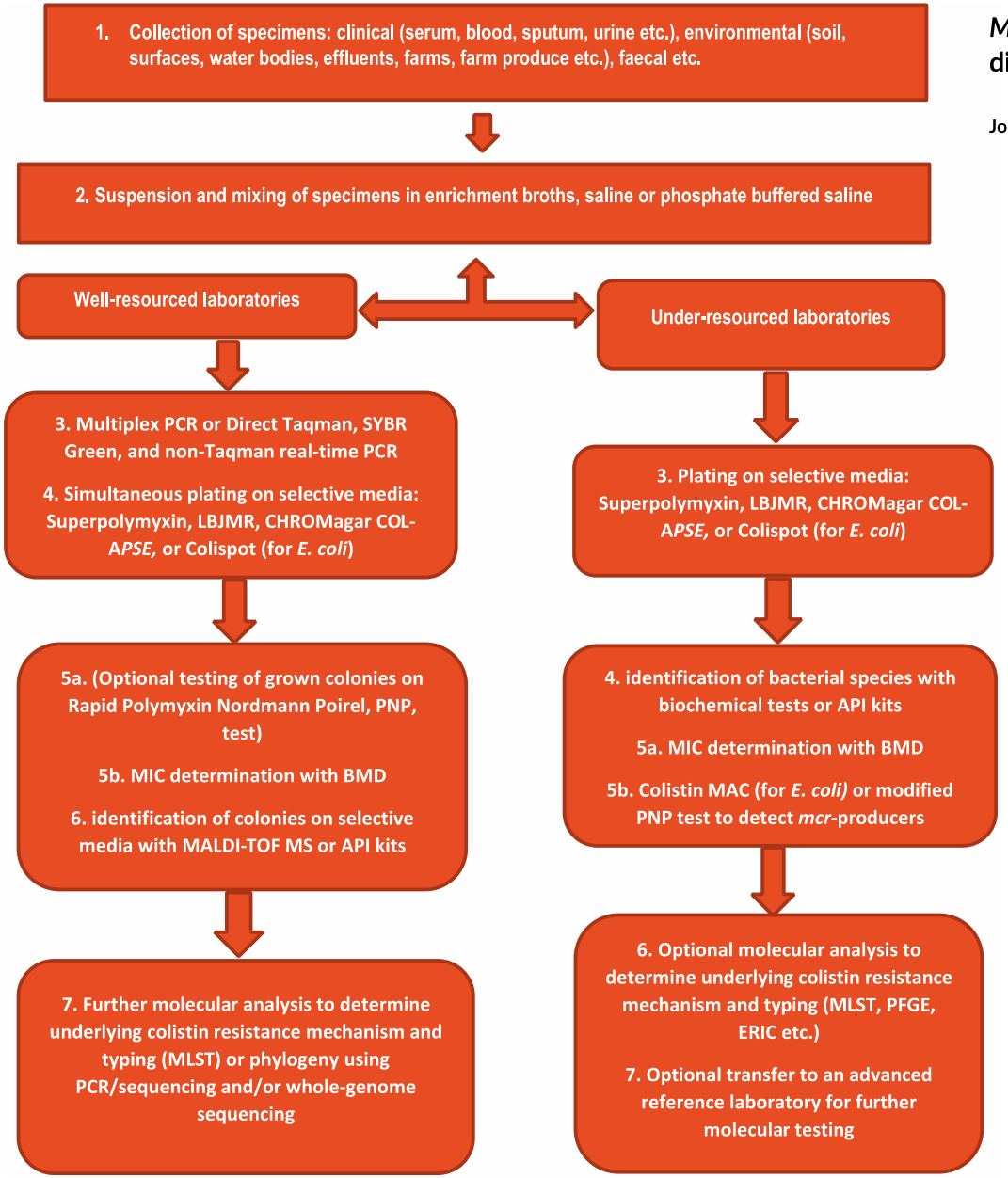
Xiaoming Wang[1], Yao Wang[1], Ying Zhou[1], Jiyun Li[2], Wenjuan Yin[2], Shaolin Wang[1], Suxia Zhang[2], Jianzhong Shen[1], Zhangqi Shen[1] and Yang Wang[2]

J Antimicrob Chemother 2018; **73**: 1791–1795
doi:10.1093/jac/dky111 Advance Access publication 17 April 2018

Journal of Antimicrobial Chemotherapy

Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*

Yong-Qiang Yang[1,2], Yun-Xia Li[1,2], Chang-Wei Lei[1,2], An-Yun Zhang[1,2] and Hong-Ning Wang[1,2]*



Mcr colistin resistance gene: a systematic review of current diagnostics and detection methods

John Osei Sekyere[1,2]

MicrobiologyOpen. 2018;e682.
https://doi.org/10.1002/mbo3.682

Rapid multiplex polymerase chain reaction for detection of *mcr-1* to *mcr-5* genes

Mathilde Lescat [a,b,c,d], Laurent Poirel [a,b,e,*], Patrice Nordmann [a,b,e,f]

Table 1
Amino acid identity of MCR polymyxin resistance determinants.

MCR determinant	Amino acid identity level			
	MCR-1	MCR-2	MCR-3	MCR-4
MCR-2	80.7			
MCR-3	32.5	31.7		
MCR-4	34.0	35.0	49.0	
MCR-5	36.1	35.3	34.7	33.7

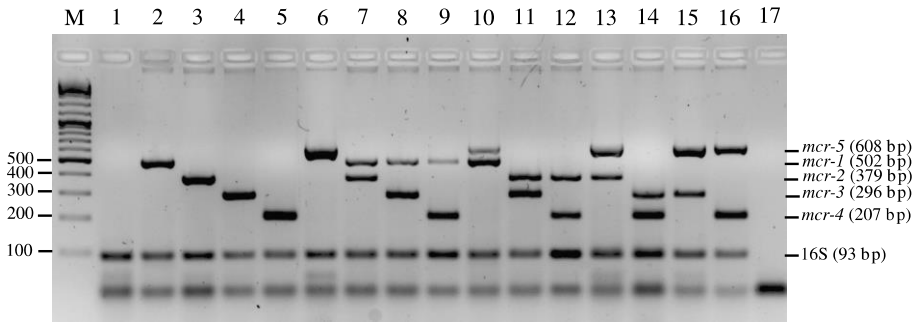


Fig. 1. Agarose gel electrophoresis (2.5%) used for the separation of multiplex PCR products. Lanes: 1, negative control (susceptible *E. coli* isolate); 2, *mcr-1*–positive isolate; 3, *mcr-2*–positive isolate; 4, *mcr-3*–positive isolate; 5, *mcr-4*–positive isolate; 6, *mcr-5*–positive isolate; 7, mix of DNA of *mcr-1*– and *mcr-2*–positive isolates; 8, mix of DNA of *mcr-1* and *mcr-3*–positive isolates; 9, mix of DNA of *mcr-1*– and *mcr-4*–positive isolates; 10, mix of DNA of *mcr-1* and *mcr-5* isolates; 11, mix of DNA of *mcr-2*– and *mcr-3*–positive isolates; 12, mix of DNA of *mcr-2*– and *mcr-4*–positive isolates; 13, mix of DNA of *mcr-2*– and *mcr-5*–positive isolates; 14, mix of DNA of *mcr-3*– and *mcr-4*–positive isolates; 15, mix of DNA of *mcr-3*– and *mcr-5*–positive isolates; 16, mix of DNA of *mcr-4*– and *mcr-5*–positive isolates; and 17, negative control (water). M = molecular size marker (GeneRuler™, 100-bp DNA Ladder Plus; Thermo Fisher Scientific, USA). The size of each PCR product is indicated in base pairs.

Multisite Evaluation of Cepheid Xpert Carba-R Assay for Detection of Carbapenemase-Producing Organisms in Rectal Swabs

M. Tato,[a] P. Ruiz-Garbajosa,[a] M. Traczewski,[b] A. Dodgson,[c] A. McEwan,[c] R. Humphries,[d] J. Hindler,[d] J. Veltman,[e] H. Wang,[f] R. Cantón[a]

TABLE 1 Xpert Carba-R assay results by target for clinical and contrived specimens

Xpert Carba-R assay result	Clinical specimens (n = 383)	Contrived specimens (n = 250)	All specimens (n = 633)
Positive (single and/or combined targets)	42	107	149
IMP-1	0	25	25
VIM	2	24	26
NDM	2	23	25
KPC	13	19	32
OXA-48	20	15	35
VIM + OXA-48	4	0	4
NDM + KPC	1	0	1
IMP-1 + NDM	0	1	1
Negative	341	143	484

TABLE 3 Overall Xpert Carba-R performance versus that of the reference method (culture plus sequencing) for combined clinical and contrived specimens

	Reference method (culture plus sequencing)		
Xpert Carba-R assay	No. positive	No. negative	Total No.
Positive	142	7	149
Negative	6	478	484
Total	148	485	633

TABLE 2 Results from the Xpert Carba-R assay and the reference method (culture plus sequencing) by individual target for combined clinical and contrived specimens

Xpert Carba-R assay	Reference method (culture plus sequencing)						
	IMP-1	VIM	NDM	KPC	OXA-48	Negative	Total
IMP-1	26	0	0	0	0	0	26
VIM	0	29	0	0	0	1	30
NDM	0	0	26	0	0	1	27
KPC	0	0	0	29	0	4	33
OXA-48	0	0	0	0	38	1	39
Negative	1	2	0	1	2	3,004	3,010
Total	27	31	26	30	40	3,011	3,165

Evaluation of a Loop-Mediated Isothermal Amplification-Based Assay for the Rapid Detection of Plasmid-Encoded Colistin Resistance Gene *mcr-1* in *Enterobacteriaceae* Isolates

Can Imirzalioglu,[a] Linda Falgenhauer,[a] Judith Schmiedel,[a] Said-Elias Waezsada,[a] Konrad Gwozdzinski,[a] Nicole Roschanski,[b] Uwe Roesler,[b] Lothar Kreienbrock,[c] Arthur P. Schiffmann,[d] Alexandra Irrgang,[e] Annemarie Käsbohrer,[e,f] Rolf Bauerfeind,[g] Eugen Domann,[a] Trinad Chakraborty[a]

April 2017 Volume 61 Issue 4 e02326-16 Antimicrobial Agents and Chemotherapy

Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals

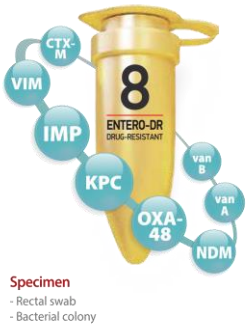
Sergio García-Fernández[1], María-Isabel Morosini[1,2]*, Francesc Marco[3,4], Desirèe Gijón[1,2], Andrea Vergara[3,4], Jordi Vila[3,4], Patricia Ruiz-Garbajosa[1,2] and Rafael Cantón[1,2]

J Antimicrob Chemother 2015; **70**: 1047–1050

Allplex™ Entero-DR Assay

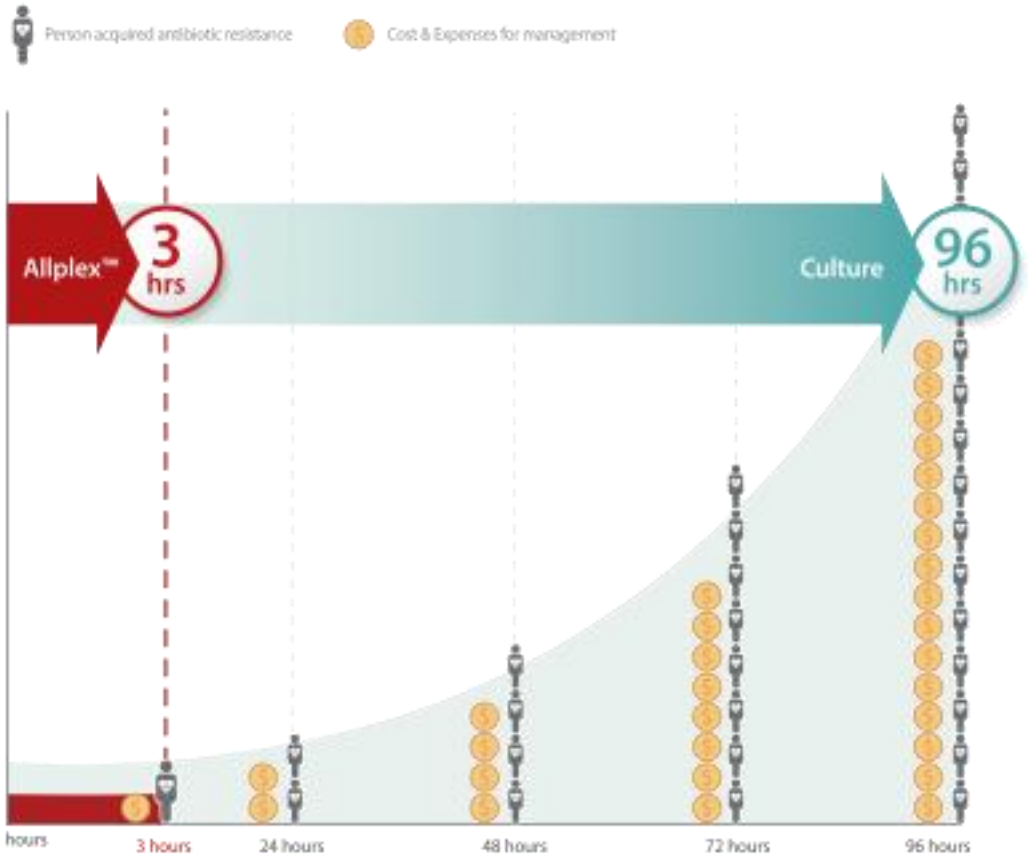
Compatible instrumentation (CE-IVD Marked)

- **Automated Extraction & PCR Setup**
  NIMBUS IVD (Hamilton)
  STARlet IVD (Hamilton)
- **Automated Extraction**
  NucliSENS® easyMAG® (BioMérieux)
- **Real-time PCR**
  CFX96™ (Bio-Rad)



Specimen
- Rectal swab
- Bacterial colony

For effective management of CPE/VRE/ESBL infections



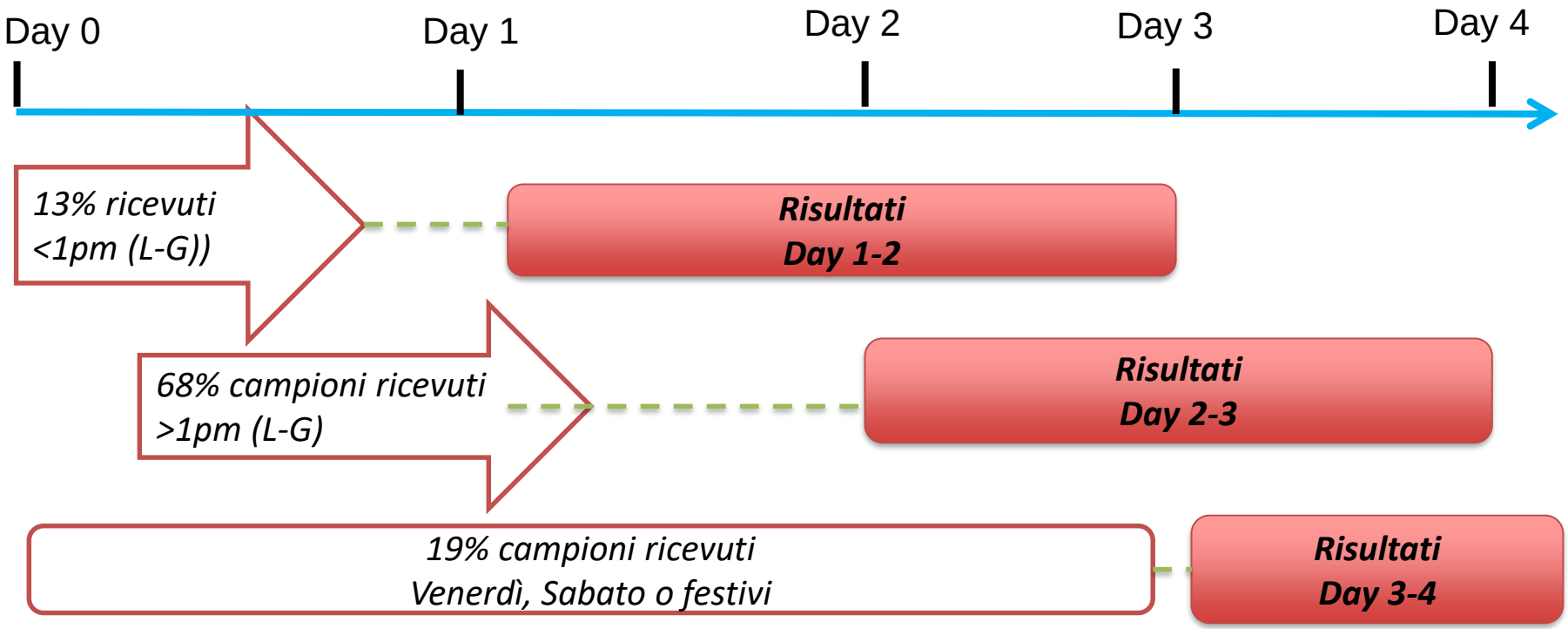
Allplex™ Entero-DR Assay detects antibiotic resistance within 3hrs whereas conventional method requires maximum 96 hrs. Allplex™ Entero-DR Assay significantly descreases the spread of antibiotic resistant bacteria.

Decrease
- Hygiene management costs and expenses
- Cases and outbreaks of infections
- Disability and mortality rates

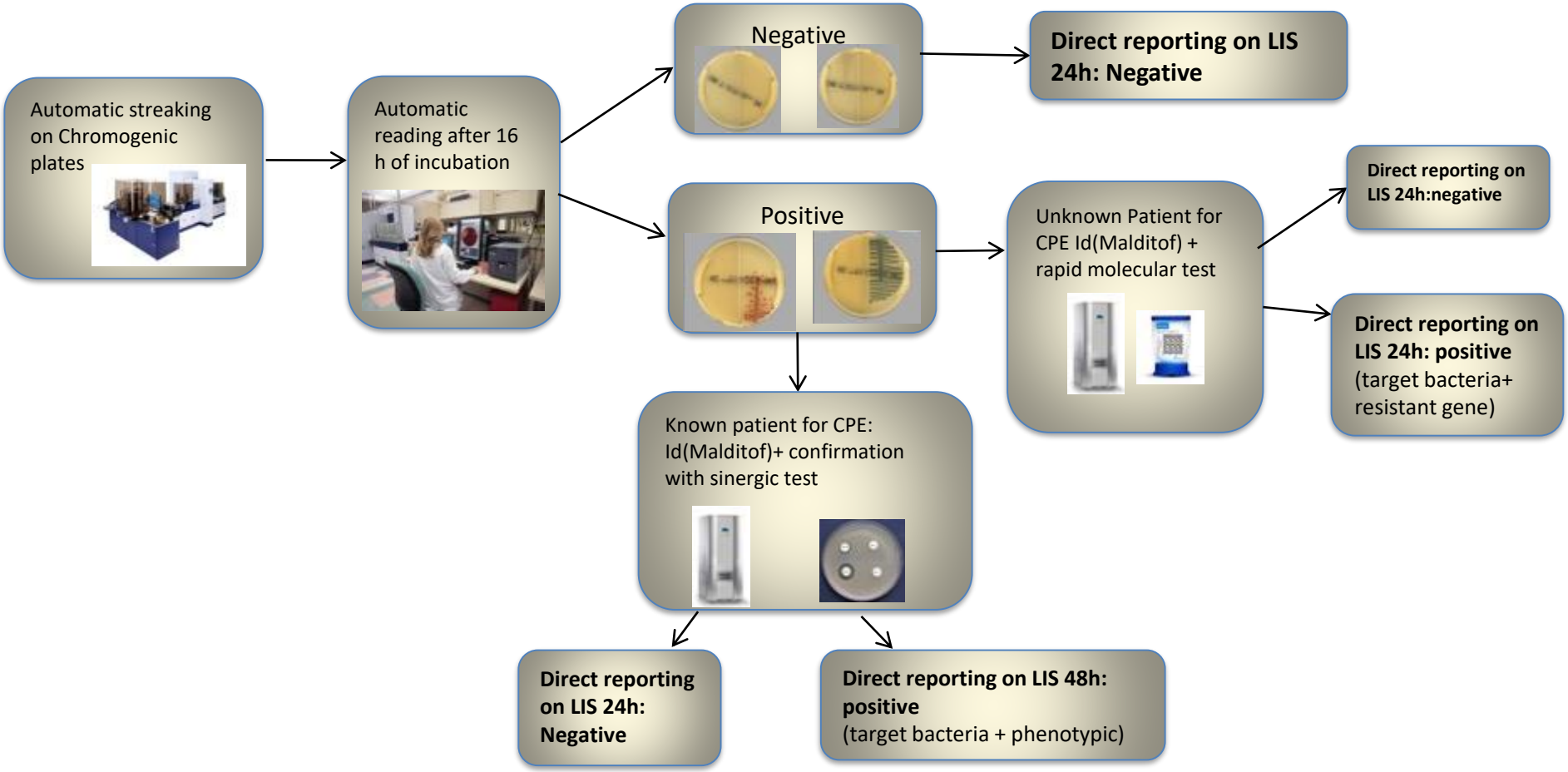
Increase
- Efficient control of patients
- Appropriate treatments
- Efficient management of infections

Analisi dei Dati
Maggio – Giugno 2015

- 84 campioni per 78 pazienti
- 60% dei risultati TAT >2 giorni



CPE Screening algorithm: from image analysis to sample reporting



Laboratory-Based and Point-of-Care Testing for MSSA/MRSA Detection in the Age of Whole Genome Sequencing

Alex van Belkum[1] and Olivier Rochas[2]*

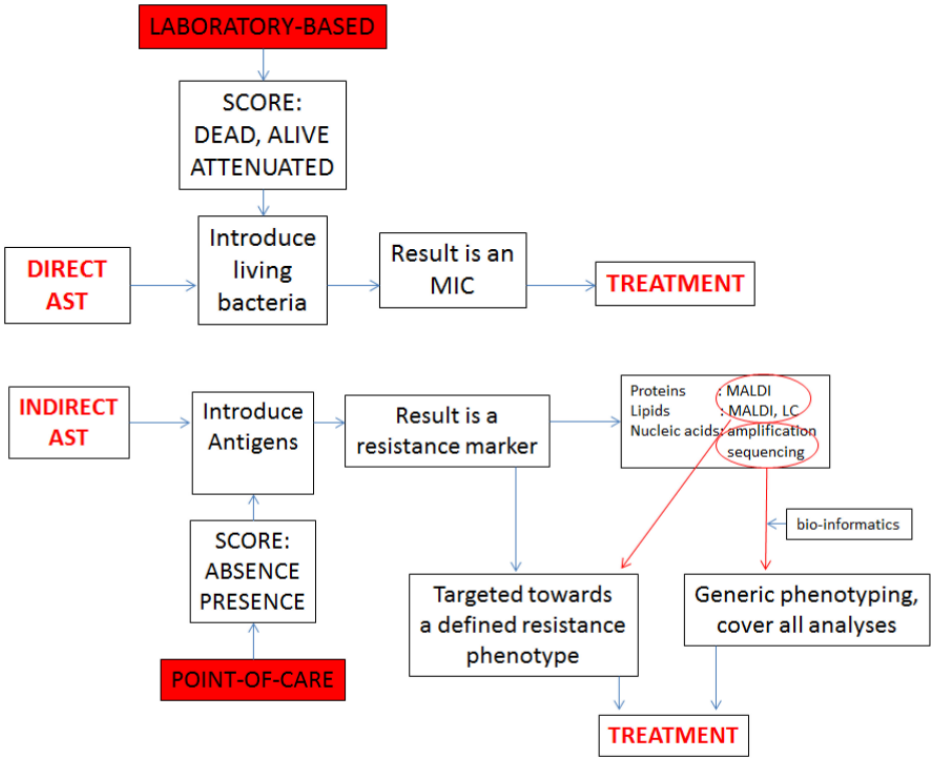


FIGURE 1 | Comparison between direct AST and indirect AST, showing the difference between the laboratory based and PoC protocols. Whereas the laboratory exercise generates precise minimum inhibitory concentrations (MICs), the indirect approach generates markers that need to be associated with therapeutic modalities through bio-informatics.

frontiers in Microbiology

MINI REVIEW
published: 29 June 2018
doi: 10.3389/fmicb.2018.01437

TABLE 1 | Global review of future and commercial PCR tests for meticillin-resistant and -susceptible strains of *Staphylococcus aureus*.

Company	Status	Concise product description	Duration of test
Abacus Diagnostica, Finland	In development	Rapid DNA testing with proprietary GenomEra CDX-technology for identification of MRSA	50 min
AdvanDx, United States	FDA approved	*Staphylococcus* QuickFISH filter *in situ* hybridization test for positive blood culture liquid	20 min
Akonni Biosystems, United States	In development	TruArray MRSA, qualitative test for detection of SA and MRSA	Non-specified
Atlas Genetics, United Kingdom	In development	Mixed technology linking NAT and immunology for MRSA, Dual MRSA/MSSA	0.5 h
Autoi/mmun Diagnostika, Germany	CE certified	Automated AID Scanner, line probe Western blot probe assay after PCR amplification, 100 strips per hour	4 h
Biocartis, Belgium	In development	Idylla platform for multiplex real-time PCR assay for rapid detection of bloodstream infections	2 h
BioFire, United States	FDA approved, new tests in development	FDA approved syndromic panels for respiratory, gastro-intestinal, and meningitis/encephalitis associated pathogens; the BCID test also covers *mec*A. Sample in—result out strategy	1 h
BD, United States	FDA approved, new tests in development	Platform BD Max. MRSA + MSSA + *mec*A test	<3 h
Cepheid, United States (acquired by Danaher)	FDA approved for HAI with MRSA/SA	Validated for positive blood culture. Xpert test format. MRSA, SA Nasal Complete, MRSA/SA SSTI, MRSA/SA BC	2 h
Coyote Biosci, United States, China	In development	Platform Mini 8 RT PCR; throat swab/Blood sample—MRSA	10–30 min
Curetis AG, Germany	CE marked, precise status not very clear	Platform Univero; >100 pathogens and resistance genes, P55 Application focuses on pneumonia, 21 pathogens, and 19 resistance markers, 40-plex. i60 ITI Application Cartridge (23 organisms and 19 resistance genes)	4–5 h
DXna, United States	CE marked	GeneSTAT portable RT PCR platform, MRSA/MRCoNS in development for 2017	1 h
Epoch Biosciences, Elitech Group	FDA approved	Triplex Real Time Amplification tests using minor groove binding DNA probes	1 h
Genesig	RUO	Quantitative PCR for various targets among which MRSA; 16 samples per run	90–120 min
GenMark, United States	In development	Platform ePlex. Electronic sensor technology, DNA hybridization, and electrochemical detection	4 h
Genspeed, Austria	In development	Straightforward PCR with hybridization confirmation, combination of microfluidics, miniaturized opto-electronics, and automation	100 min
GFC Diagnostics	In development	Microscreen enzymatic-colorigenic DNA hybridization test on Safetube device	Non-specified
Great Basin Scientific, United States	Early stage	Whole blood, multiplexed nucleic-acid based assay using an opto-fluidic device; announced for 2021	Non-specified
Grenier Bio-One, United States	CE marked, not FDA cleared	PCR-based chip-probe Genspeed platform. Genspeed MRSA distinguishes MRSA/MRSE or *mec*A/C positive *S. haemolyticus*	1.5 h
Hain, Germany	CE marked for many tests	PCR/hybridization platform. GenoType, FluoroType and GenoQuick technologies, MRSA, CoNS	2.5 h
Icubate, United States	RUO	Random access multiplex PCR disposable test cassette for pathogens and resistances. Portfolio: gram + MSSA, *S. epidermidis*, MRSA	Non-specified
ID Biomedical, Corp., Vancouver	Early stage	Velogene rapid MRSA identification assay	2 h
Linear Diagnostics, Ltd.	In development	Detection of aligned substrate or PCR fragment via polarized light	Non-specified
Magnomics, Portugal	In development	Chip DNA extraction, amplification, and magnetic detection. Primary for veterinary application	1 h
Mobidiag, Finland	CE marked	Novodiag and Amplidiag product line. Sepsis, 60 bacterial species, 13 fungi, and *mec*A in one assay	3.5 h
Nanosphere Inc, United States	FDA cleared	DNA amplification-hybridization. Verigene BC-GP and BC-GN. Gold Nanoparticle Technology with oligo-hybridization to target DNA, narrow temperature range	2–2.5 h

Laboratory-Based and Point-of-Care Testing for MSSA/MRSA Detection in the Age of Whole Genome Sequencing

Alex van Belkum[1] and Olivier Rochas[2]*

European Journal of Clinical Microbiology & Infectious Diseases (2018) 37:2091–2096
https://doi.org/10.1007/s10096-018-3343-2

ORIGINAL ARTICLE

A 5-year study of the performance of the Verigene Gram-positive blood culture panel in a pediatric hospital

Chairut Vareechon[1] · Javier Mestas[1] · Claudia M. Polanco[1] · Jennifer Dien Bard[1,2]

Abstract

High accuracy of direct from positive blood culture molecular panels is imperative, particularly for the detection of resistance determinants as it allows for antimicrobial optimization prior to conventional susceptibility testing. In this study, we provide extensive data since implementation of the Verigene Gram-positive blood culture panel (BC-GP) in 2013. Within 5 years, 1636 blood culture bottles positive for a Gram-positive organism were tested on the BC-GP panel. The BC-GP panel identified 1520 Gram-positive organisms in 1636 (92.9%) blood cultures tested. For positive blood cultures, we observed 96.4% (806/834) concordance to the species level. Compared with conventional antimicrobial susceptibility testing, the positive percent agreement (PPA) of methicillin-resistant SA (MRSA) (50) and methicillin-resistant SE (MRSE) (365) was 100%. The *mecA* gene was detected in two methicillin-susceptible *Staphylococcus aureus* (MSSA) and one methicillin-susceptible *S. epidermidis* (MSSE) with a negative percent agreement (NPA) of 99.1% (221/223) and 99.2% (120/121), respectively. The PPA and NPA for vancomycin-resistant *Enterococcus faecium* (VRE) was 100%. The BC-GP panel demonstrated excellent performance and clinicians can confidently de-escalate antimicrobial therapy in the absence of *mecA* and *vanA/B* gene.

Table 4 Detection of resistance determinants using the BC-GP Panel

	Result values[a]				% (95% CI) for[b]	
Identification (resistance marker)	TP	FP	TN	FN	PPA	NPA
Staphylococcus aureus (*mecA*)	50	2	221	0	100 (91–100)	99.1 (96–100)
Staphylococcus epidermidis (*mecA*)	365	1	120	0	100 (99–100)	99.2 (95–100)
Enterococcus faecium (*vanA/vanB*)	5	0	12	0	100.0 (46–100)	100.0 (70–100)
Enterococcus faecalis (*vanA/vanB*)	0	0	84	0	ND	100.0 (95–100)
Total	420	3	437	0	100 (99–100)	99.3 (98–100)

[a] *TP*, true positive; *FP*, false positive; *TN*, true negative; *FN*, false negative

[b] *PPA*, positive percent agreement; *NPA*, negative percent agreement; *95% CI*, 95% confidence interval; *ND*, not detected

microorganisms

MDPI

Article

Comparison of four commercial screening assays for detection of blaKPC, blaNDM, blaIMP, blaVIM and blaOXA48 from rectal secretion collected by swabs.

Francesca Del Bianco [1,*], Manuela Morotti [1], Silvia Zannoli [1], Giorgio Dirani [1], Michela Fantini [1], Maria F Pedna [1], Patrizia Farabegoli [1] and Vittorio Sambri [1,2]

[1] Unit of Microbiology, The Great Romagna Hub Laboratory, 47822 Pievesestina (FC), Italy
[2] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, 40126 Bologna, Italy

A total of 1015 non-duplicated rectal swab specimens were prospectively collected using ESwab™ (COPAN Italia S.p.A., Brescia, Italy). The samples were transported to the Laboratory upon collection, processed within 24 hours and reported in 48 hours.

Table 1. *Detailed results of study specimens*

Assay		Routine screening tests			
	Assay results	Positive	Negative	Total	
Allplex Entero-DR assay	Positive	25	17	42	
	Negative	0	940	940	
	Total	25	957	982	
	Invalid				22
Amplidiag CARBAR+MCR kit	Positive	20	11 [a]	31 [a]	
	Negative	4	949	953	
	Total	24	959	983	
	Invalid				32
AusDiagnostics MT CRE EU assay	Positive	19	5	24	
	Negative	0	810	810	
	Total	19	815	834	
	Invalid				3
EasyScreen ESBL/CPO Detection Kit	Positive	22	5	27	
	Negative	0	751	751	
	Total	22	756	778	
	Invalid				40

[a] A specimen was KPC positive for routine screening, while with Amplidiag kit resulted positive for KPC and VIM targets.

Table 2. *Assay performance*

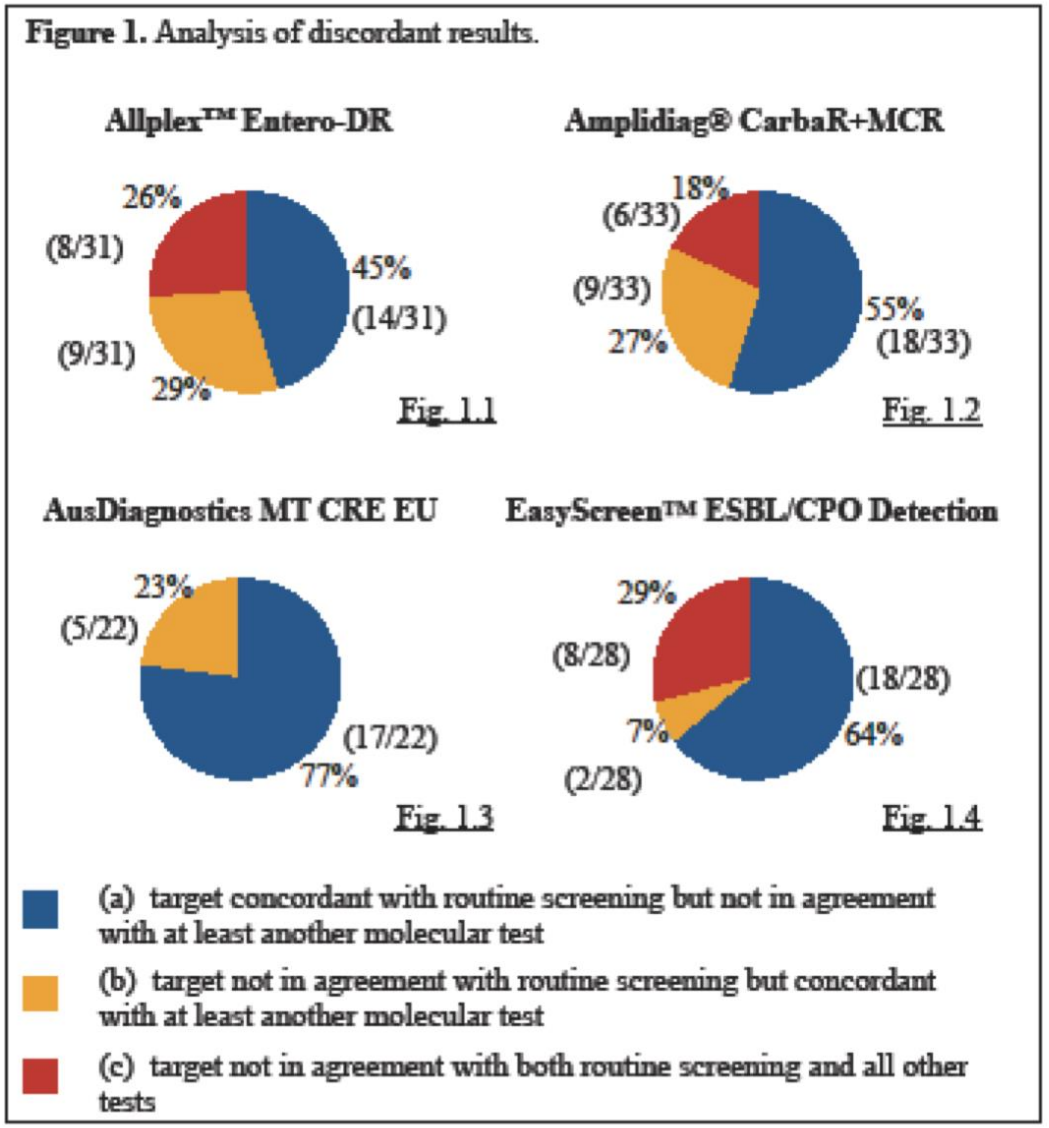
Assay	Sensitivity (%[95%CI])	Specificity (%[95%CI])	PPV (%[95%CI])	NPV (%[95%CI])	Overall % agreement (%[95%CI])	Kappa statistic
Allplex Entero-DR assay	100 (86.28- 100)	98.22 (97.17-98.96)	59.52 (47.86-70.20)	100	98,27 (97.24- 98.99)	0,74
Amplidiag CARBAR+MCR kit	83.33 (62.62-95.26)	98.85 (97.96-99.43)	64.52 (49.59-77.07)	99.58 (98.98-99.83)	98.48 (97.50-99.14)	0.72
AusDiagnostics MT CRE EU assay	100 (82.35-100)	99.39 (98.57-99.80)	79.17 (61.33-90.10)	100	99.40 (98.61- 99.81)	0.88
EasyScreen ESBL/CPO Detection Kit	100 (84.56-100)	99.34 (98.46-99.78)	81.48 (64.75-91.33)	100	99.36 (98.51-99.79)	0,89

Table 3. *Main characteristics of four commercial molecular kits for detecting carbapenemase genes in rectal swabs*

	Allplex Entero-DR Assay	**Amplidiag CARBAR+MCR kit**	**Ausdiagnostics MT CRE EU Assay**	**EasyScreen ESBL/CPO Detection Kit**
Sample throughput	up to 94 tests/ batch	up to 64 tests/ batch	24 up to 64 tests/ batch	up to 80 tests / batch
Hands on time	45min	1h	20 min. [a]	3h
Assay run time	4 h	5h	2h [a]	6h
Extraction control	Yes	yes	yes	yes
PCR control	Yes	yes	yes	yes
intrinsic control	Yes	no	yes	yes
Other targets	vanA; vanB; CTX-M	AcOXA; MCR 1/2; GES-CPO;	SME; OXA-23,51,58-like; CTX-M group 1 and group 9; GES	TEM; DHA; CTX-M; CMY SHV; OXA 23; 51-like
Traceability	Yes	yes	Depending on DNA extraction system	no

[a] without extraction step.

Figure 1. *Analysis of discordant results*



When I say “we”……. I mean THEM