Methods for Active Screening for Colonisation by ESBL and carbapenemases

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- Presentation is based on:
  - Our recent review paper (Gazin et al, J. Clin Microbiol 50: 1140-6, 2012)
  - Information provided by certain authors
  - Information (or lack of…) provided by certain companies
  - Poster presentations at ECCMID 2012

Agenda

- Background
- Culture-based methods for screening of patient samples
  - ESBL
    - Non-chromogenic media
    - Chromogenic media
  - Carbapenemases
    - Non-chromogenic media
    - Chromogenic media
- Molecular-based methods for screening of patient samples
  - In house methods
  - Commercial methods
- Conclusions and critical notes

Classification of β-Lactamases

Class A

- PC
- SHV-1, TEM-1, 2
- **SHV->1**
- **TEM->2**
- **CTX-M**
- PER
- VEB
- CARB
- RTG
- CepA
- IMI
- SME
- NMC
- IND
- **KPC**
- GES
- BIC

Class B

- IMP
- **VIM**
- KHM
- SPM
- GIM
- SIM
- **NDM**
- AIM
- DIM
- BEL

Class C

- **AmpC**
- **CMY**
- ACT
- DHA
- ACC
- FOX

Class D

- OXA-1, 10,
- OXA-11, 15
- OXA-23/27 24/40 **48,** 51/66/69 **58**, 143

ESBL

Carbapenemases

Metallo-β-Lactamases (MBLs)

Bush & Jacoby, Antimicrob Agents Chemother 2010; 54: 969 – 76
Patel & Bonomo, Expert Rev Anti Infect Ther 2011; 9: 555 - 70

Screening for Colonization: Why? Where? What?

- **Why?**
  - Infection control?
  - Decolonization?
  - Monitoring ESBL or carbapenemase status of critically ill patients
- **Where?**
  - Fecal samples?
  - Rectal swabs?
  - Other human samples?
- **What?**
  - Phenotypic resistance in isolates?
  - Resistance genes ?
    - In isolates?
    - In samples?

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  - Commercial methods

Culture-Based Detection of ESBL-Harbouring *Enterobacteriaceae*

- **Non-chromogenic selective media:**
  - Culture media (with cefotaxime and/or ceftazidime)
    - MacConkey agar
    - Drigalski agar
  - Commercial ready-to-use media plates
    - BLSE agar (AES Chemunex, France)
    - EbSA ESBL (AlphaOmega, the Netherlands)

- **Chromogenic selective media:**
  - ChromID ESBL (bioMérieux, France)
  - Brilliance ESBL (Oxoid, UK)
  - CHROMagar ESBL and CHROMagar CTX (CHROMagar, France); (Colorex ESBL, RambaCHROM ready-to-use media plates)

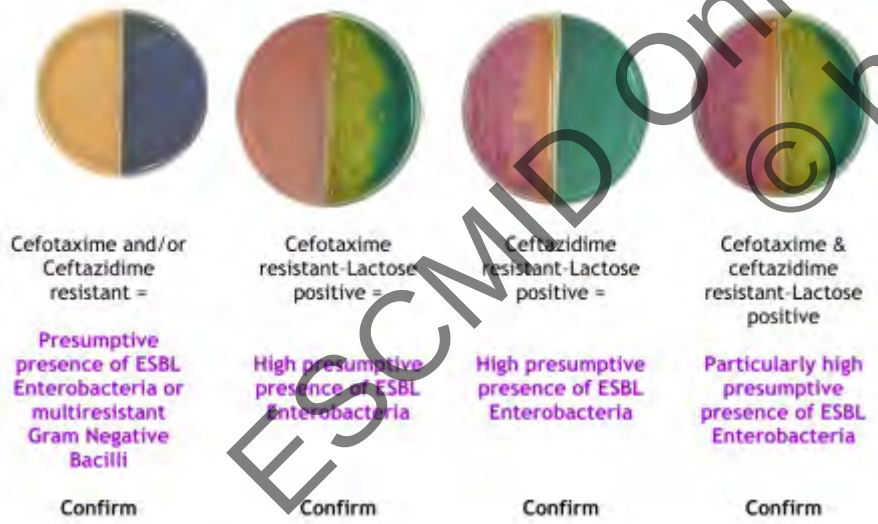
Commercial Ready-to-Use Media Plates

BLSE agar

(AES Chemunex, France)

MacConkey + ceftazidime

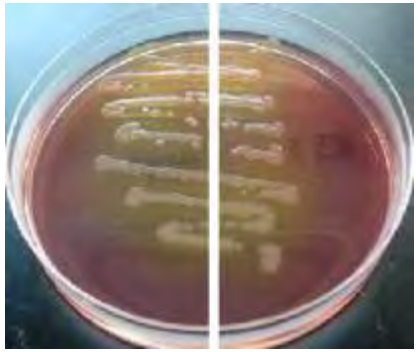
Drigalski + cefotaxime



EbSA-ESBL agar

(AlphaOmega B.V., The Netherlands)

MacConkey + ceftazidime



MacConkey + cefotaxime

+ cloxacillin

+ vancomycin

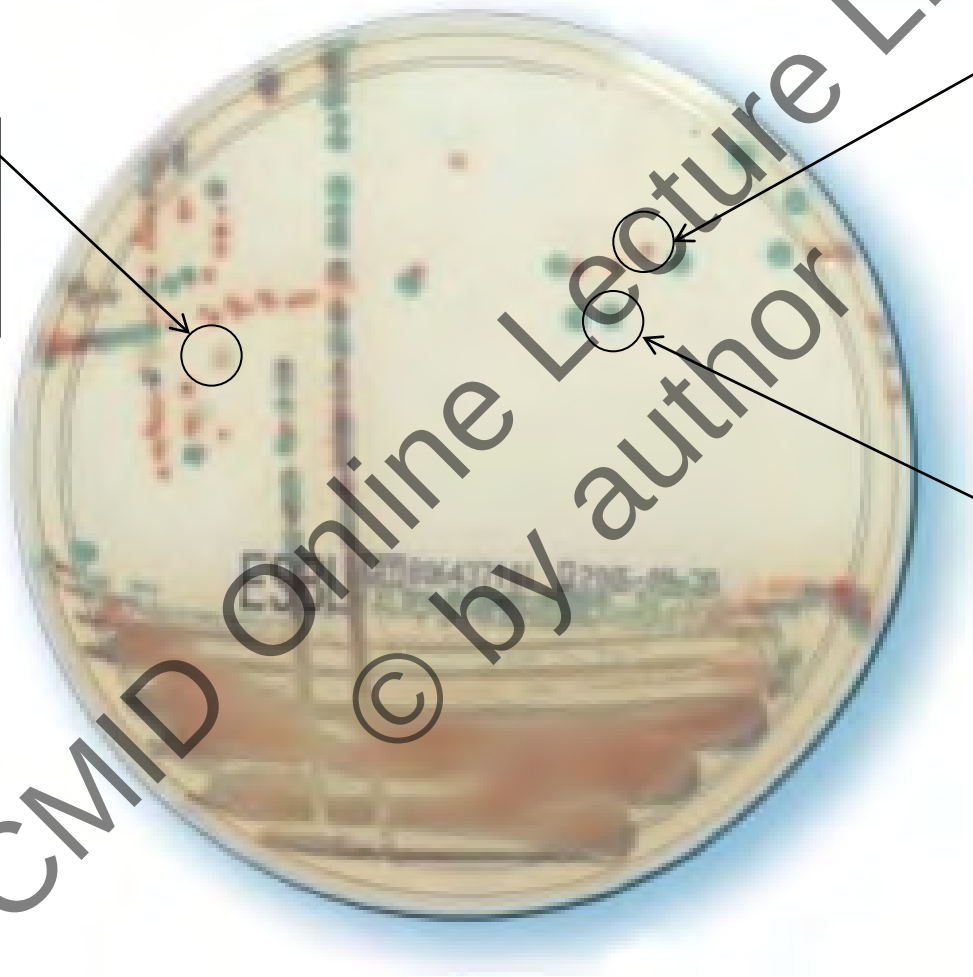
Chromogenic Media/plates for Detection of ESBL-Harbouring *Enterobacteriaceae*

Chromogenic media	Antibiotic	Enzyme	***E. coli***	***Klebsiella, Enterobacter Serratia Citrobacter***	***Proteus Morganella Providencia***
chromID ESBL	cefpodoxime	β-glucuronidase	pink to burgundy		
		β-glucosidase		green/blue to browny-green	
		tryptophan deaminase			dark to light brown
Brilliance ESBL	cefpodoxime	β-glucuronidase + β-galactosidase	blue		
		β-glucuronidase	pink		
		β-galactosidase		green	
		tryptophan deaminase			brown halo
CHROMagar ESBL	Not disclosed		dark pink to reddish		
				metallic blue	
					brown halo

Characteristics of Colonies of non-fermenting Gram-Negative Bacteria on Chromogenic Media

Chromogenic media	***Pseudomonas***	***Acinetobacter***	***Stenotrophomonas***
chromID ESBL	colorless or naturally reddish/brownish (pyocyanin pigmentation)	Colorless or white	May grow
Brilliance ESBL	colorless or naturally reddish/brownish (pyocyanin pigmentation)	Colorless or white	Mostly inhibited
CHROMagar ESBL	green/brown or white	Colorless or white	No information

ChromID ESBL

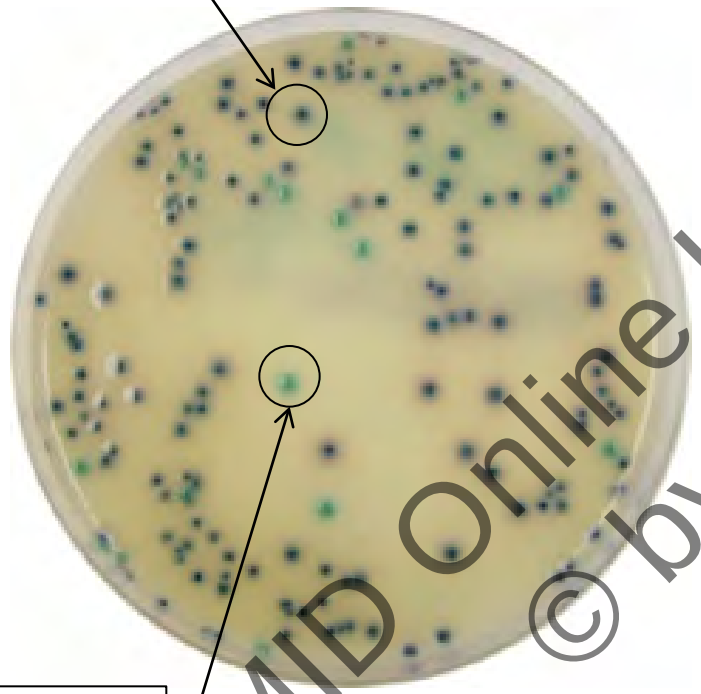


Proteus
Morganella
Providencia

E. coli

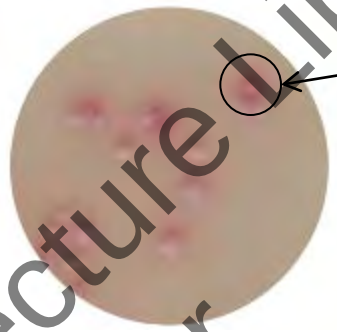
Klebsiella
Enterobacter
Serratia
Citrobacter

Brilliance ESBL

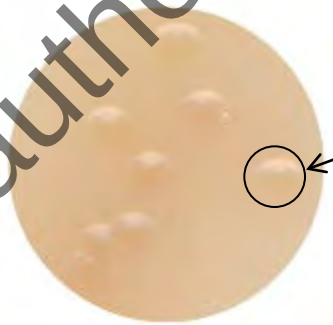


E. coli

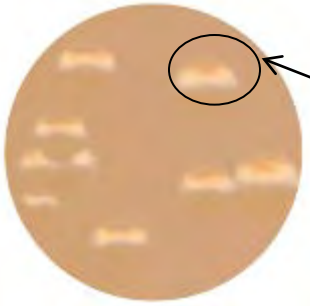
Klebsiella
Enterobacter
Serratia
Citrobacter



E. coli



Proteus
Morganella
Providencia



Acinetobacter,
other resistant
bacteria

Analytical Performance of EbSA and ChromID

	Strains tested (n)	Proportion of ESBL producers in total tested strains (%)	Media	Sensitivity %	Specificity %
Group 1	*E. coli* (334) *Klebsiella* spp. (124) *Proteus* spp (42) *Salmonella* spp. (3) *Shigella* spp. (2)	291/505 (57.6)	chromID ESBL	97.3	93.9
			EbSA	96.6	93.9
Group 2	Enterobacter spp. (90) *Citrobacter* spp (30) *M. morganii* (9) *S. marcescens* (6) *Providencia* spp. (2)	65/137 (47.4)	chromID ESBL	98.5	44.3
			EbSA	96.9	78.6

AmpC

→ EbSA (containing cloxacillin) had best overall performance as screening test

Overdevest IT et al, (2011) J. Clin. Microbiol. 49:519-522.

Analytical Performance of Brilliance ESBL and ChromID ESBL

TABLE 1. Species and resistance mechanism distribution of the 200 challenge strains tested on the two chromogenic media

Group and resistance mechanism	Species	No. of isolates growing on:		No. of isolates tested
		BM chromID	OX Brilliance	
Enterobacteria				
ESBL	*E. coli*	45	45	45
	K. pneumoniae	22	22	22
	E. aerogenes	20	20	20
	E. cloacae	5	5	5
	C. freundii	3	3	3
	K. oxytoca	3	3	3
Carbapenemase	*K. pneumoniae*	2	2	2
AmpC cephalosporinase	*E. coli*	9	9	12
	E. aerogenes	11	11	11
	C. freundii	6	5	6
	M. morganii	2	3	3
	K. pneumoniae	2	2	2
KI-OXY penicillinase	*K. oxytoca*	8	8	8
OXA-30 penicillinase	*E. coli*	3	3	6
Susceptible (wild type)	*E. coli*	0	0	4
	K. pneumoniae	0	0	2
	E. aerogenes	0	0	1
	K. oxytoca	0	0	1

Huang TD et al, (2010) Diagn. Microbiol. Infect. Dis. 70:565-567.

Clinical Performance of Culture-Based Media

Gazin and Paasch et al, (2012) J. Clin. Microbiol. 50:1140-1146

Chromogenic Medium	Samples (n)	Proportion of samples harboring ESBL producers in total tested samples (%)	Incubation time h	Comparator(s)	Sensitivity %	Specificity %	PPV %	NPV %	Ref
ChromID ESBL	Fecal (344) Respiratory tract (134) Wound, urine, vaginal, blood (50)	59/528 (11)	24	Brilliance ESBL MAC + CAZ (30 µg)	94.9 **86,4%**	95.5	70.8	98.2	(1)
	Fecal (500)	41/500 (8.2)	24	MAC + CAZ (1 µg/ml) MAC + CTX (1 µg/ml)	100	94.8	63	100	(2)
	Stool (186) Urine (48) Sputum (12) Wound (10)	17/256 (6.6)	24	CHROMagar ESBL	88.2	92.9	46.9	99.1	(3)
	Rectal swab (468) Urine (255) Pulmonary aspiration (42)	32/765 (4.2)	24 48	BLSE	88 94	94.4 90.5	38.7 28.4	99.6 99.9	(4)
	Fecal (561) Lower respiratory tract (63) Wound, ear-nose-throat (20)	37/644 (5.7)	18−24	MAC + CAZ (2 µg/ml)	97.7	90.4	ND	ND	(5)
CHROMagar ESBL	Stool (186) Urine (48) Sputum (12) Wound (10)	17/256 (6.6)	24	ChromID ESBL	100	93.3	51.5	100	(3)
Brilliance ESBL	Fecal (344) Respiratory tract (134) Wound, urine, vaginal, blood (50)	59/528 (11)	24	ChromID ESBL MAC + CAZ (30 µg)	94.9	95.7	73.7	99.3	(1)

MAC: MacConkey agar; CAZ:ceftazidime; CTX:cefotaxime; ND: not described

1: Huang TD et al, (2010) Diagn. Microbiol. Infect. Dis. 70:565-567
2: Paniagua R et al, (2011) Diagn. Microbiol. Infect. Dis. 67:376-379
3: Saito R et al, (2010) Lett. Appl. Microbiol. 51:704-706.
4: Réglier-Poupet H et al, (2010) J. Med. Microbiol. 57:310-315
5: Glupczynski Y et al, (2007) J. Clin. Microbiol. 45:501-505

Conclusions

- Performance of media depends on resistance mechanisms, bacterial species, samples, time of incubation, reference method, definition of true positives, …
- Concerning low specificity, need additional confirmation of the ESBL phenotype and organism identification
- If longer incubation (48 h), increased sensitivity, but decrease of specificity and PPV
- **Sensitivity:**
  - Missing CTX-M ESBLs on non-chromogenic media with ceftazidime
  - Superior recovery of CTX-M-type ESBLs on chromogenic media with cefpodoxime
  - Missing colorless (on ChromID) and atypically pigmented *E. coli* (on Brilliance)
- **Specificity**
  - High for EbSA (cloxacillin) and CHROMagar CTX (inhibition of AmpC)
  - May decrease due to growth of AmpC, K1-OXY *K. oxytoca* and some OXA *E. coli* hyperproducers
  - May decrease due to non-fermentative Gram-negative bacilli

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- Molecular-based methods for screening of patient samples
  - In house methods
  - Commercial methods

Culture-Based Detection of Carbapenemase-Harbouring *Enterobacteriaceae*

- **Non-chromogenic home-made selective media:**
  - Tryptic soy broth or agar / MacConkey agar / Drigalski agar
    - with carbapenems diluted or used as disks or Etest strips;
    - combined with inhibitor test:
      - Phenylboronic acid (KPC inhibitor)
      - EDTA (MBL inhibitor)
  - Drigalski agar medium with carbapenem, cloxacillin and zinc sulfate (SUPERCARBA)
- **Chromogenic commercial selective media:**
  - ID Carba (bioMérieux, France) – prototype (launched in June 2012?)
  - Brilliance CRE (Oxoid, UK)
  - CHROMagar KPC (CHROMagar, France); Colorex KPC (ready-to-use media plates)
  - HardyCHROM Carbapenemase (Hardy Diagnostics, USA)

Rectal Screening of KPC and MBL Carbapenemase-Producing *Enterobacteriaceae*

55 rectal swab suspensions inoculated on MacConkey agar + MER disk (10µg)

alone ≥ 24mm = negative for carbapenemase production

+ PBA (phenylboronic acid, 20mg/ml): ≥ 5mm difference from MER = KPC producer

+ EDTA (0,1M): ≥ 5mm difference from MER = MBL producer

+ PBA + EDTA: ≥ 5mm difference from MER = KPC + MBL co-producer

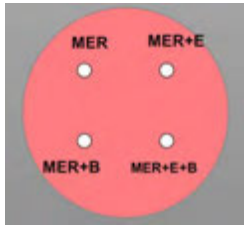
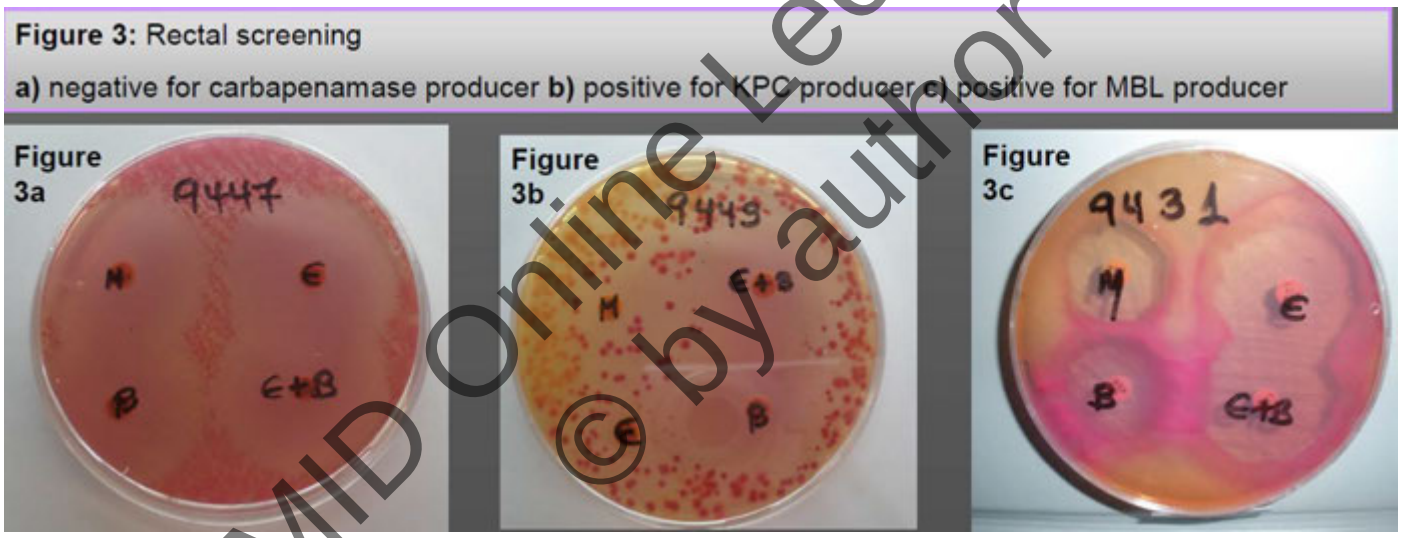


Figure 3: Rectal screening

a) negative for carbapenamase producer **b)** positive for KPC producer **c)** positive for MBL producer



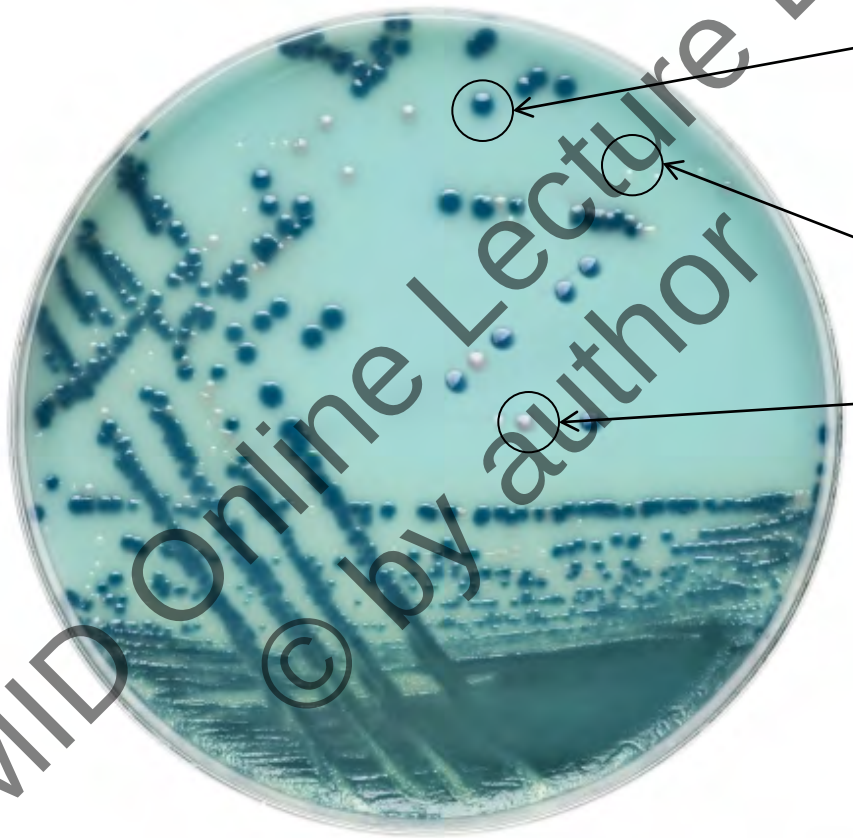
Method	Result			
	Negative	MBL	KPC	MBL+KPC
PCR	34	3	9	9
ERT disc test	35	3	8	9
MER disc test	36	2	8	9

→ Sensitivity 90% Specificity 100%

Chromogenic Media/plates for Detection of Carbapenemase-Harbouring *Enterobacteriaceae*

Chromogenic media	Antibiotic	Enzyme	***E. coli***	***K**lebsiella, **E**nterobacter **S**erratia **C**itrobacter*
ID Carba	Not disclosed		pink/red	blue-green
Brilliance CRE	Not disclosed		pale pink	blue
CHROMagar KPC	Not disclosed		Dark pink to reddish	blue

Brilliance CRE



Klebsiella
Enterobacter
Serratia
Citrobacter

Acinetobacter

E. coli

We need to agree on the colours…

- **Evaluation of Brilliance CRE at ECCMID 2012:**
  - Poster 1715:
    - "… a pale pink colour was observed for *E. coli…*"
  - Poster 1716:
    - "… *E. coli* colonies were brownish…"

Analytical Performance: Levels of Detection of Three Screening Media

TABLE 1. Levels of detection for the three CRE screening plates for detection of carbapenem-resistant *Enterobacteriaceae*

Bacterial strain	Species	*bla* type	MIC (μg/ml)			Level of detection (CFU/ml) on the following screening plate[a]:			Reference
			Imipenem	Meropenem	Ertapenem	MacI	MacD	Chrom	
490 (ST 258)	*K. pneumoniae*	KPC-3	32	32	64	1.1×10^2	1.1×10^3	1.1×10^2	11
9	*K. pneumoniae*	KPC-3	0.5	1	1.5	4.1×10^2	ND	4.1×10^3	16
14	*K. pneumoniae*	KPC-3	>32	>32	>32	1×10^2	1×10^2	1×10^2	16
533	*Enterobacter cloacae*	KPC-2	4	2	12	1.1×10^2	ND	1.1×10^2	3
360	*Escherichia coli*	KPC-2	1	1.5	2	9.7×10^1	ND	9.7×10^4	6
2438	*E. coli*	KPC-2	12	12	>32	6.5×10^1	2×10^6	6.5×10^1	6
1679	*E. coli*	KPC-2	4	2	12	8.9×10^1	ND	8.9×10^1	6
2112	*E. coli*	KPC-3	1	1.5	0.75	8.3×10^6	ND	ND	5
2565	*K. pneumoniae*	IMP-1	2	32	4	1.4×10^4	ND	1.4×10^6	MOSAR1144
2577	*K. pneumoniae*	VIM-1	32	32	32	1.1×10^2	1.1×10^5	1.1×10^2	MOSAR1156

[a] Chrom, CHROMagar-KPC plates; MacI, MacConkey agar with imipenem at 1 μg/ml; MacD, MacConkey agar plates with standard imipenem, meropenem, and ertapenem 10-μg paper disks; ND, no detection.

Conclusion:

- **All media perform well for detection of high-level carbapenem resistence**
- **MacConkey agar with disks lack sensitivity if lower MICs for carbapenems**

Adler et al, (2011) J. Clin. Microbiol. 49:2239-2242

Clinical Performance: Evaluation of Three Media for Detection of Carbapenem-Resistant *Enterobacteriaceae* from Rectal Swabs

TABLE 2. Summary of CRE screening plate performances

Method[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Mean turnaround time (range), days
MacI	84.9	94.3	82.3	95.2	92.1	2.8 (2–4)
MacD	75.8	89.6	69.5	92.2	86.3	2.8 (2–4)
Chrom	84.9	88.7	70	95	87.8	3.0 (2–4)

[a] Chrom, CHROMagar-KPC plates; MacI, MacConkey agar with imipenem (1 μg/ml); MacD, MacConkey agar plates with standard imipenem, meropenem, and ertapenem 10-μg paper disks.

Conclusions:

- Carbapenem disks on MacConkey plates was inferior

- Both CHROMagar KPC and MacConkey with imipenem diluted at 1 μg/ml showed similar sensitivity, but MacConkey was the most specific method

Sensitivity of Colorex KPC and ID Carba for Detection of *Enterobacteriaceae* with NDM-1 Carbapenemase from Stool Samples

		ID Carba	Colorex KPC
Number of **samples**	37	37 (**100%**)	36 (**97%**)
Number of **isolates**	64	56 (**88%**)	41 (**64%**)

Perry JD et al, (2011) J. Antimicrob. Chemother. 66:2288-2294

Why Did Colorex KPC Fail to Detect Many Isolates of *Enterobacteriaceae* with NDM Carbapenemases?

Table 1. Enterobacteriaceae with NDM carbapenemase and other isolates recovered from 200 stool samples using two chromogenic media

	Either medium	Colorex KPC	ID Carba
Enterobacteriaceae with the NDM-1 enzyme			
Escherichia coli	30	12	30
Enterobacter cloacae	21	21	17
Citrobacter freundii	4	1	4
Citrobacter braakii	1	1	1
Citrobacter sp. (novel species)	3	2	2
Klebsiella pneumoniae	3	2	2
Providencia rettgeri	2	2	0
total	64	41	56
Other carbapenemase producers			
Acinetobacter baumannii[a]	3	3	0
Aeromonas caviae[a]	1	1	0
Pseudomonas putida[b]	1	1	0
Comamonas testosteroni[c]	1	1	0
Stenotrophomonas maltophilia[c]	4	3	4
Carbapenemase-negative isolates			
Escherichia coli	9	2	9
Acinetobacter baumannii	3	3	1
Pseudomonas aeruginosa	1	1	0
Morganella morganii	1	1	1
Total isolates other than carbapenemase-producing Enterobacteriaceae	24	16	15

[a] bla_{NDM-1} confirmed by PCR.
[b] bla_{IMP} carbapenemase confirmed by PCR.
[c] Metallo-β-lactamase detected by diagnostic discs, but uncharacterized by PCR assays.

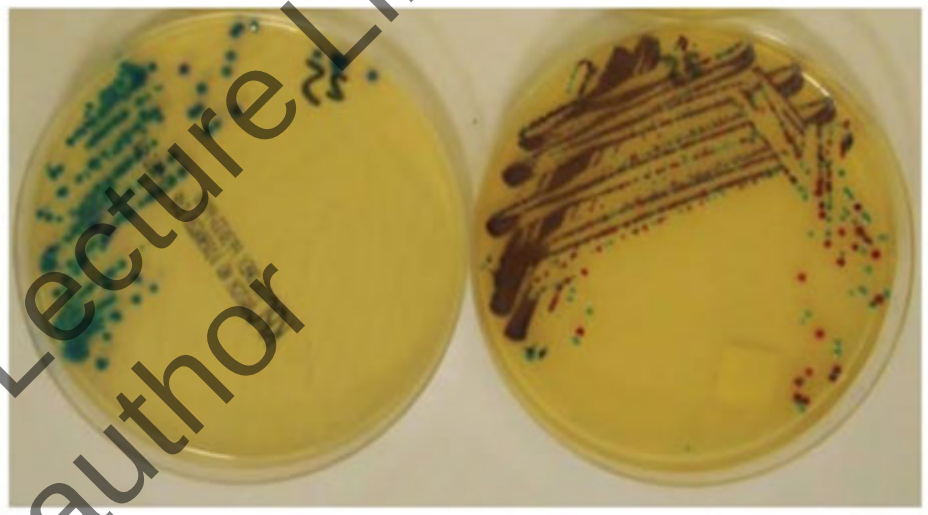


Figure 1. Pure growth of blue colonies of *C. freundii* with the NDM-1 enzyme on Colorex KPC medium (left). On ID Carba (right) the same specimen yields *C. freundii* as green colonies and *E. coli* as red colonies; both species produced NDM-1 carbapenemase.

Conclusion: Meropenem susceptible (MICs ≤ 2 µg/ml) NDM-1 producing *Enterobacteriaceae* were inhibited on Colorex KPC

Perry et al, J Antimicrob Chemother 2011; 66:2288-94

Evaluation of chromID CARBA, bioMérieux

- Two batches of chromID CARBA: one freshly prepared and one close to the expiry date
- Inoculated with 194 isolates
- Sensitivity OXA-48 carbapenemase:
  - Freshly prepared: 67%
  - Close to expiry date: 100%
- Sends bizarre message…
- Underscores stability problems with these media

JCM Accepts, published online ahead of print on 22 February 2012
J. Clin. Microbiol. doi:10.1128/JCM.06477-11

Detection of carbapenemase producers in *Enterobacteriaceae* using a novel screening medium

Patrice Nordmann*, Delphine Girlich, and Laurent Poirel

Service de Bactériologie-Virologie, INSERM U914 "Emerging Resistance to Antibiotics", Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, K.-Bicêtre, France

SUPERCARBA = Drigalski agar + cloxacillin + carbapenem + zinc sulfate

Evaluation of SUPERCARBA, CHROMagar KPC, and ChromID for Detection of Carbapenemase-producing *Enterobacteriaceae*

Table 2. Sensitivity and specificity of SUPERCARBA, ChromID ESBL, and CHROMagar KPC media[a]

	SUPER CARBA	ChromID ESBL	CHROMagar KPC
SN (%)[a]	95.6	87.7	40.3
SP (%)	82.2	24.2	85.5
SN class A[b]	100	100	66.7
SN class B	90	98	55.8
SN class D	100	70	13.6

Conclusions:

- **Detects OXA-48, non-ESBL producing strains**
- **More specific than ChromID ESBL**

Conclusions

- **Home-made MacConkey agar plates:**
  - With carbapenems disks: low sensitivity and specificity
  - With carbapenems diluted: better sensitivity, low specificity
  - Many validation and quality control issues (stability of supplement, etc)
- **chromID ESBL, bioMérieux:**
  - Fails to detect OXA-48 producers (in the absence of co-production of ESBL)
  - Lack of specificity (ESBL producers co-selected)
- **chromID CARBA, bioMérieux:**
  - Good sensitivity and specificity (so far…)
- **CHROMagar KPC or Colorex KPC, CHROMagar:**
  - Only detects high level carbapenem resistance
- **Brilliance CRE, Oxoid:**
  - Good sensitivity and specificity (so far…)

General Conclusions

- You need to test and train your ability to recognise colors…
- Need additional confirmation of the resistance mechanism and organism identification
- Performance of media depends on level of carbapenem resistance, hydrolysis of other beta-lactam (spectrum), presence of other resistance mechanisms, geographic location, bacterial species, samples, time of incubation, reference method, definition of true positives, …
- Makes comparison of studies and media very difficult
- Provide guidance on how to properly evaluate the performence of the screening media
- Need independant investigators evaluating these media

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DNA Amplification Based Methods for ESBL and CPE Detection

- **In house methods:**
  - Singlex or Multiplex PCR, amplicon detection by
    - Gel electrophoresis / sequencing (Wickramasinghe et al, JAC, 2012)*
    - Array hybridization (Ensor et al, JAC, 2007; Leinberger et al, JCM, 2010)
    - Electrospray ionization – mass spectrometry (Endimiani et al, JAC, 2010)
  - Real-time PCR, amplicon detection by
    - TaqMan assay (Naas et al, AAC, 2011)*
    - Real-time pyrosequencing (Naas et al, AAC, 2007)

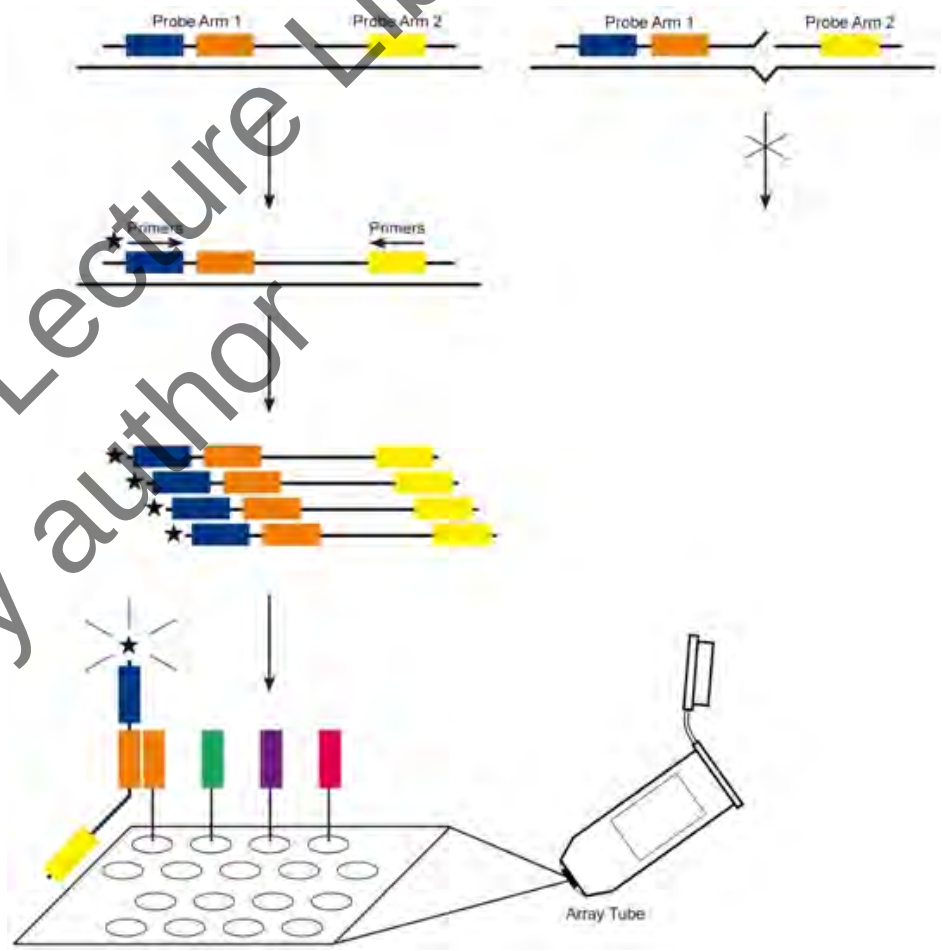
*Tested on faecal samples

- **Commercial methods:**
  - Tube microarray
    - Check-Points assays, Check-Points Health, The Netherlands
    - Identibac AMR-ve assays, Alere GmbH, Germany
  - Enzyme linked immunosorbent assay (ELISA)
    - Hyplex assays, Amplex Diagnostics, Germany*

*Tested on blood, urine, pus and sputum samples

Check-Points Assays

- Ligation-mediated amplification
- Hybridization on tube array on the basis of a unique probe ‘ZIP code’
- Visualization colorimetricaly using streptavidin-horseradish peroxidase and tetramethyl benzidine
- TAT: 6 hrs



Check-Points

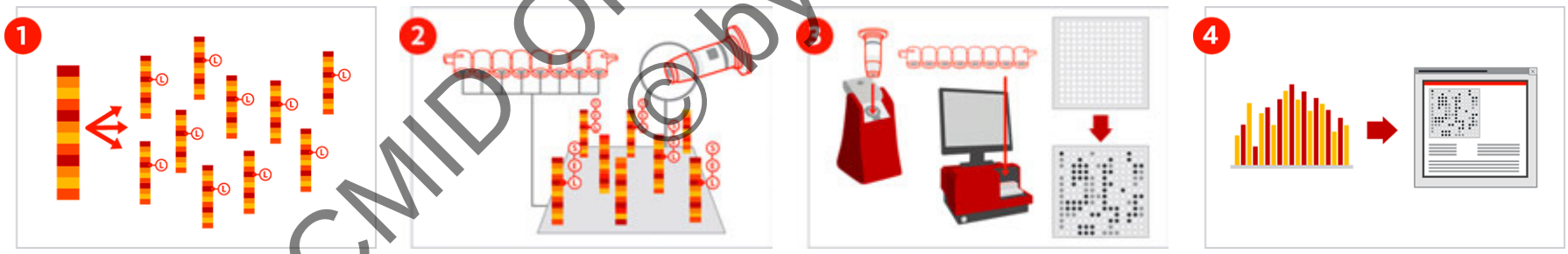
Detection Spectrum of Check-Points Assays

- **Check-ESBL**
  - Non-ESBL TEM and SHV
  - ESBLs: SHV, TEM, and CTX-M types
- **Check-KPC ESBL**
  + Carbapenemase: KPC
- **Check-MDR CT101**
  + Carbapenemases: KPC, NDM
  + AmpCs: CMY, DHA, FOX, MOX, ACC, MIR, ACT
- **Check-MDR CT102**
  + Carbapenemases: KPC, NDM, VIM, IMP, OXA-48
- **Check-MDR CT103:** evaluation in progress
  + Carbapenemases: KPC, NDM, VIM, IMP, OXA-48
  + AmpCs: CMY, DHA, FOX, MOX, ACC, MIR, ACT

Check-Points

Identibac AMR-ve Assays

- Amplification by linear multiplex-PCR
- Hybridization on microarray of biotin-labeled amplicons
- Visualization colorimetricaly by streptavidin-horseradish peroxidase conjugation with seramun green staining
- TAT: 8 hrs



IDENTIBAC. Alere™

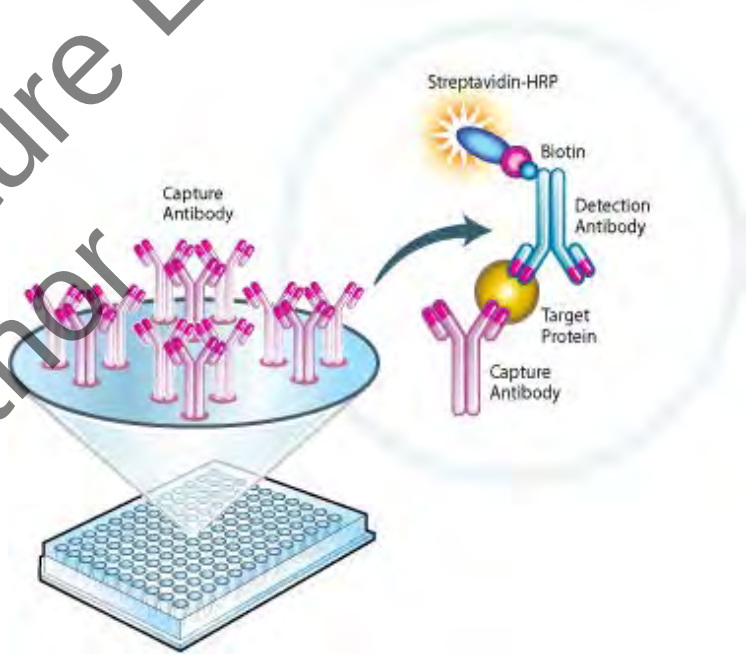
Detection Spectrum of Identibac AMR-ve Assays

- **Identibac AMR-ve Array Tube**
  - β-lactams (ESBLs)
  - Aminoglycosides
  - Trimethoprim
  - Sulphonamides
  - Tetracyclines
  - Quinolones
- **Identibac AMR-ve Array Tube, updated version**
  - β-lactams (ESBLs)
  - Aminoglycosides
  - Trimethoprim
  - Sulphonamides
  - Tetracyclines
  - Quinolones
  - Chloramphenicols
  - Streptogramins
  - Macrolides/erythromycin

IDENTIBAC. Alere™

Hyplex Assays

- Amplification by multiplex-PCR
- Hybridization on ELISA-microwell plates by immobilized reverse probes
- Visualization colorimetricaly by peroxidase-conjugated antibodies
- TAT: around 8 hrs



Detection Spectrum of Hyplex Assays

- **Hyplex ESBL ID**
  - ESBL: SHV, TEM, CTX-M, OXA
- **Hyplex MBL ID**
  - Metallo-β-lactamases: VIM, IMP
- **Hyplex Superbug ID, updated Hyplex MBL ID**
  - Metallo-β-lactamases: VIM, IMP, NDM-1
  - Carbapenemases: KPC, OXA-48

Analytical Evaluations of Check-Points Assays

Assay type	Samples (n)	Comparators	Sensitivity %	Specificity %	Ref
Check-ESBL	212	PCR/sequencing	95 (ESBL)	100 (ESBL)	(1)
Check-KPC ESBL	106	PCR/sequencing Analytical isolelectrical focusing	92	100 (SHV, CTX-M, KPC) 96 (TEM)	(2)
	125	PCR/sequencing	95	100	(3)
	344	PCR/sequencing	97 (ESBL)	98 (ESBL)	(4)
	638	Susceptibilty testing	98 (ESBL)	99 (ESBL)	(5)
Check-MDR CT101	182	PCR/sequencing	100	100	(6)
Check-MDR CT102	144	PCR/sequencing Analytical isolelectrical focusing	99	100	(7)
	64	PCR/sequencing	100 (carbapenemases)	96 (carbapenemases)	(8)

(1) Cohen Stuart *et al*., (2010), J. Antimicrob. Chemother. 65:1377-1381;
(2) Endimiani *et al*., (2010), J. Clin. Microbiol., 48:2618-2622;
(3) Naas *et al*., (2010), Antimicrob. Agents Chemother., 54:3086-3092;
(4) Platteel *et al*., (2011), Clin. Microbiol. Infect., 17:1435-1438;
(5) Willemsen *et al*., (2011), J. Clin. Microbiol., 49:2984-2987;
(6) Bogaerts *et al*., (2011), Antimicrob. Agents Chemother., 55:4457-4460;
(7) Naas *et al*., (2011), J. Clin. Microbiol., 49:1608-1613;
(8) Woodford *et al*., (2011), J. Antimicrob. Chemother., 66:2887-2888

Analytical Evaluations of Identibac AMR-ve Assays

Assay type	Samples (n)	Comparators	Sensitivity %	Specificity %	Ref
Identibac AMR-ve Array Tube (6 antibiotic classes)	119	PCR Susceptibility testing	–	–	(1)
	15	PCR Enterobacterial Resistance Virulence array	–	–	(2)
Identibac AMR-ve Array Tube (9 antibiotic classes)	30	PCR/sequencing	93 (ESBL)	50 (ESBL)*	(3)

*Low specificity % due to the assay inability to distinguish extended-spectrum from narrow-spectrum β-lactamases

(1) Batchelor *et al*., (2008). Int. J. Antimicrob. Ag., 31:440-451;
(2) Walsh *et al*., (2010). Int. J. Antimicrob. Ag., 35:593-598;
(3) Gazin *et al*., in preparation

Analytical Evaluations of Hyplex Assays

Assay type	Samples (n)	Comparators	Sensitivity %	Specificity %	Ref
hyplex® ESBL ID	73	Vitek 2 ESBL E-test	100	100	(1)
hyplex® MBL ID	326	E-test PCR	99	98	(2)

(1) Paniara *et al*., (2008), ECCMID, P881
(2) Avlami *et al*., (2010), J. Microbiol. Methods, 83:185-187

Commercial Screening Methods for Detection of Intestinal Carriage of MDR-GNB Under Development in R-GNOSIS

Company	Test	Sample prep.	TAT / No of samples	Timing
Genewave (France)	LOC platform with automated 20-plex PCR	Commercial kit	5 hrs 2 / day	October 2012
Check-Points (The Netherlands)	Real-time PCR	Commercial kit (e.g. EasyMag, Qiagen)	1.5 hrs 96	July 2012

R-GNOSIS

Conclusions

- DNA amplification based assays are still rather slow, not user-friendly and mostly reliant on conventional culture
- Great medical need to develop rapid POCT for detection of ESBLs and CPE
- Several companies and research projects are working on such POCTs

THANK YOU

PLEASE COMMENT ON MY PRESENTATION