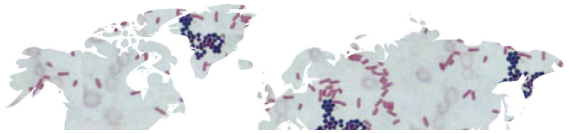
Rapid identification of multidrug resistance in gram negatives

UNIVERSITÉ DE FRIBOURG SUISSE
UNIVERSITÄT FREIBURG SCHWEIZ



Emerging Antibiotic Resistance Unit

hôpital fribourgeois
freiburger spital

Prof. Patrice Nordmann

the WHITE HOUSE PRESIDENT BARACK OBAMA

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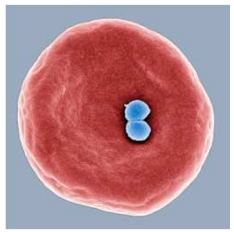
For Immediate Release September 18, 2014

FACT SHEET: Obama Administration Takes Actions to Combat Antibiotic-Resistant Bacteria



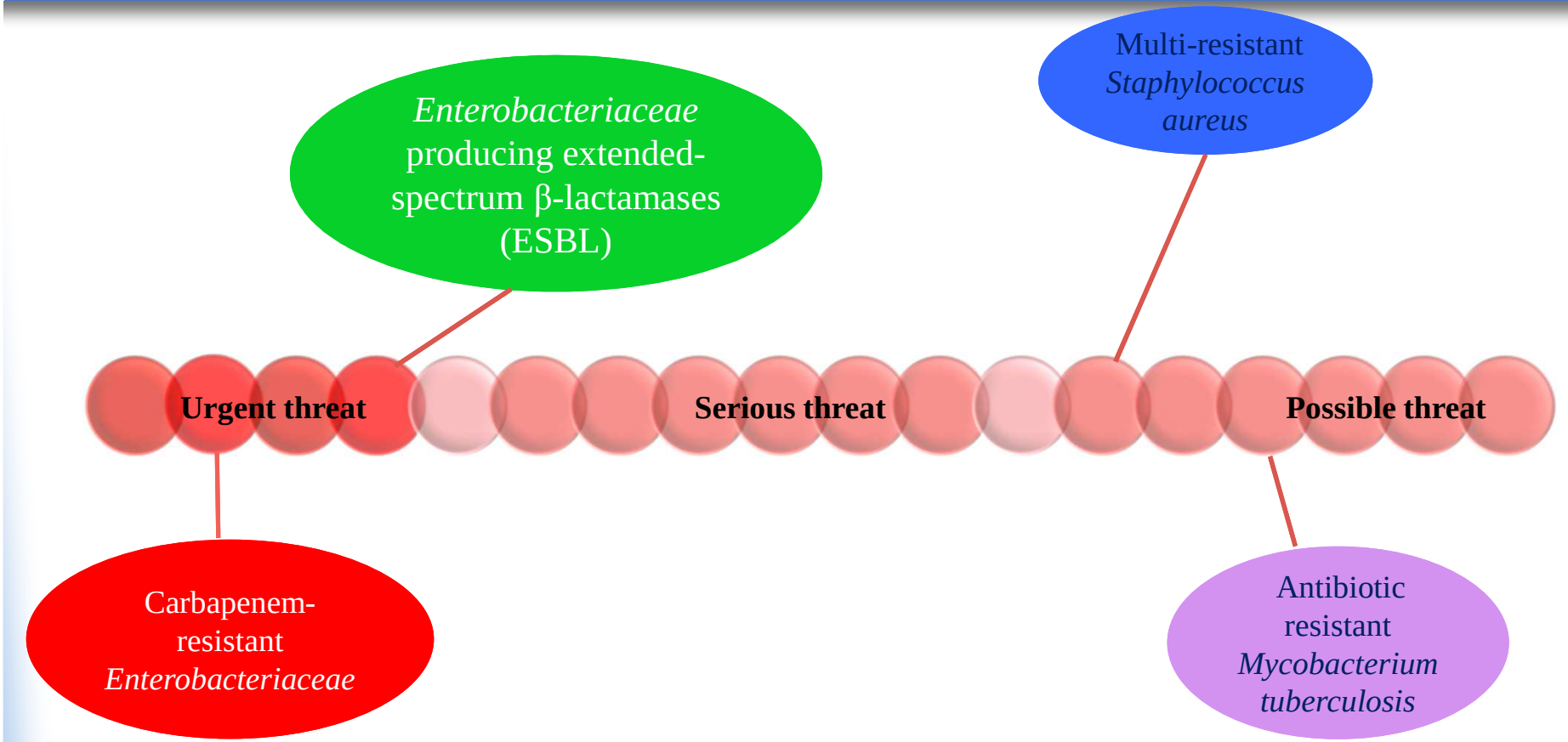
NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

MARCH 2015

The goals of the *National Action Plan* include:

1. Slow the Emergence of Resistant Bacteria and Prevent the Spread of Resistant Infections.
2. Strengthen National One-Health Surveillance Efforts to Combat Resistance.
3. Advance Development and Use of Rapid and Innovative Diagnostic Tests for Identification and Characterization of Resistant Bacteria.
4. Accelerate Basic and Applied Research and Development for New Antibiotics, Other Therapeutics, and Vaccines.
5. Improve International Collaboration and Capacities for Antibiotic-resistance Prevention, Surveillance, Control, and Antibiotic Research and Development.

Emerging Resistance threats, CDC – USA-2013

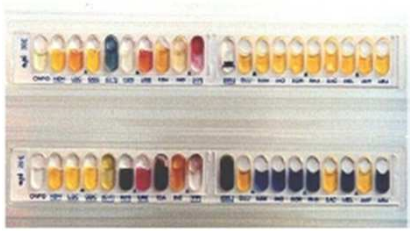


Classical scheme for diagnostic in microbiology

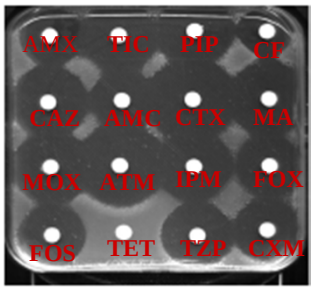
Direct examination
Gram staining

Culture
18h (*E. coli*)
to three weeks
(Mycobacteria)

Phenotypic identification



Antibiogram



From 48 h Pasteur's Microbiology to Rapid Diagnostic testing (30 min)

Clinical sample

+ 24 h

In-vitro culture (*E. coli*)

+ 24 h

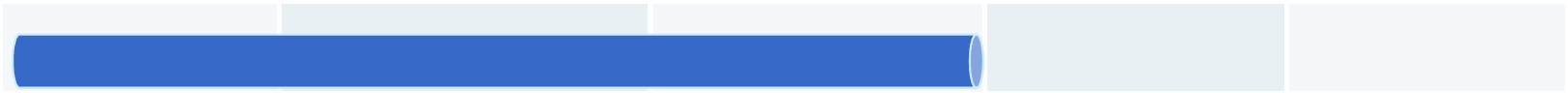
Antibiotic susceptibility testing

A culture change…. without culture !

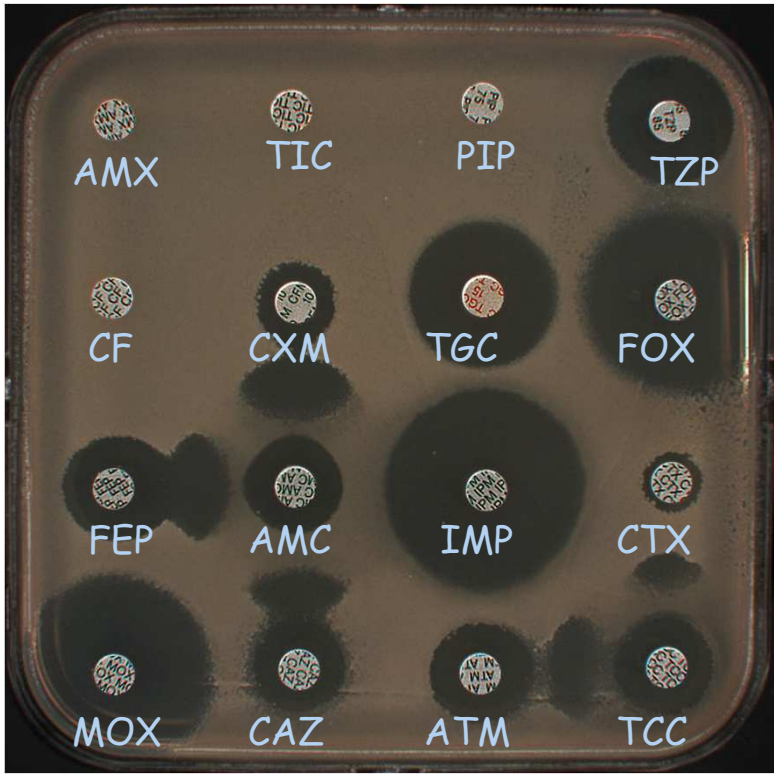
Broad-spectrum ß-lactamases in gram negatives

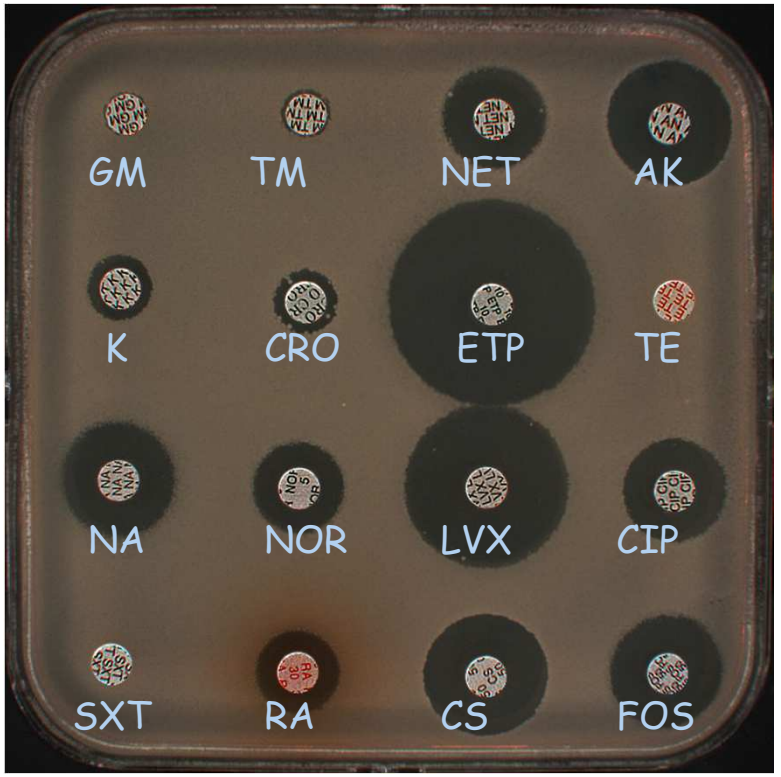
Penicillins Cephalosporins Carbapenems

Extended-spectrum ß-lactamases (ESBL); CTX-M

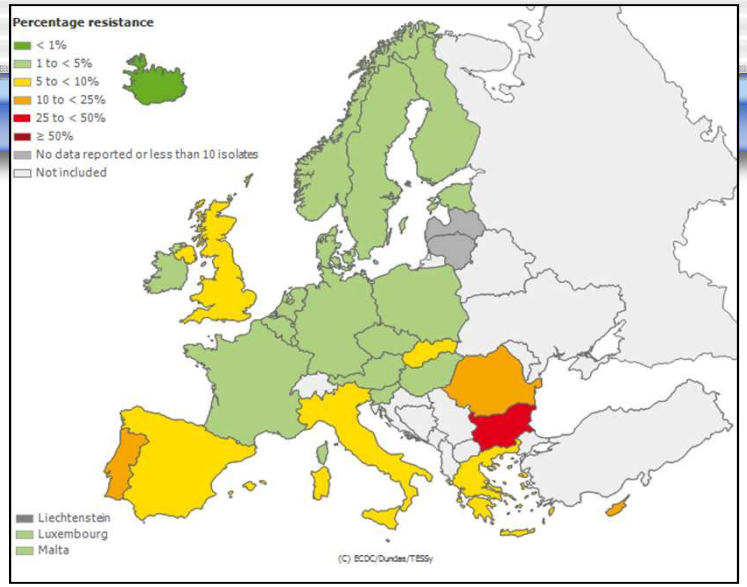


Multidrug resistance of ESBL-producing *Escherichia coli*

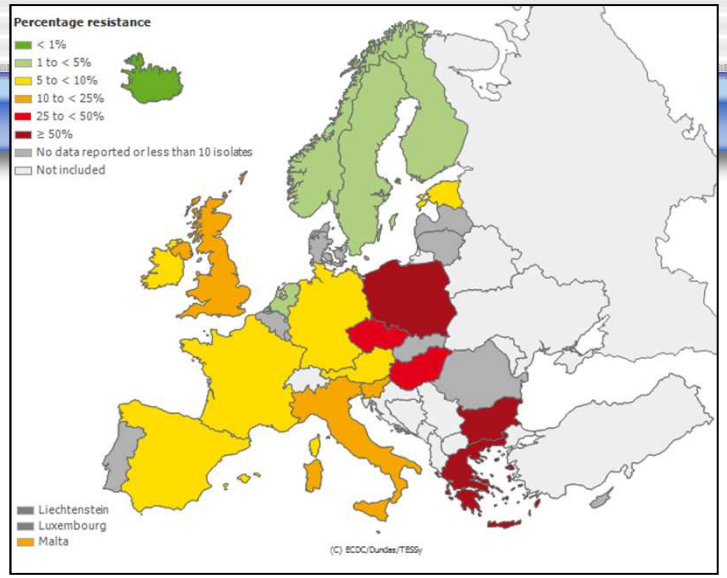




Resistance to expanded-spectrum cephalosporins. *Enterobacteriaceae* in Europe

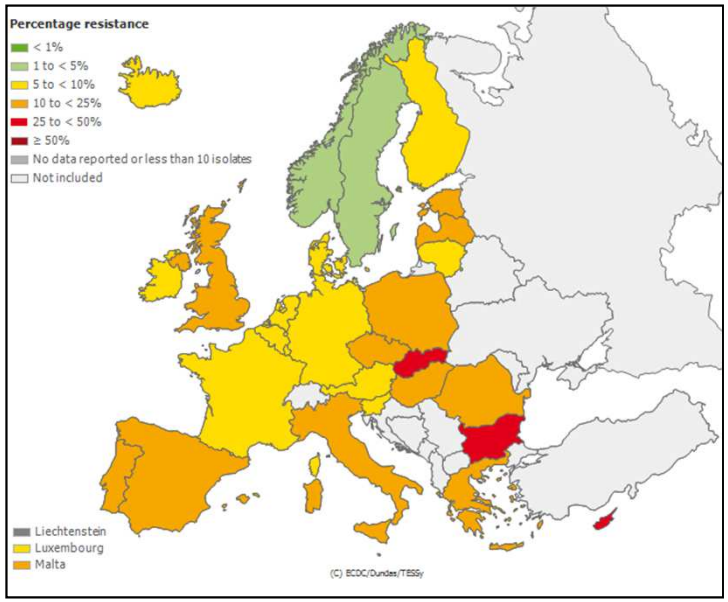


2005

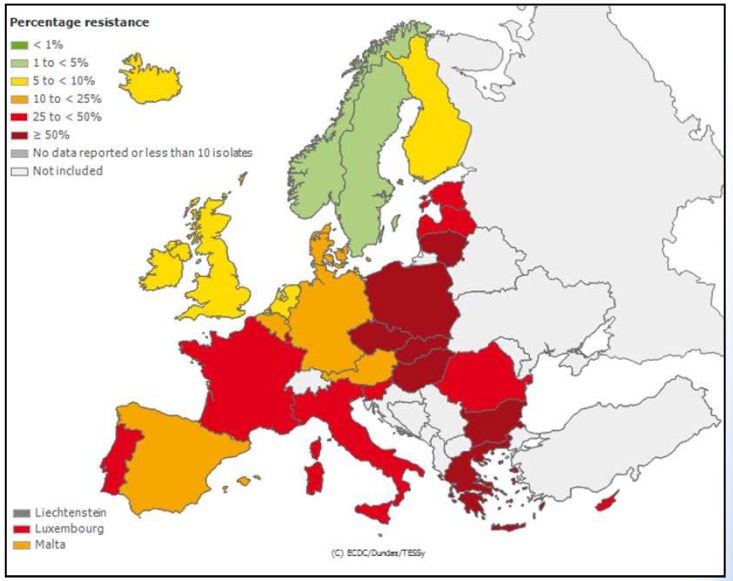


E.coli

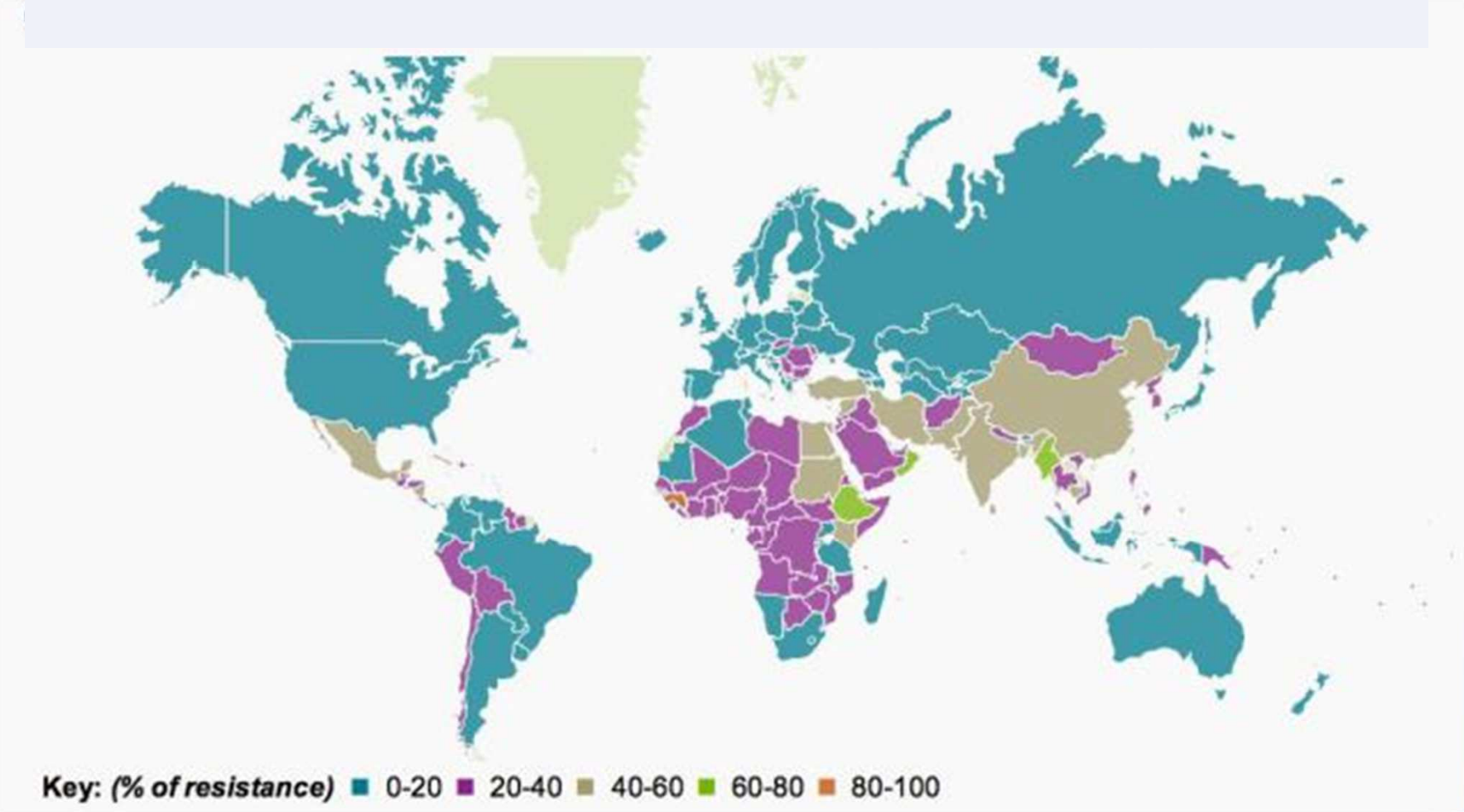
K. pneumoniae



2011

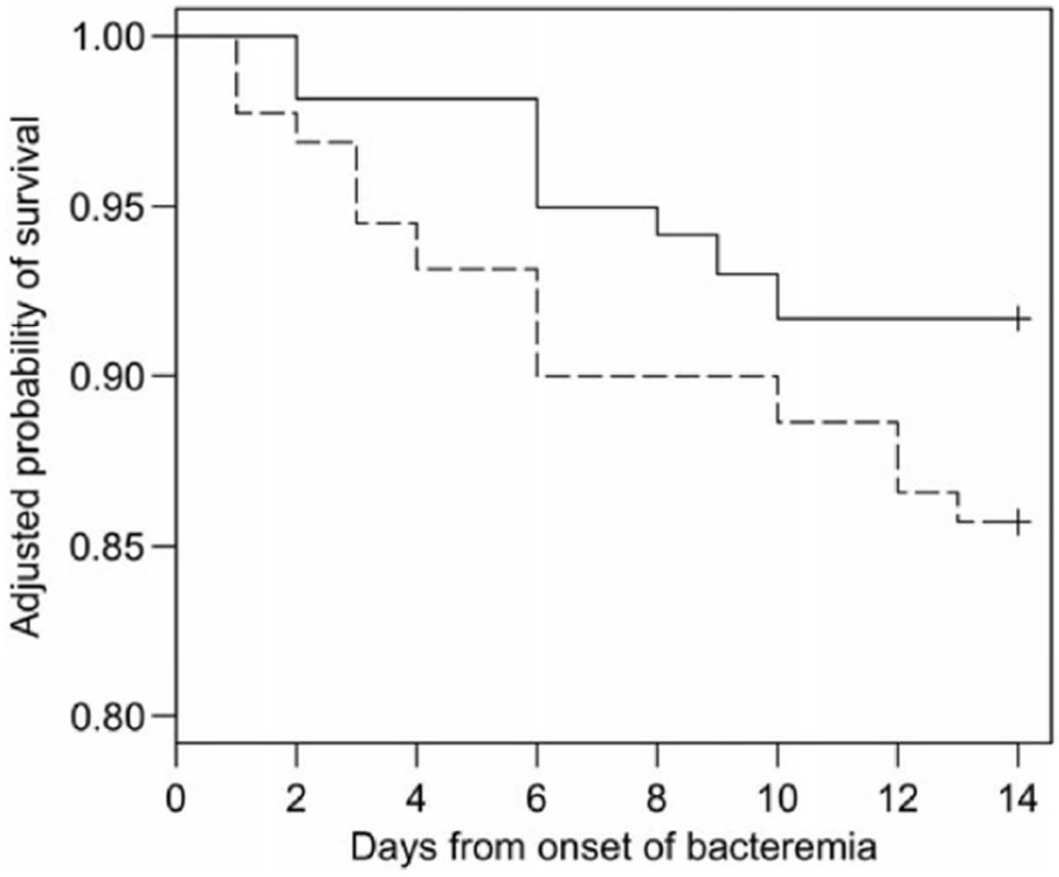


Global resistance rates for *Escherichia coli* to third generation cephalosporins: mostly CTX-M



Review on Antimicrobial Resistance, J O'Neill, 2014 UK Prime Minister

Probability of survival of patients with ESBL bacteremia

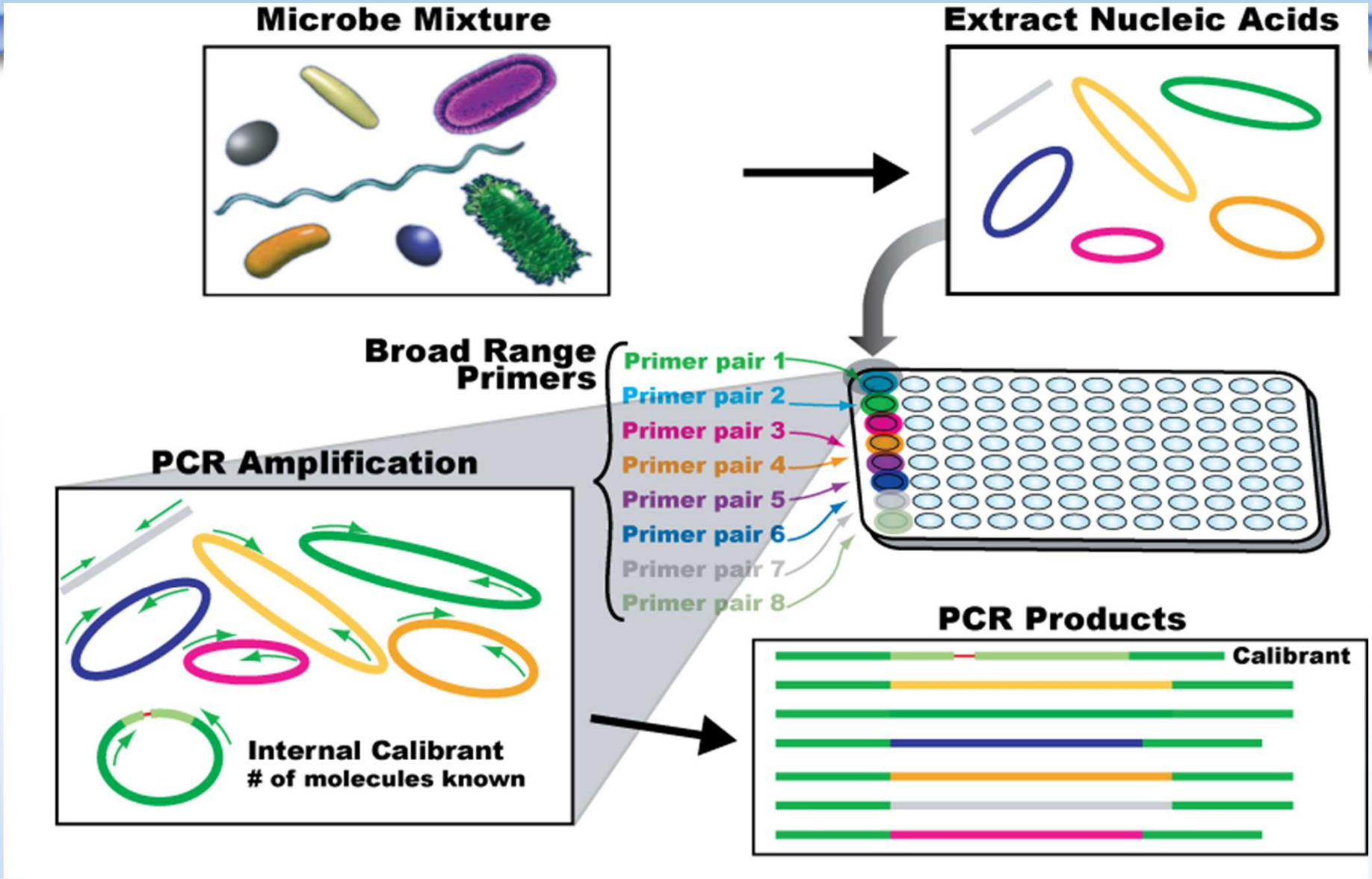


Carbapenem

Pip/Tazo, later carbapenem

Clin Infect Dis 2015, 60:1319–25

Sample Prep and Broad Range PCR



Detection of ESBLs : molecular biology

Principles:

- Amplification by PCR +/- sequencing
- Hybridization on DNA chips

Advantages:

- Quick and reliable result when performed directly on colony

Disadvantages:

- Cost +++ / Expertise
- Detect only known ESBL genes

Molecular detection of ATB resistance gene from clinical samples

Journal of Antimicrobial Chemotherapy (2009) **64**, 986–989
doi:10.1093/jac/dkp336
Advance Access publication 10 September 2009

JAC

Rapid detection of CTX-M-producing Enterobacteriaceae in urine samples

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Service de Bactériologie-Virologie, INSERM U914: 'Emerging Resistance to Antibiotics', Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, Faculté de Médecine, Université Paris-Sud, 94275 Le Kremlin-Bicêtre, France

Received 24 June 2009; returned 27 July 2009; revised 18 August 2009; accepted 19 August 2009

Objectives: CTX-M extended-spectrum β-lactamases (ESBLs) are emerging worldwide. Fast and reliable detection techniques may become mandatory for implementing proper treatment and infection control measures. Here, a bla_{CTX-M}-specific LightCycler real-time PCR (LC-PCR) assay based on hybridization probes was developed.

Methods: Urine samples positive for Gram-negative bacilli as revealed by Gram staining were collected over a 3 month period at Bicêtre hospital, France. Aliquots of these urine samples were frozen for subsequent molecular analysis, and the bacteria were cultured and identified by standard bacteriological techniques (biochemical tests, disc diffusion antibiogram and synergy testing). LC-PCR and standard PCR followed by sequencing was performed on all ESBL-positive and on 70 randomly chosen ESBL-negative urine samples.

Results: Over the study period, 810 urine samples were collected from 655 patients. Thirty-six ESBL-producing Enterobacteriaceae, mostly *Escherichia coli* (77%), were identified from 29 patients, of which half were outpatients. Twenty-five urine samples (19 patients) were found to be positive for bla_{CTX-M} genes using the LC-PCR assay. The bla_{CTX-M} genes belonged to the $bla_{CTX-M-1}$, $bla_{CTX-M-9}$ and $bla_{CTX-M-2}$ groups (68%, 24% and 8%, respectively). Standard PCR and sequencing of the entire bla_{CTX-M} genes confirmed the LC-PCR results; 17 CTX-M-15, 6 CTX-M-9 and 2 CTX-M-2. Among the remaining ESBLs, eight were of the TEM type and three of the SHV type.

Conclusions: The LC-PCR assay represents a powerful tool for rapid identification of CTX-M producers in urine samples.

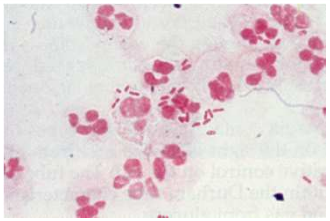


Table 1. Summary of NAD devices that target antimicrobial resistance determinants in Gram-negative bacteria

Test name (supplier)	Turnaround time to result	Technology	Analytical specificity/ sensitivity	Description, applications and regulatory status
Gram-Negative Blood Culture Test (Nanosphere)	2 h	PCR amplification followed by hybridization to gold nanoparticle-conjugated capture probes immobilized on a glass slide	not available	• FDA-approved kit for use on positive blood cultures • detection of 9 bacterial species/genera and 6 associated β-lactamases (KPC, NDM, CTX-M, VIM, IMP, OXA) • limitation in sample throughput
Blood Culture Identification Panel (Biofire Diagnostics)	2 h	Nested multiplex PCR amplification and subsequent detection by melt curve analysis	88%–100%/85%–100%[17,27]	• FDA-approved fully integrated test for use on positive blood cultures • detection of 24 bacterial and fungal pathogens (10 Gram-negatives) and 3 resistance determinants (KPC associated with Gram-negatives) • limitation in sample throughput
Unyvero™ P50 Pneumonia (Curetis)	4 h	Multiplex end-point PCR and amplicon detection by hybridization to oligo probes spotted on membrane arrays	72.3%–100%/55%–100%[16]	• CE-IVD-marked fully integrated test for use on respiratory samples • detection of 17 bacterial and fungal pathogens in addition to 22 antibiotic resistance genes, including bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{DHA}, bla_{EBC}, bla_{OXA-51} and bla_{KPC}, as well as fluoroquinolone resistance mutations (*gyrA83*, *gyrA87*, *parC*) and class 1 integron markers (*int1*, *sul1*)
GeneXpert MDRO Assay (Cepheid)	<1 h	Multiplex real-time PCR	99%–99.4%/93.4%–100%[14]	• designed for detecting patients colonized with carbapenemase producers from rectal swabs • detection of 3 carbapenemases, KPC, NDM and VIM

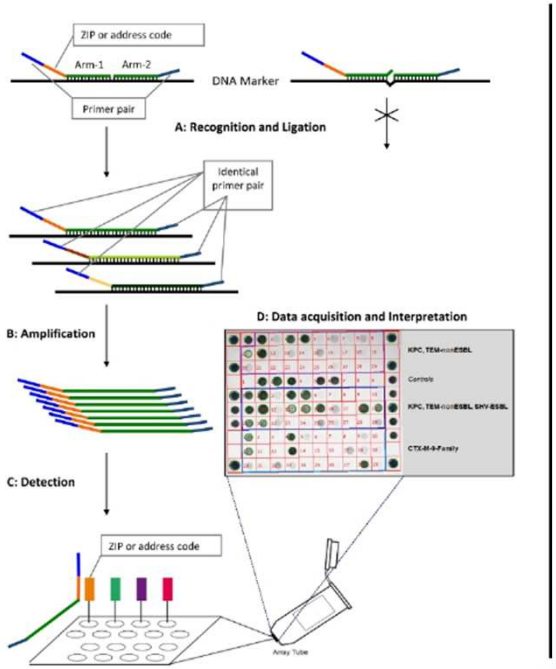
Rapid molecular detection of ESBL producers

- Accurate molecular ESBL assay, with results in < 7 h

- Tracking tool for outbreak management
  - includes molecular typing of ESBL

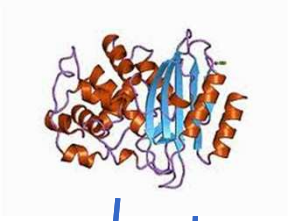
- Identifies ESBL from non-ESBL:
  - for CTX-M, TEM & SHV

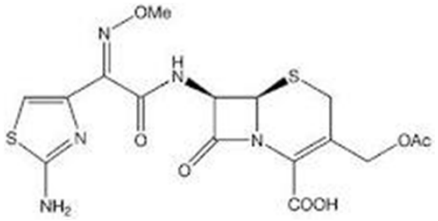
Problems: Cost ++ and diversity of ESBLs



The diagnostic test for biochemical detection of ESBL producer; the rapid ESBL NP test

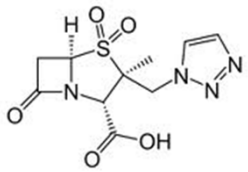
ESBL

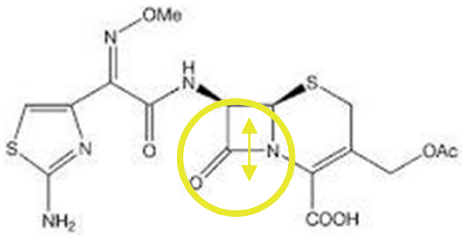




+

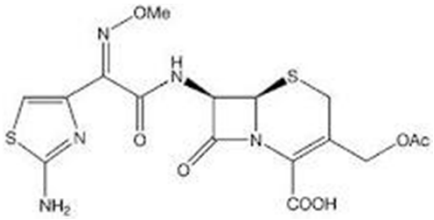
Tazobactam





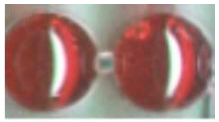
Acid production

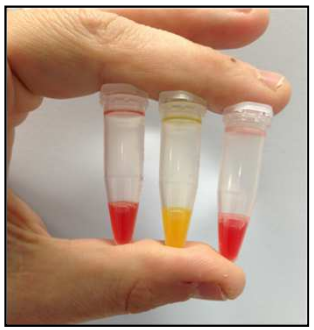
pH



No acid production

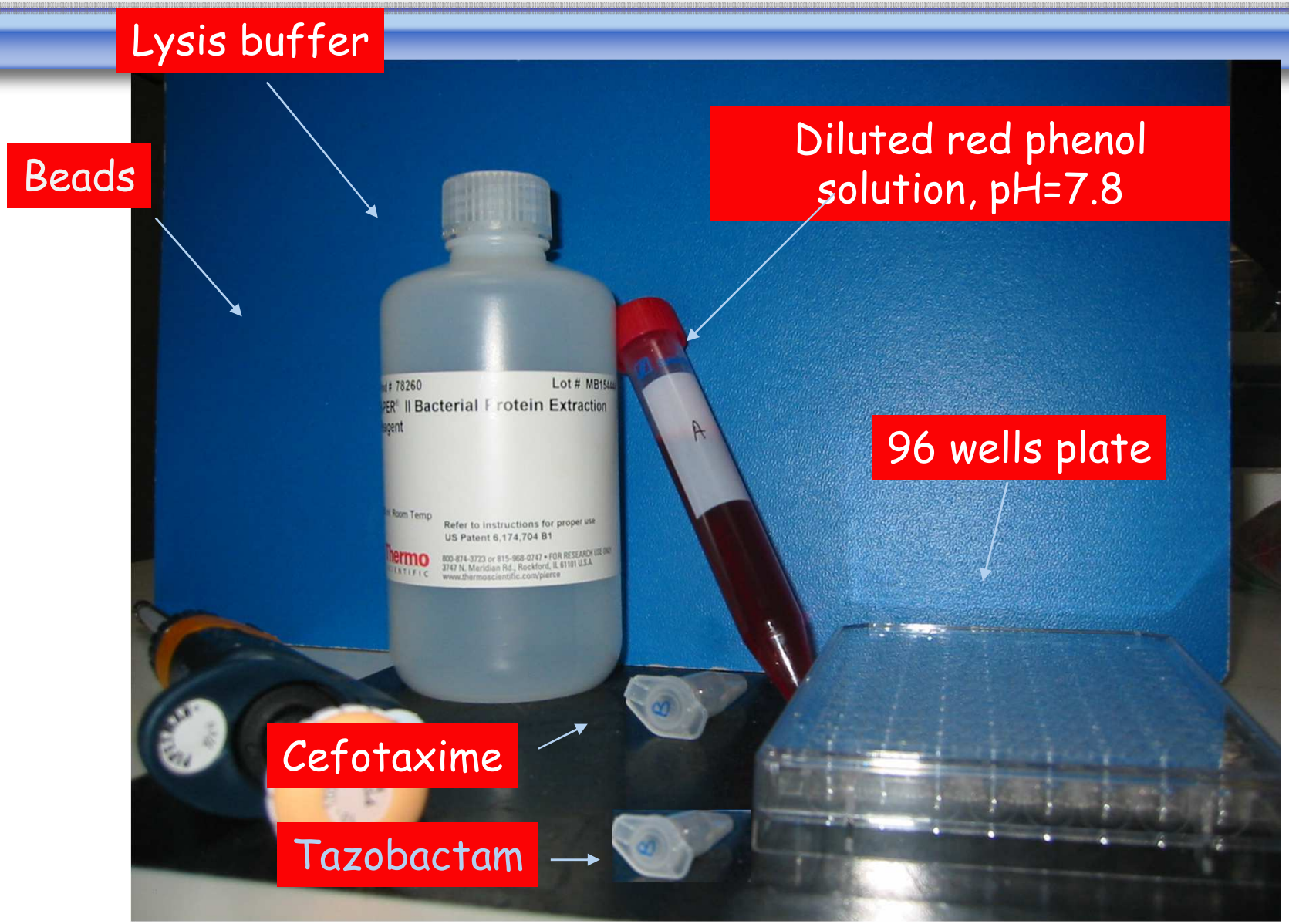
= pH





Nordmann *et al.*, JCM 2012

The ESBL NP test; the kit



The ESBL NP test

① Rapid; less than 1 h

② Sensitive ;94-100%

③ Specific:100%

④ Cheap: 4-5 euros

⑤ Easy-to-handle

⑥ Implementable worldwide

JCM
Journals.ASM.org

Rapid Detection of Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* from Urine Samples by Use of the ESBL NDP Test

Laurent Dortet,[a,b] Laurent Poirel,[a,c] Patrice Nordmann[a,b,c,d]

INSERM U914, Le Kremlin-Bicêtre, France[a]; Centre National Associé-Centre de Référence des Résistances aux Antibiotiqus, Le Kremlin-Bicêtre, France[b]; Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland[c]; Hôpital Fribourgeois-hôpital Cantonal, Fribourg, Switzerland[d]

From June to September 2012, 500 urine samples were recovered from patients with urinary tract infections (UTI) due to Gram-negative bacilli (≥10^4 leukocytes/ml and ≥10^5 Gram-negative isolates/ml) who visited the University hospital Bicêtre (France). They were challenged with extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) using the rapid diagnostic ESBL NDP test. Results of the ESBL NDP test were compared to the results of the double-disc susceptibility test (DDST) performed on solid-agar plates and molecular identification of the β-lactamase genes. Among the 450 nonduplicate urine samples, 11.3% were positive for ESBL-E by using the DDST, the ESBL determinants being mostly of the CTX-M type (CTX-M-15) according to molecular testing. Results of the ESBL NDP test were obtained within 15 min. The sensitivity and specificity of the ESBL NDP test were 98% and 99.8%, respectively, whereas the positive and negative predictive values of this test were 98% and 99.8%, respectively. A perfect correlation between cefotaxime resistance and positivity of the ESBL NDP test was observed. Therefore, the ESBL NDP test offers a powerful tool for a rapid identification of ESBL-E and associated resistance to expanded-spectrum cephalosporins. It may be useful in particular for guiding first-line antibiotic therapy.

October 2014 Volume 52 Number 10 Journal of Clinical Microbiology p. 3701–3706

Rapid Detection of ESBL-Producing *Enterobacteriaceae* in Blood Cultures

Laurent Dortet, Laurent Poirel, Patrice Nordmann

We rapidly identified extended-spectrum β-lactamase (ESBL) producers prospectively among 245 gram-negative bacilli–positive cultured blood specimens using the Rapid ESBL Nordmann/Dortet/Poirel test and direct bacterial identification using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. This combination identified ESBL-producing *Enterobacteriaceae* within 30 min and had high predictive values.

concomitantly from blood cultures by using enhanced MALDI-TOF procedures.

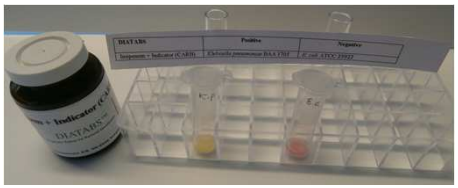
The Study

During November 2012–May 2013, we studied a single blood culture positive for GNB from each of 245 patients hospitalized at the Bicêtre hospital, a 950-bed hospital located in a suburb of Paris. Positivity of blood cultures was detected by using the BacT/Alert system (bioMérieux, La

Biochemical identification of ESBL producers

Sensitivity	+ + +	+ +	+ + +
Specificity	+ +	+ +	+ + +

B-lacta Test



Rapid ESBL Screen

A rapid detection of resistance to third generation cephalosporins for *Enterobacteriaceae* based on the cleavage of a chromogenic substrate; ESBL, +/_ AmpC, KPC…

Comparison of three biochemical tests for rapid detection of extended-spectrum-β-lactamase-producing *Enterobacteriaceae*

L Poirel, J.Fernández, and P. Nordmann,
J Clin Microbiol, 2015, in press

2005...

CLINICAL MICROBIOLOGY REVIEWS, Apr. 2005, p. 306–325 Vol. 18, No. 2
0893-8512/05/$08.00+0 doi:10.1128/CMR.18.2.306–325.2005

Metallo-β-Lactamases: the Quiet before the Storm?

Timothy R. Walsh,[1]* Mark A. Toleman,[1] Laurent Poirel,[2] and Patrice Nordmann[2]

Department of Pathology and Microbiology, University of Bristol, Bristol, United Kingdom,[1] and Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre, France[2]

2012...

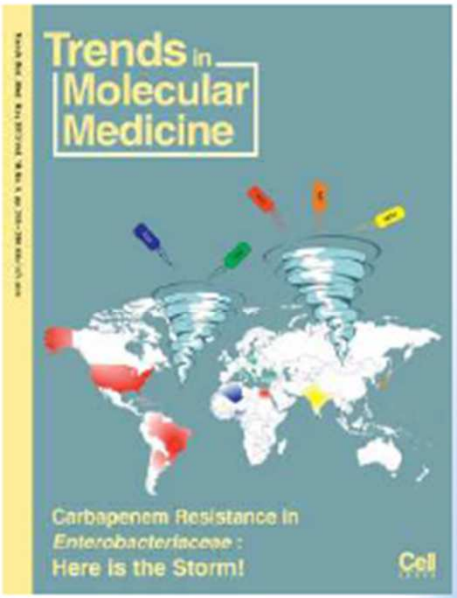
Review

Cell PRESS

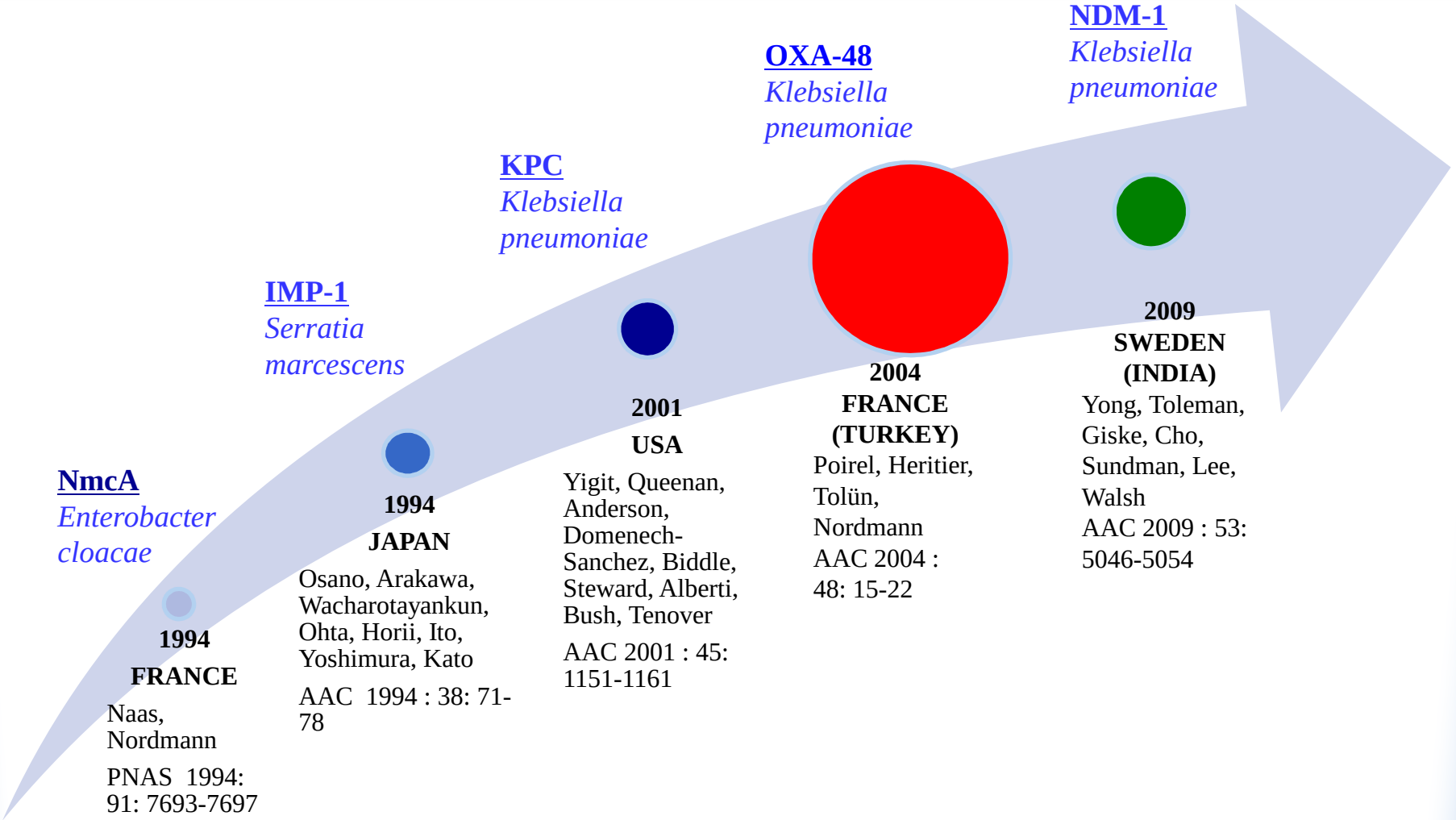
Carbapenem resistance in *Enterobacteriaceae*: here is the storm!

Patrice Nordmann, Laurent Dortet 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, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France



Emergence of carbapenemases in *Enterobacteriaceae*



KPCs; Klebsiella Pneumoniae Carbapenemase

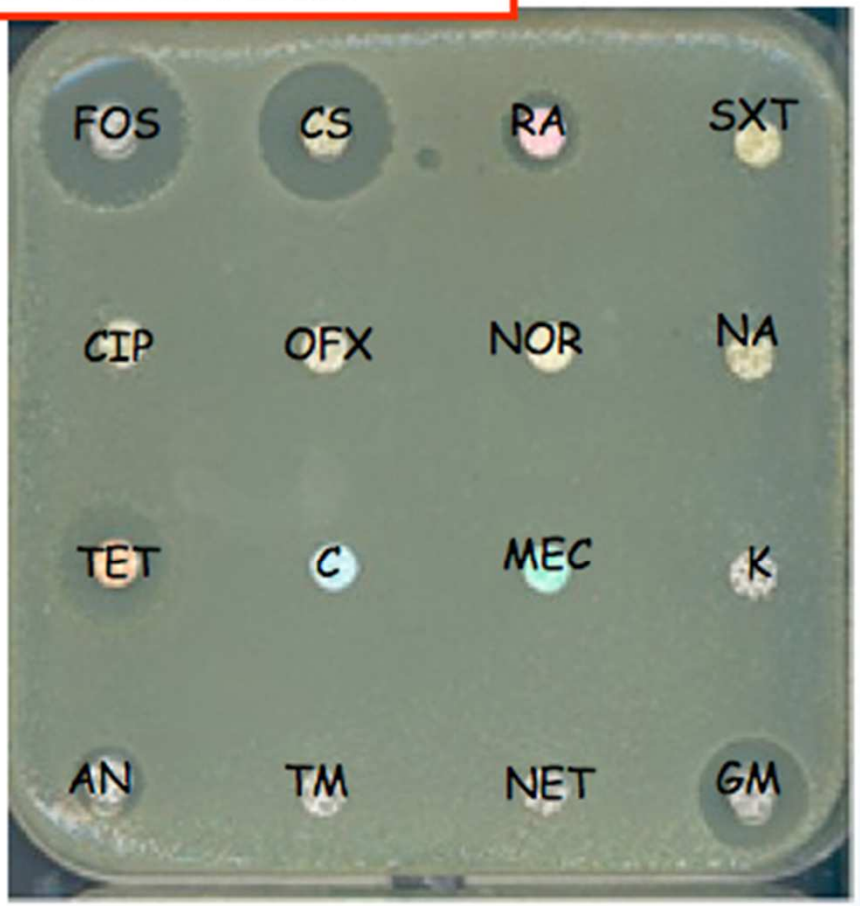
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2001, p. 1151–1161 Vol. 45, No. 4
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.4.1151–1161.2001

Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of *Klebsiella pneumoniae*

HESNA YIGIT,[1] ANNE MARIE QUEENAN,[2] GREGORY J. ANDERSON,[1] ANTONIO DOMENECH-SANCHEZ,[3] JAMES W. BIDDLE,[1] CHRISTINE D. STEWARD,[1] SEBASTIAN ALBERTI,[4] KAREN BUSH,[2] AND FRED C. TENOVER[1]*

Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; The R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey 08869[2]; and Unidad de Investigacion, Hospital Son Dureta, Andreu Doria, Palma de Mallorca, 07014,[4] and Área de Microbiología, Universidad de las Islas Baleares, Crta. Valldemossa, Palma de Mallorca, 07071,[3] Spain

Received 19 September 2000/Returned for modification 21 November 2000/Accepted 23 January 2001



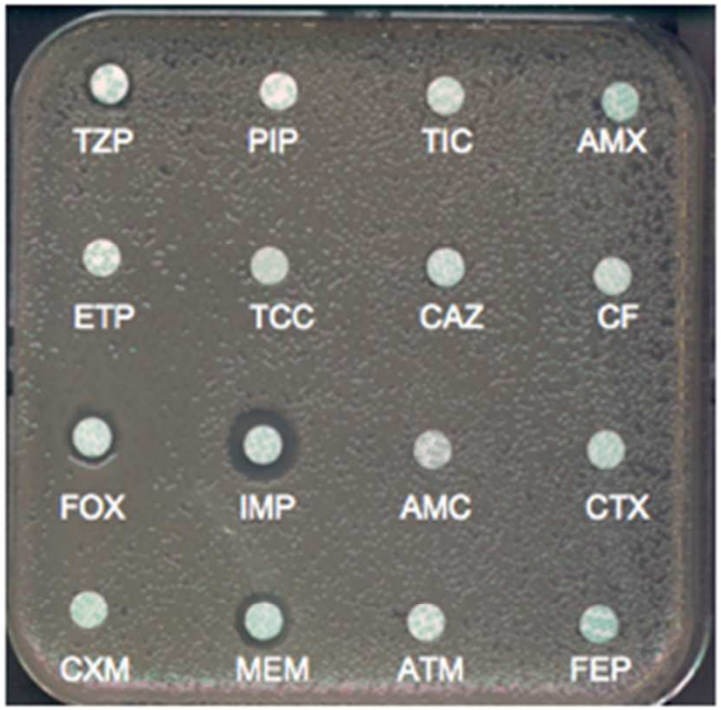
AAC,2009; 53 5046-5054

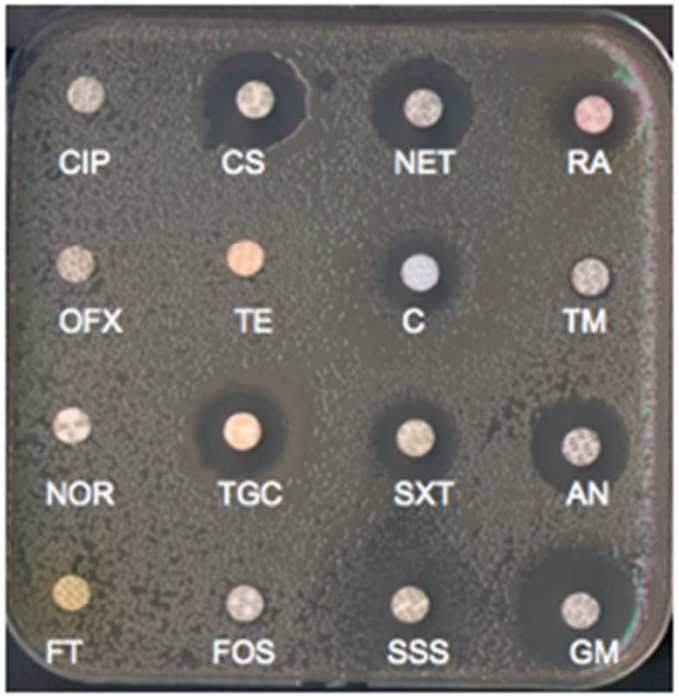
Characterization of a New Metallo-β-Lactamase Gene, *bla*$_{NDM-1}$, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in *Klebsiella pneumoniae* Sequence Type 14 from India[▽]

Dongeun Yong,[1,2] Mark A. Toleman,[2] Christian G. Giske,[3] Hyun S. Cho,[4] Kristina Sundman,[5] Kyungwon Lee,[1] and Timothy R. Walsh[2]*

Yonsei University College of Medicine, Research Institute of Antimicrobial Resistance, Seoul, Republic of Korea[1]; Department of Medical Microbiology, Cardiff University, Cardiff, United Kingdom[2]; Clinical Microbiology, MTC—Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden[3]; Yonsei University College of Life Science and Biotechnology, Seoul, Republic of Korea[4]; and Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden[5]

OXA-48 + CTX-M-15

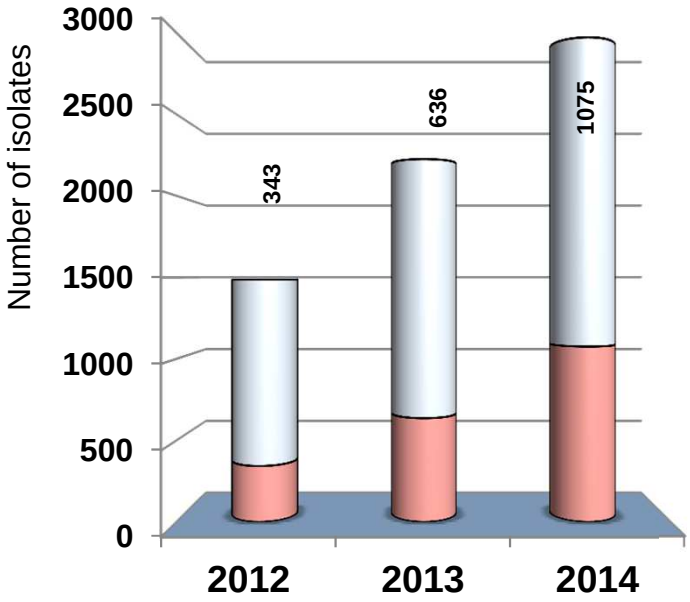


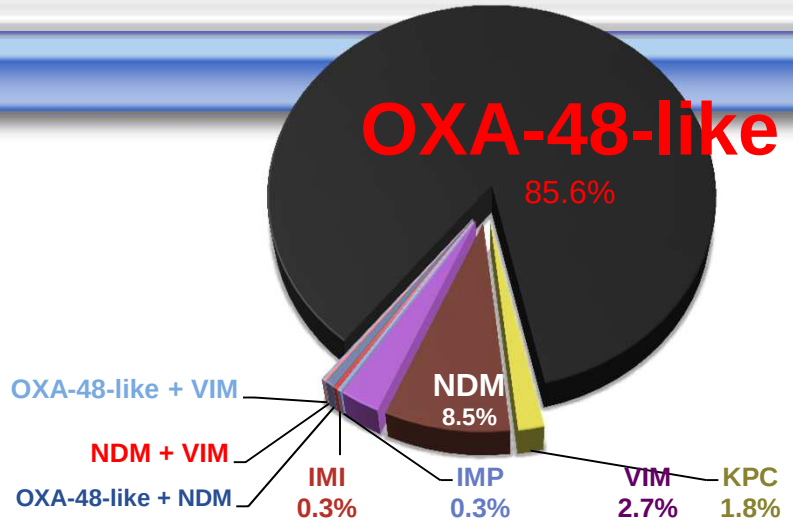


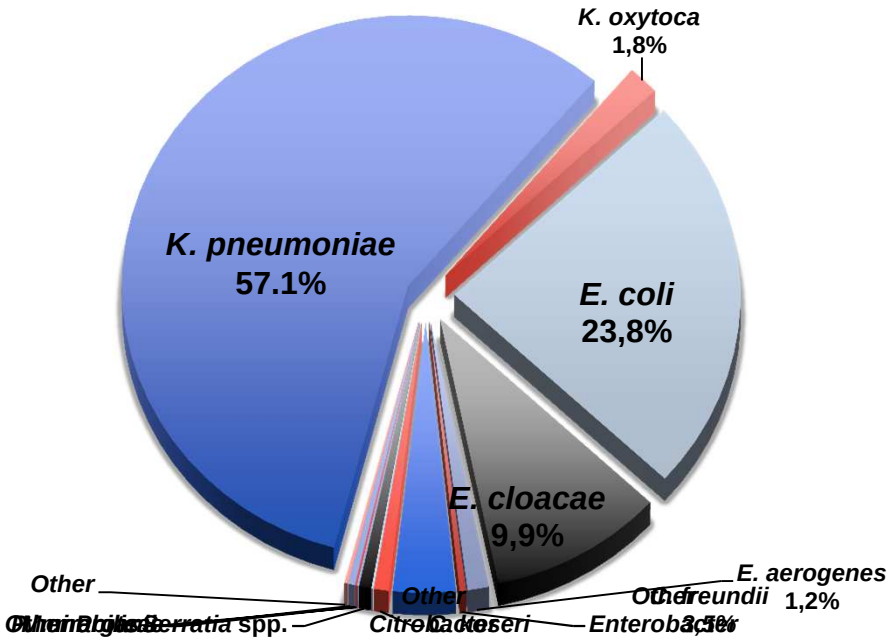
K. pneumoniae

Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae.*
Poirel L, Héritier C, Tolün V, Nordmann P. Antimicrob Agents Chemother. 2004 Jan;48(1):15-22

Carbapenemases – France, 2014

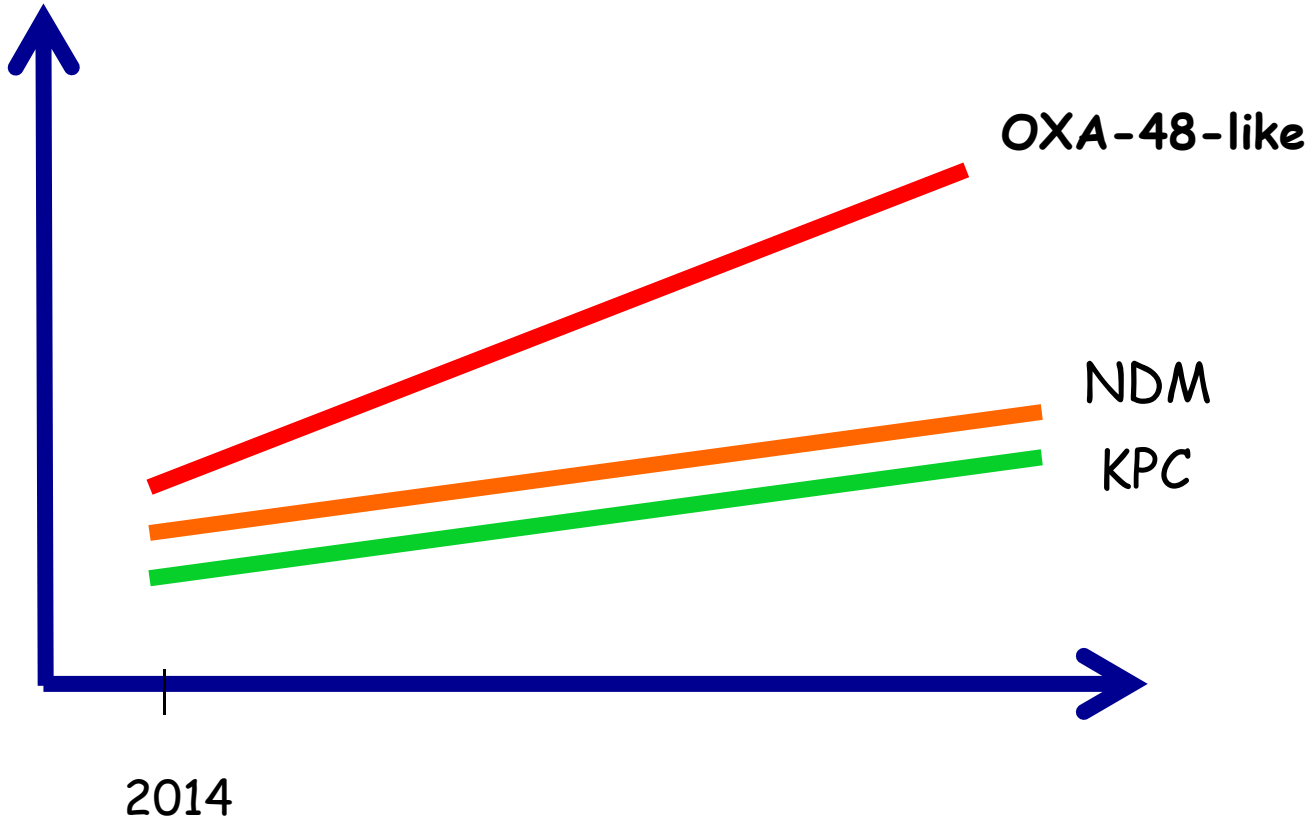




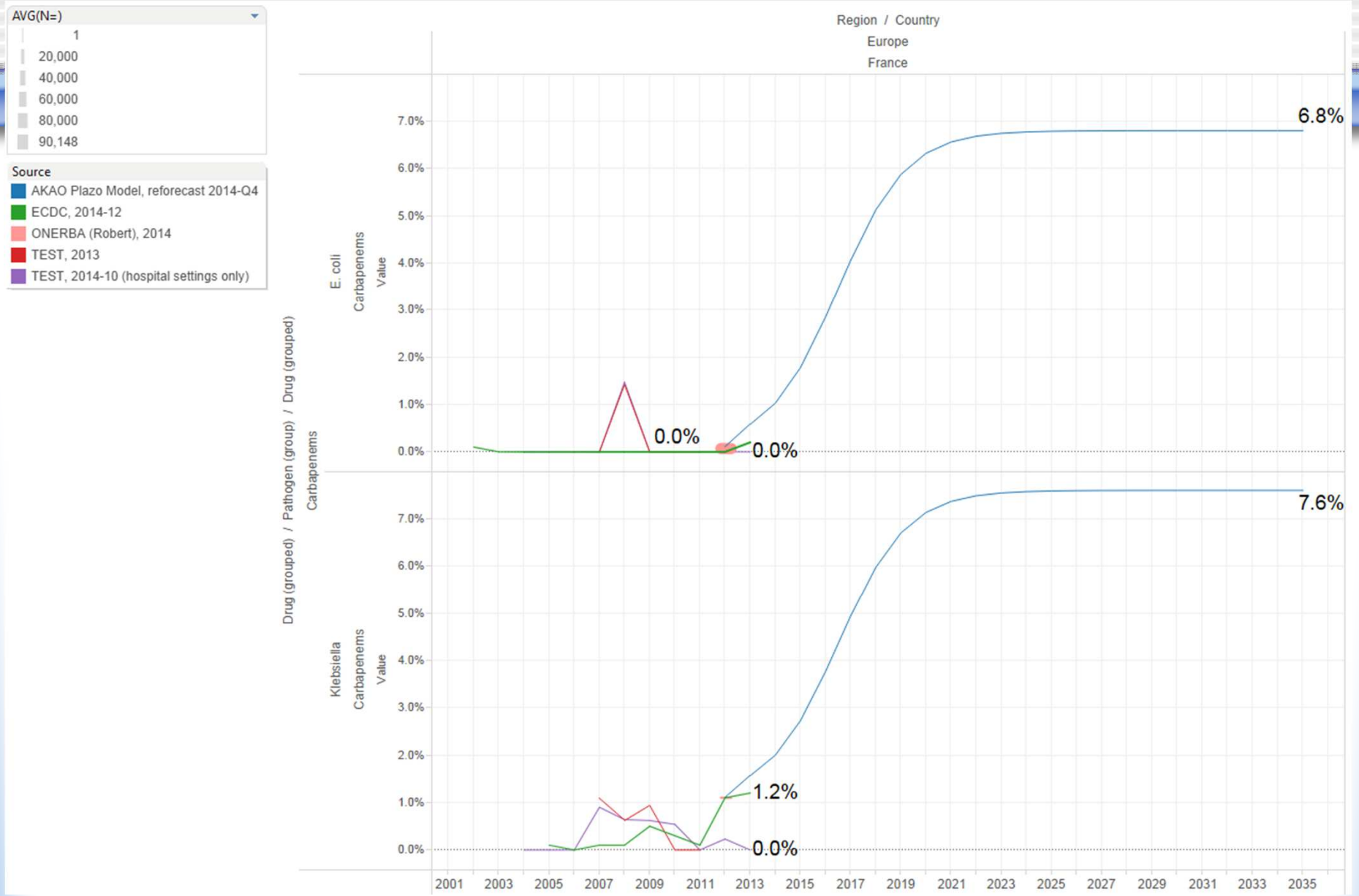


CNR Antibiotic R,
Nordmann, Dortet personal communication

Future spread of carbapenemase producers in Europe



Carbapenemase producers, France; the future



Molecular biology : PCR-based techniques

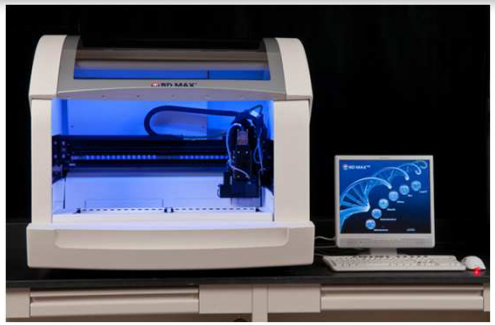
- **Real-Time PCR :**

  - Check-MDR Real-Time PCR
  - Detect the presence of the carbapenemase gene
  - 4-5 h
  - Cost +++

- **Specific PCR +/- sequencing :**

  - OXA-48-like / KPC / VIM / IMP / NDM
  - 3 to 5 h
  - Expertise ++
  - Cost +

Molecular detection of carbapenemase genes



BD

BIOFIRE

HAIN LIFESCIENCE

ELSEVIER

Diagnostic Microbiology and Infectious Disease

Volume 77, Issue 3, November 2013, Pages 179–194

Review

Non-phenotypic tests to detect and characterize antibiotic resistance mechanisms in Enterobacteriaceae

Agnese Lupo[a], Krisztina M. Papp-Wallace[b, c], Parham Sendi[a, d], Robert A. Bonomo[b, c, e], Andrea Endimiani[a]

Cepheid

The example of the Xpert MDRO cartridge

Cartridge detects four carbapenem resistance gene families (54 genes in total)

- bla_{KPC}
- bla_{NDM}
- bla_{VIM}
- bla_{OXA-48}

- Sample : Rectal Swabs
- Result in 50 minutes

. Cost +++

Clinical Microbiology and Infection

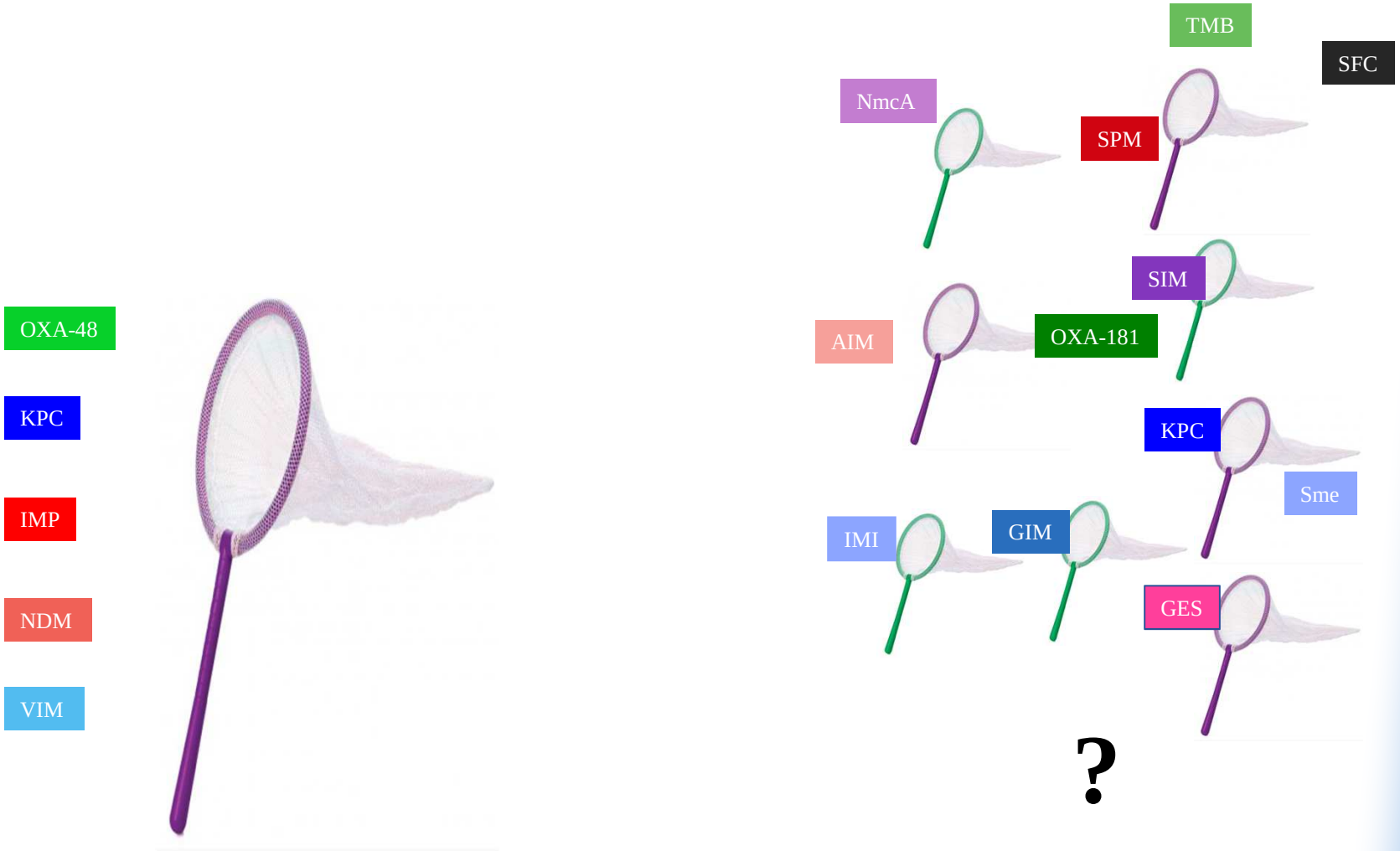
Volume 21, Issue 2, February 2015, Pages e9–e10

Letter to the Editor

Failure to detect carbapenem-resistant *Escherichia coli* producing OXA-48-like using the Xpert Carba-R assay®

J.-W. Decousser[1, 2], L. Poirel[3, 4, 5], M. Desroches[1, 2], A. Jayol[1], E. Denamur[2], P. Nordmann[3, 4, 5, 6]

Gene identification of carbapenemase producers in *Enterobacteriaceae*

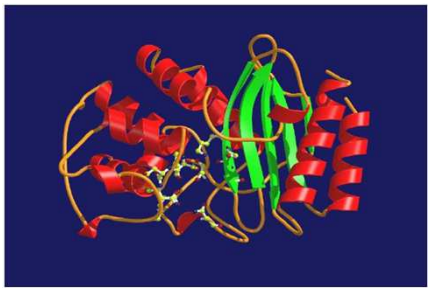


Failure to detect totally novel genes

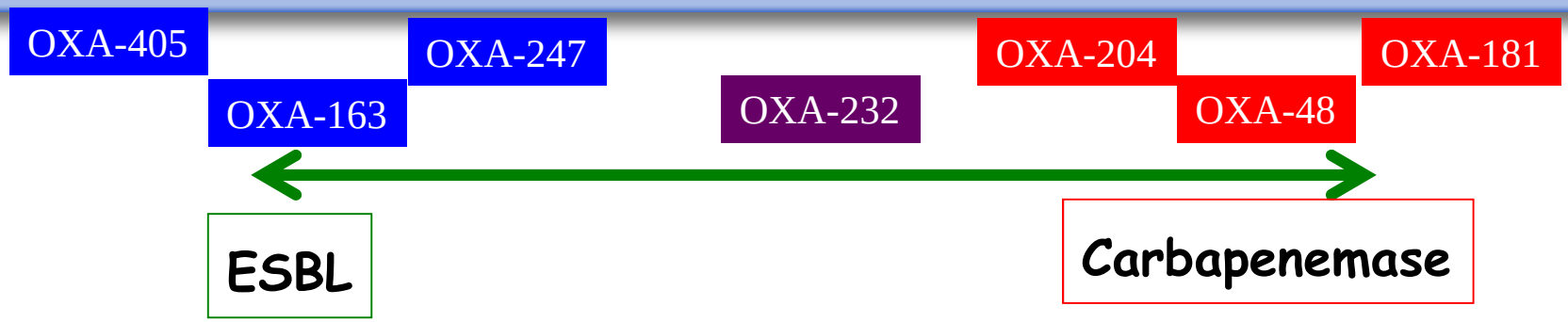
ICAAC 2014: Genetic and biochemical characterisation of FRI-1, a novel carbapenem-hydrolysing class A beta-lactamase from Enterobacter cloacae

P. Nordmann , L. Poirel, L. Dortet; Univ. of Fribourg, Fribourg, Switzerland, Hosp. de Bicetre, Le Kremlin Bicetre, France

Background: Carbapenem resistance in Enterobacteriaceae is mainly linked to carbapenemase production. The most commonly carbapenemases identified in Enterobacter cloacae are the class A ß-lactamases KPC and NMC-A/IMI, the class B (metallo-ß-lactamases) VIM, IMP, and NDM, and the class D carbapenemase OXA-48. Our study was initiated by the isolation of a carbapenem-resistant E. cloacae. **Methods:** Antimicrobial susceptibility was determined by the disk diffusion method and E-test. The production of a class A carbapenemase was assessed using the Carba NP II test aimed to identify a carbapenemase activity and its type. The genetic environment of the ß-lactamase gene was characterized by cloning and DNA sequencing. The carbapenemase was purified using ion exchange liquid chromatography, and kinetic parameters were determined by UV spectrophotometry. **Results:** E. cloacae DUB was recovered from a rectal swab sample of a patient hospitalized in France without known history of travel. This isolate was resistant to penicillins, 1st and 2nd generation cephalosporins, aztreonam and carbapenems, but remained susceptible to 3rd generation cephalosporins. It also remained susceptible to all other antibiotics, except to rifampin. Use of the Carba NP test II revealed the production of an Ambler class A carbapenemase. Cloning experiments followed by sequencing identified a gene encoding a novel class A carbapenemase, FRI-1, sharing 51-55% amino-acid sequence identity with the other class A carbapenemases. The blaFRI-1 gene was located on a ca. ~ 250 kb untypeable, transferable, but non self-conjugative plasmid carrying no other antibiotic resistance determinant. A putative LysR family regulator encoding gene at the 5' end of the gene was identified as observed upstream the NmcA and Sme carbapenemase genes. The purified FRI-1 significantly hydrolyzed penicillins, aztreonam and carbapenems, but spared 3rd generation cephalosporins. The IC50s of clavulanic acid and tazobactam were 10-fold higher than those found for KPC, IMI and SME, leading to a lower susceptibility towards ß-lactamase inhibitors. **Conclusion:** A novel plasmid-encoded Ambler class A carbapenemase was identified from E. cloacae. This enzyme adds to the variety of emerging and plasmid-encoded carbapenemases



OXA-48 type carbapenemases: more complicated than expected




```
                1        10        20        30        40        50        60        70        80        90       100
                |        |         |         |         |         |         |         |         |         |         |
OXA-48          MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-405         MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-163         MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR
OXA-247         MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTR

                        110       120       130       140       150       160       170       180       190       200
                        |         |         |         |         |         |         |         |         |         |
OXA-48          DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-405         DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-163         DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN
OXA-247         DIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEAN

                        210       220       230       240       250       260
                        |         |         |         |         |         |
OXA-48          GDYIIRAKTGYSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-405         GDYIIRAKTGYS----PKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-163         GDYIIRAKTGYDT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
OXA-247         GDYIIRAKTGSNT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
```

Heterogeneous hydrolytic features for **OXA-48**-like β-lactamases.

Oueslati S, **Nordmann** P, Poirel L.

J Antimicrob Chemother. 2015 Apr;70(4):1059-63. doi: 10.1093/jac/dku524. Epub 2015 Jan 11.

AAC

Genetic and Biochemical Characterization of OXA-405, an OXA-48-Type Extended-Spectrum β-Lactamase without Significant Carbapenemase Activity

Laurent Dortet,[a,b,c,d] Saoussen Oueslati,[a] Katy Jeannot,[b,e] Didier Tandé,[f] Thierry Naas,[a,b,c,d] Patrice Nordmann[a,b,g,h]

INSERM U 914, Le Kremlin-Bicêtre, France[a]; Associated National Reference Center for Antibiotic Resistance, Le Kremlin-Bicêtre, France[b]; Faculty of Medicine, South-Paris University, Le Kremlin-Bicêtre, France[c]; Bacteriology-Hygiene Unit, Bicêtre Hospital, Assistance Publique/Hôpitaux de Paris, Le Kremlin-Bicêtre, France[d]; Besançon Hospital, Microbiology Laboratory, Besançon, France[e]; Brest Hospital, Microbiology Laboratory, Brest, France[f]; Medical and Microbiology Unit, Department of Medicine, University Fribourg, Fribourg, Switzerland[g]; HFR-Hôpital Cantonal, Fribourg, Switzerland[h]

False positivity

Diagnostic Microbiology and Infectious Disease 82 (2015) 1–3

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Bacteriology

Intestinal carriage of *Shewanella xiamenensis* simulating carriage of OXA-48–producing Enterobacteriaceae

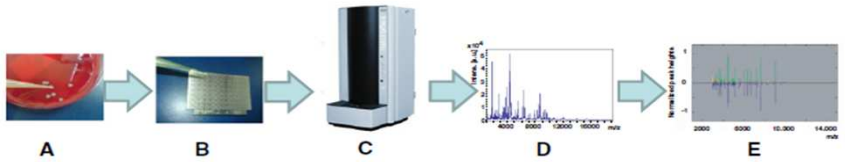
Alberto Antonelli [a,b], Domenica Maria Di Palo [a,c], Angelo Galano [a,c], Sabrina Becciani [d], Carlotta Montagnani [d], Patrizia Pecile [c], Luisa Galli [d,e], Gian Maria Rossolini [a,b,c,*]

[a] Department of Medical Biotechnologies, University of Siena, Policlinico Santa Maria alle Scotte, Viale Bracci, I-53100 Siena, Italy
[b] Department of Experimental and Clinical Medicine, University of Florence, Piastra dei Servizi, Via San Damiano, I-50134 Florence, Italy
[c] Clinical Microbiology and Virology Unit, Careggi University Hospital, Piastra dei Servizi, Via San Damiano, I-50134 Florence, Italy
[d] Department of Health Sciences, University of Florence, Viale Pieraccini, I-50139 Florence, Italy
[e] Meyer Children's University Hospital, Viale Pieraccini, I-50139 Florence, Italy

The problems with the molecular biology techniques

1. **Detection of known genes only**
2. **Lack of detection of novel genes or non frequent carbapenemase genes (NmcA, Sme…)**
3. **Difficult differentiation between ESBL and carbapenemases (OXA-48 series, GES..)**
4. **No established correlation between gene identification and enterobacterial strain producing carbapenemases in stools**
5. **A need for trained personal. Opening hours**
6. **Time consuming**
7. **Cost**
8. **Still a need for bacterial culture; full antibiogram, epidemiology**

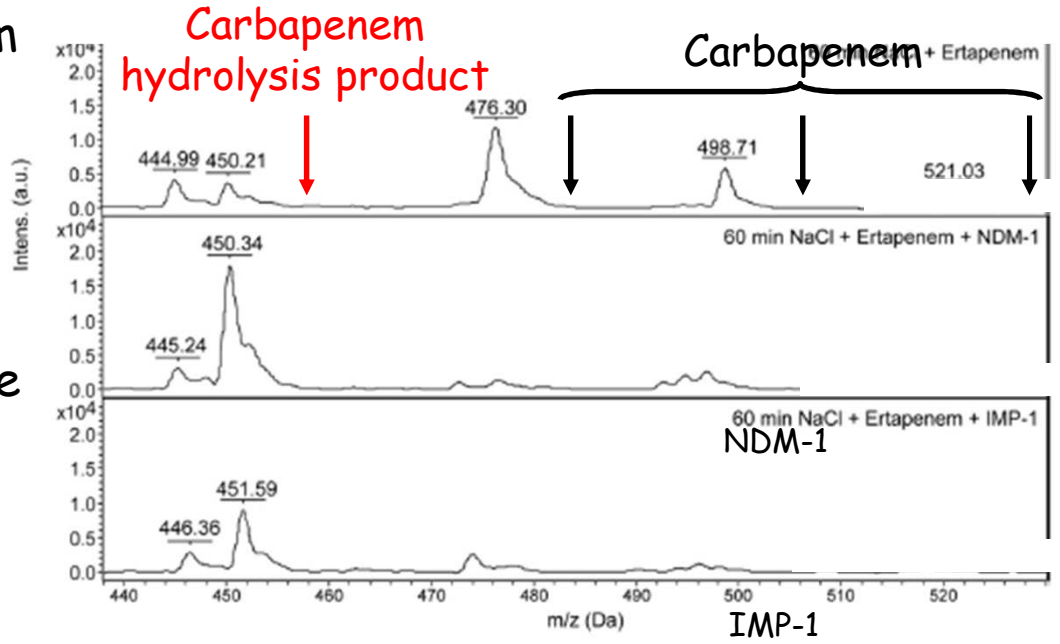
Mass spectrometry : MALDI-TOF



Hrabák et al. JCM. 2011
Burckhardt et al. JCM. 2011
Hrabák et al. JCM. 2012

Protocol :

1) Broth culture with the strain to be tested + carbapenem : 3-6h
2) Mass spectrometry
3) if carbapenemase + :
hydrolysis of the carbapenem molecule leading to a degradation product



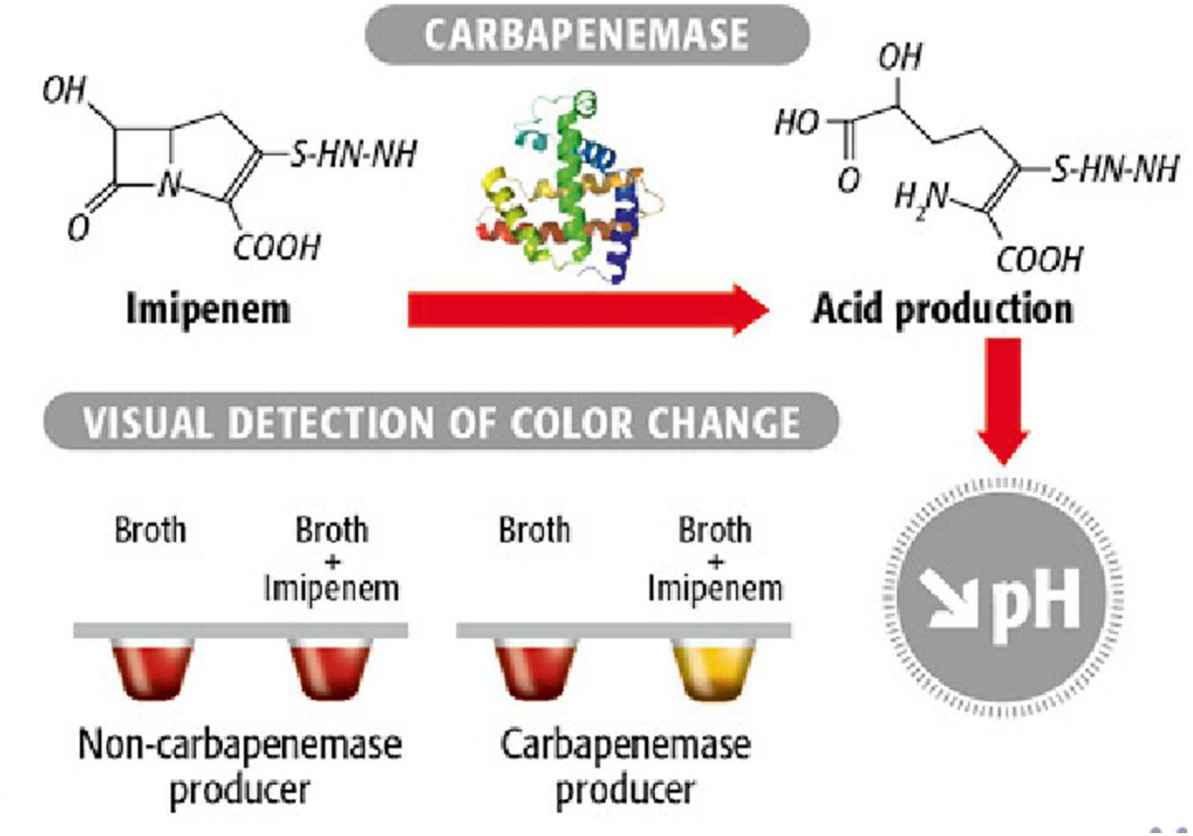
Advantages :

Specific / sensitive
Cheap if you own the machine !

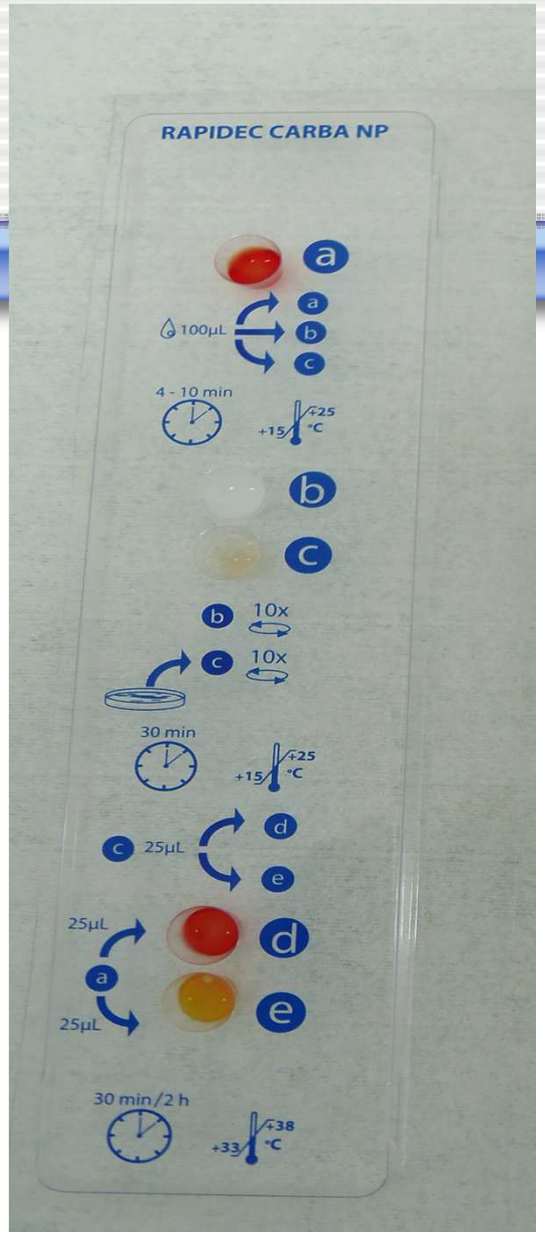
Disadvantages

Material price, result delay
Needed expertise

Biochemical identification of carbapenemase activity: the Carba NP test



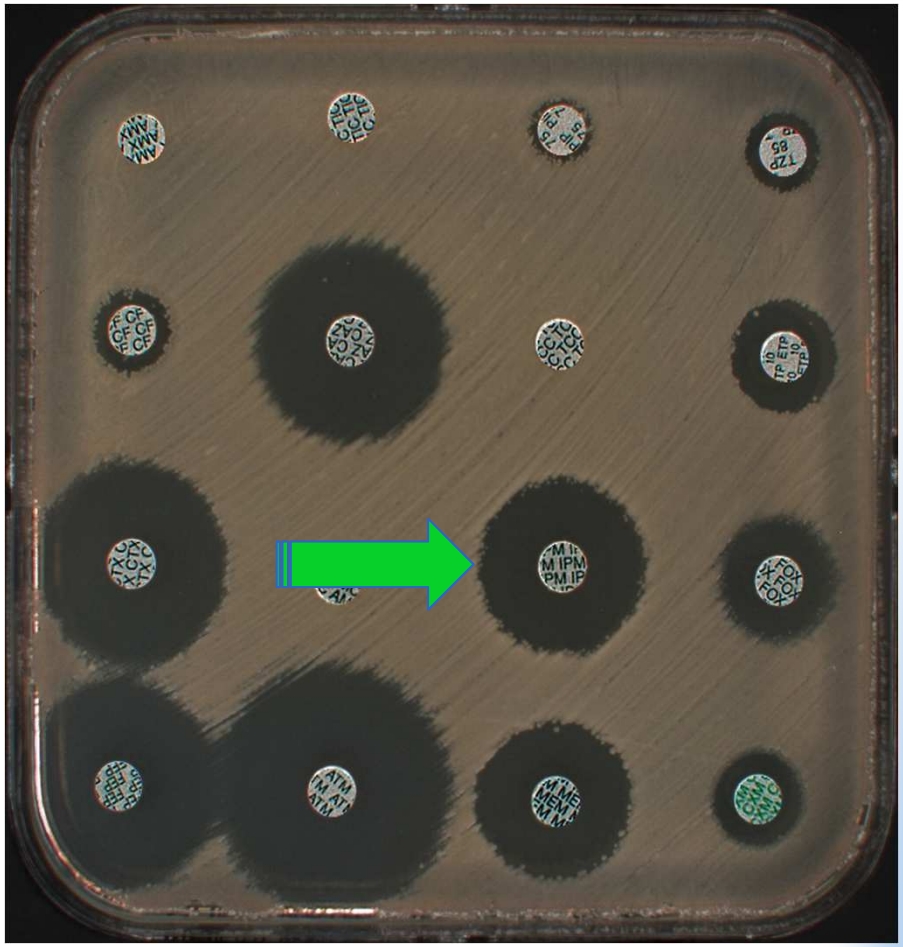
Nordmann et al., Emerg Infect Dis 2012



Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter baumannii

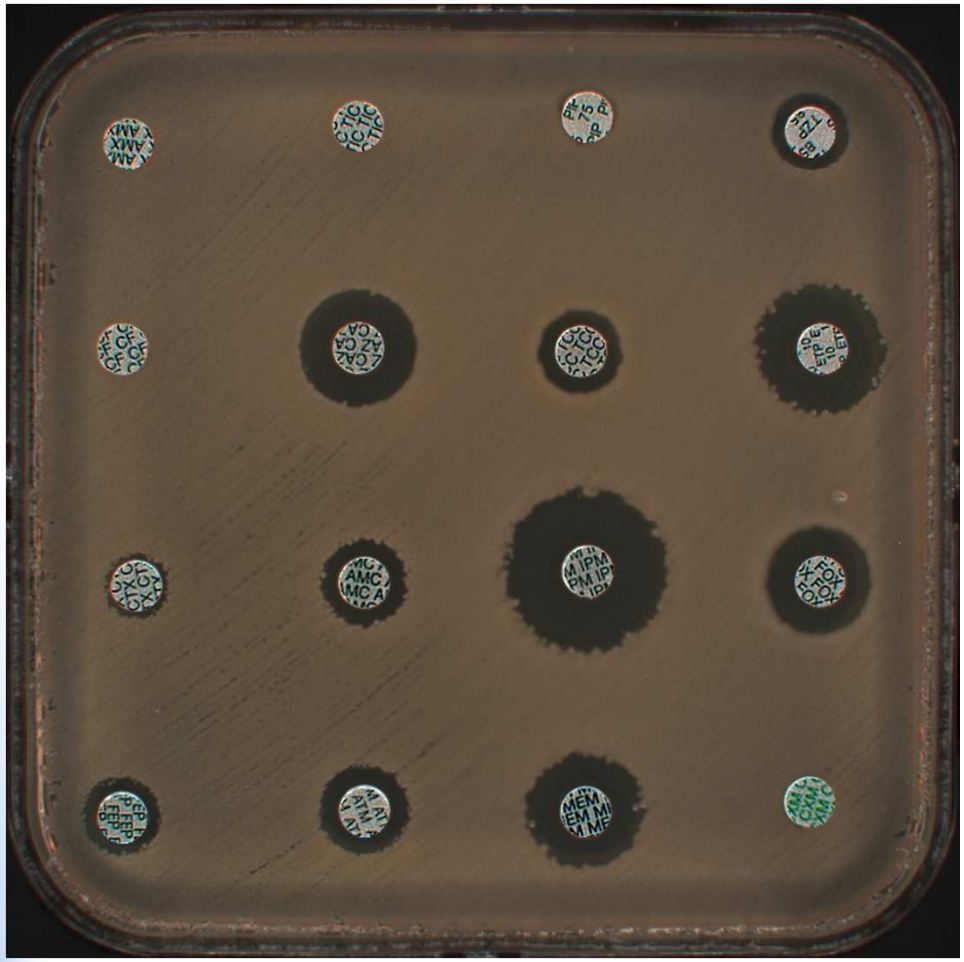
K. pneumoniae

K. pneumoniae



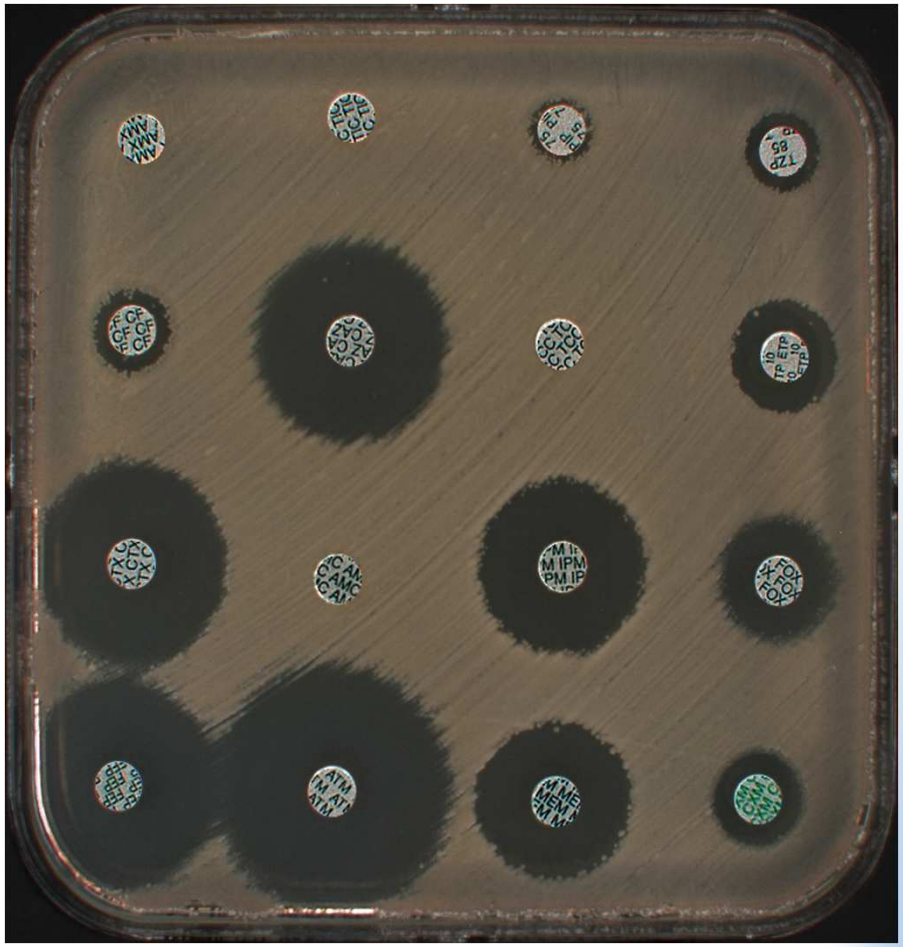
The Carba NP test

K. pneumoniae
CTX-M15 + impermeability



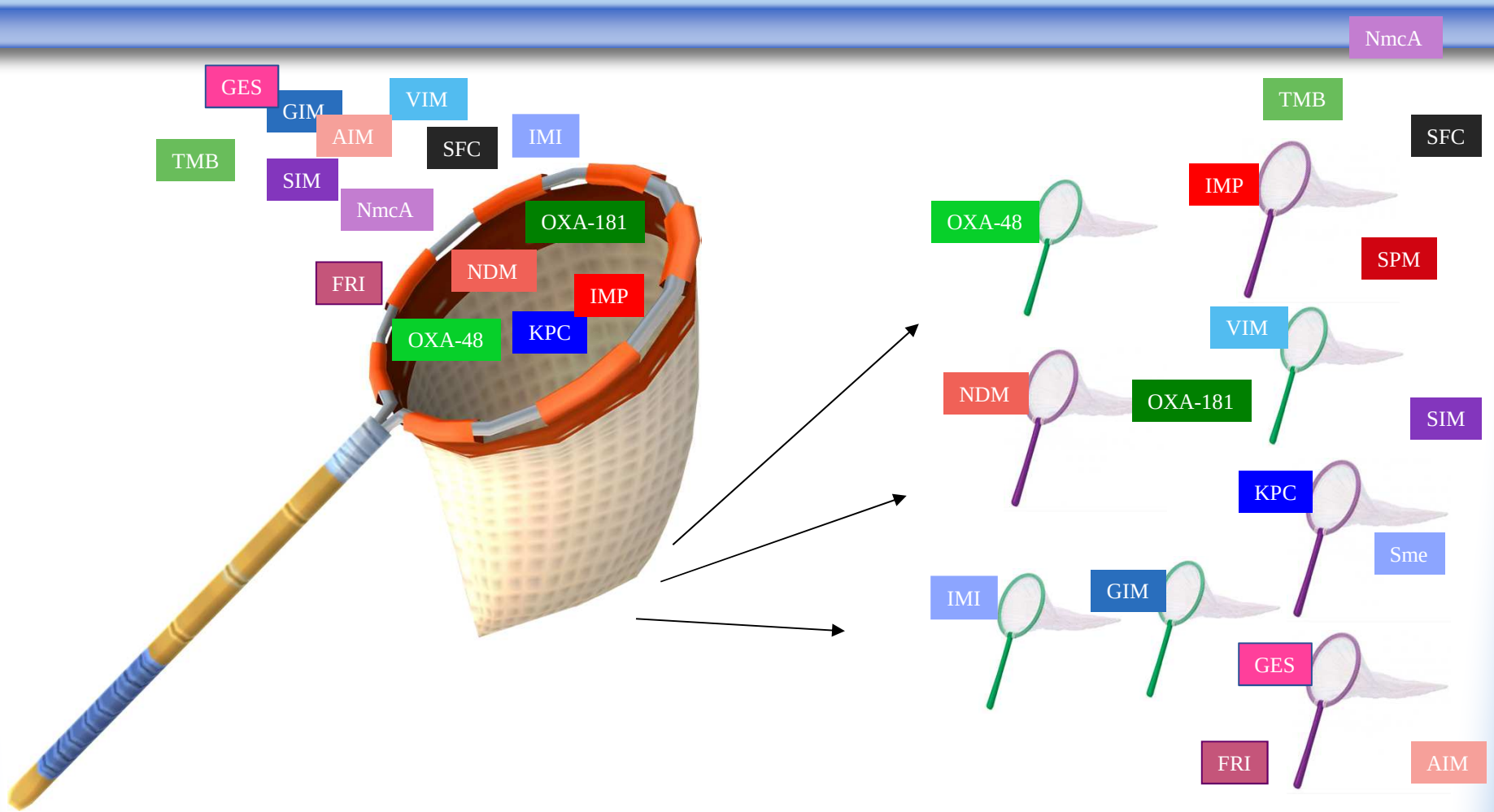
(-)

***K. pneumoniae* OXA-48**



(+) 35 min

Strategy for rapid identification of carbapenemase producers in *Enterobacteriaceae*



1. **Biochemical identification**

2. Molecular biology

Carbapenemase producers; carriage detection: outbreak

J0

Molecular biology

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4038–4043 Vol. 55, No. 9
0066-4804/11/$12.00 doi:10.1128/AAC.01734-10

Real-Time PCR for Detection of NDM-1 Carbapenemase Genes from Spiked Stool Samples[▽]

Thierry Naas,†* Ayla Ergani,† Amélie Carrër, and Patrice Nordmann

Service de Bactériologie-Virologie, INSERM U914: Emerging Resistance to Antibiotics, Hôpital de Bicêtre, 94275 Le Kremlin-Bicêtre, and Assistance Publique-Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Paris, France

J2

RAPID COMMUNICATIONS

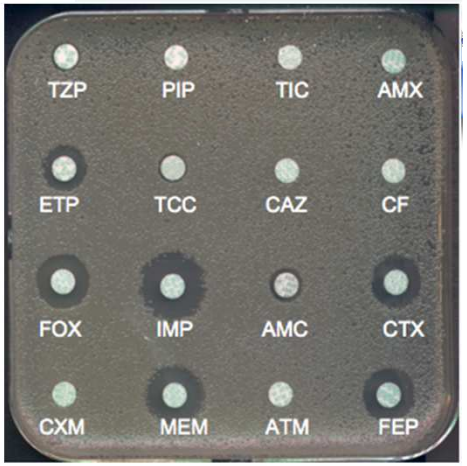
Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014

M. Monaco[1,2], T Giani[2,3], M Raffone[1,4], F Arena[3], A Garcia-Fernandez[1], S Pollini[3], Network EuSCAPE-Italy[5], H Grundmann[6], A Pantosti (annalisa.pantosti@iss.it)[1], G M Rossolini[3,7,8]

1. Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy
2. MM and TG have equally contributed to this work
3. Department of Medical Biotechnologies, University of Siena, Siena, Italy
4. Federico II University Hospital, Neaples, Italy
5. The network EuSCAPE-Italy participants are listed at the end of this article
6. Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, the Netherlands
7. Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
8. Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy

Citation style for this article:
Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S, Network EuSCAPE-Italy, Grundmann H, Pantosti A, Rossolini GM. Colistin resistance superimposed to endemic carbapenem-resistant Klebsiella pneumoniae: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill. 2014;19(42):pii=20939. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20939

Article submitted on 08 October 2014 / published on 23 October 2014



Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen*

Summary

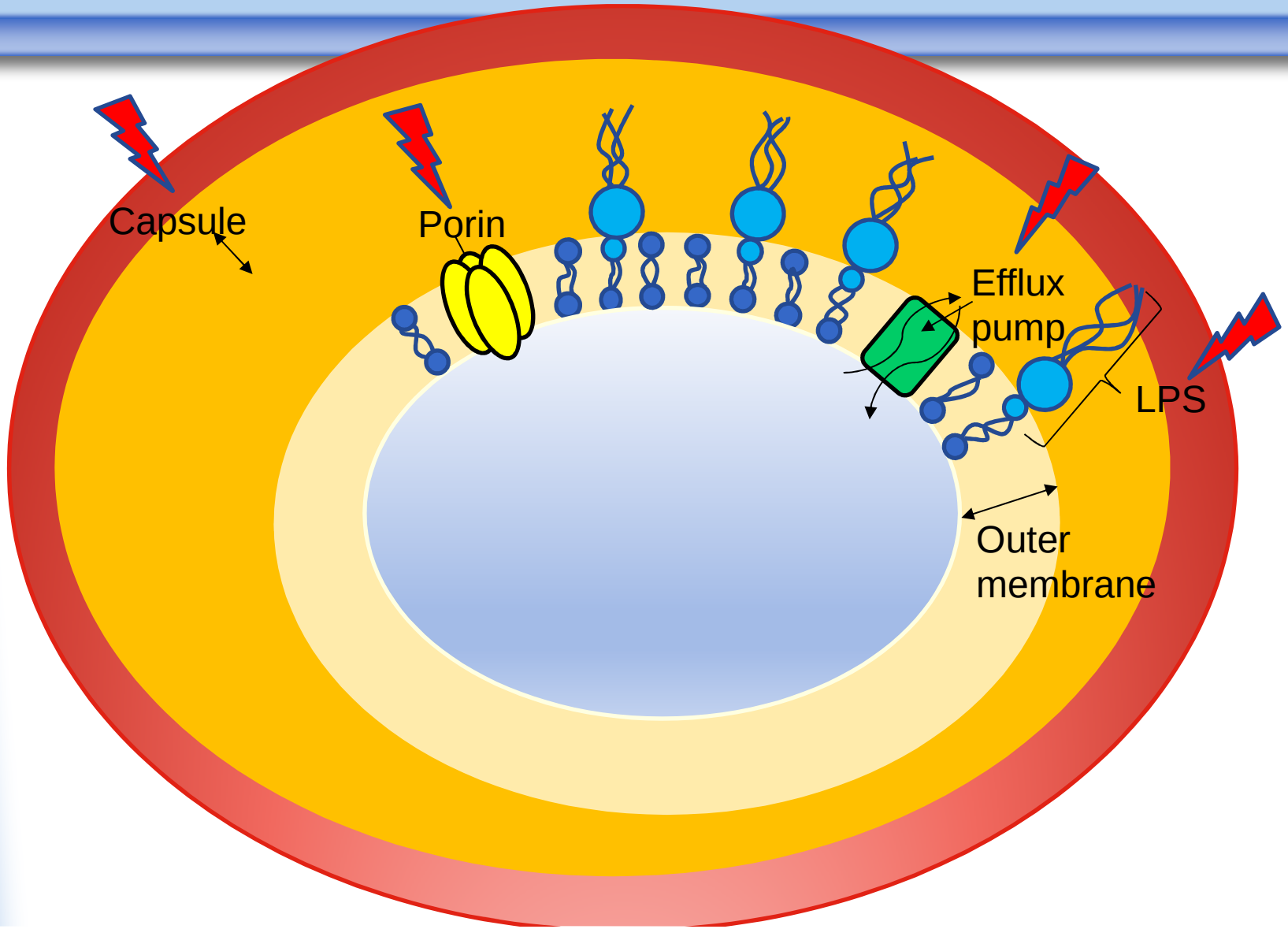
Background Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed. When an *E coli* strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.

Lancet Infect Dis 2015

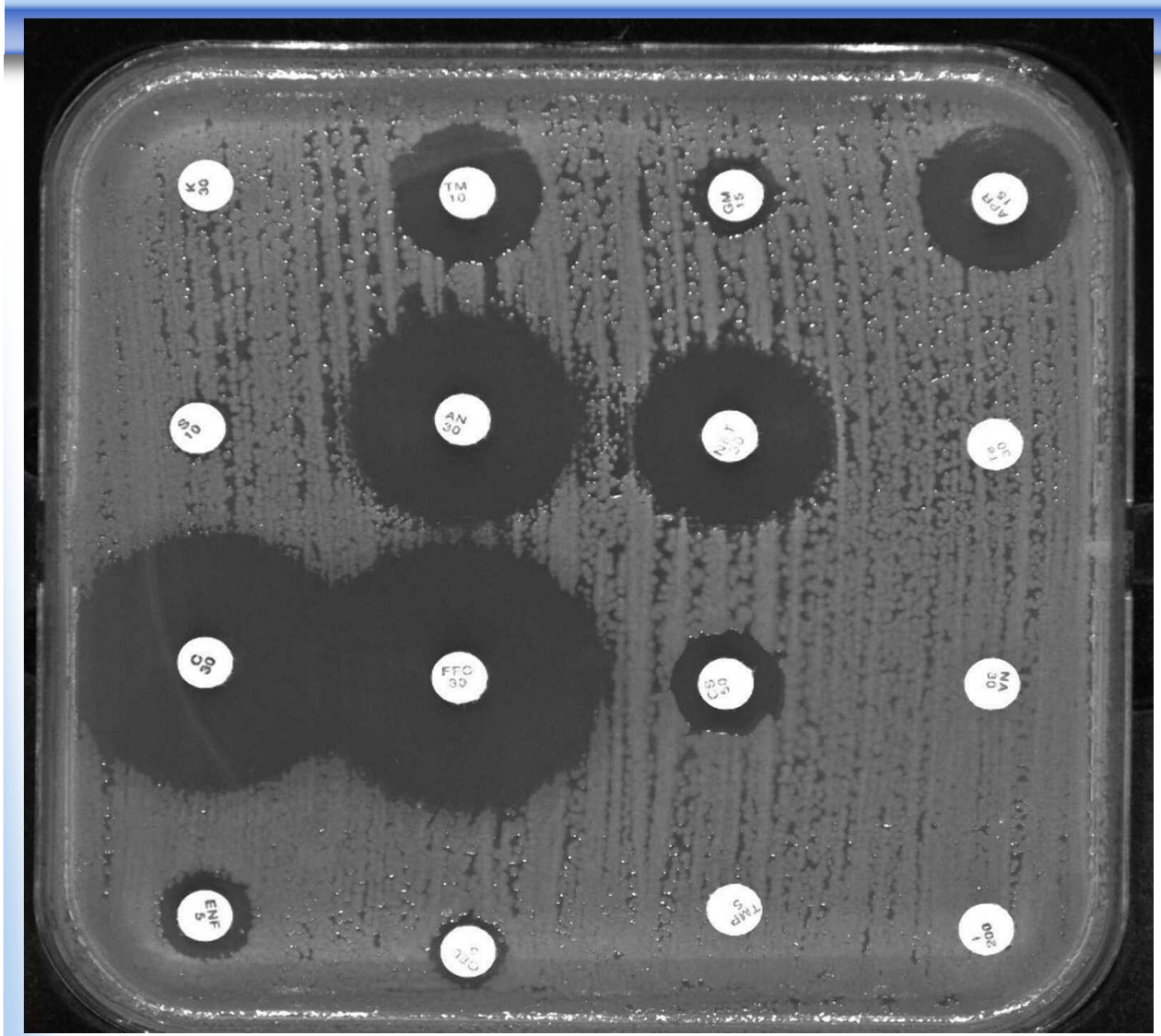
Published Online
November 18, 2015
http://dx.doi.org/10.1016/S1473-3099(15)00424-7

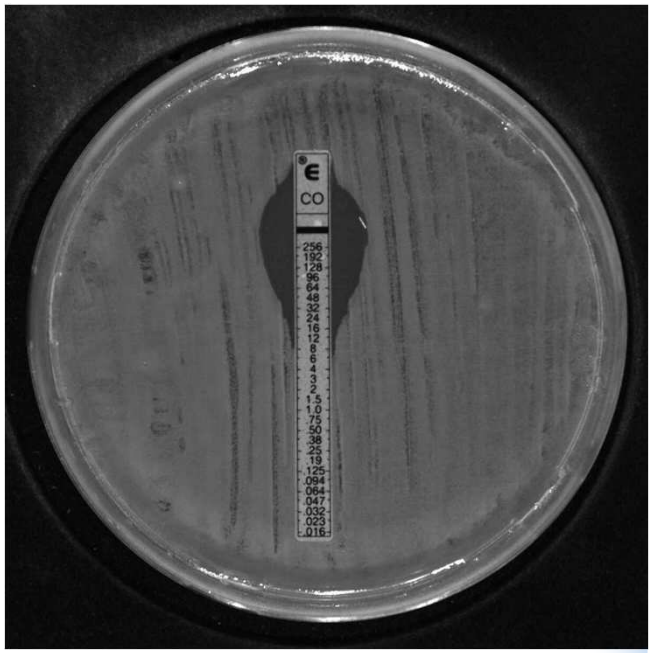
See Online/Articles
http://dx.doi.org/10.1016/

Multiple and combined mechanisms of colistin resistance

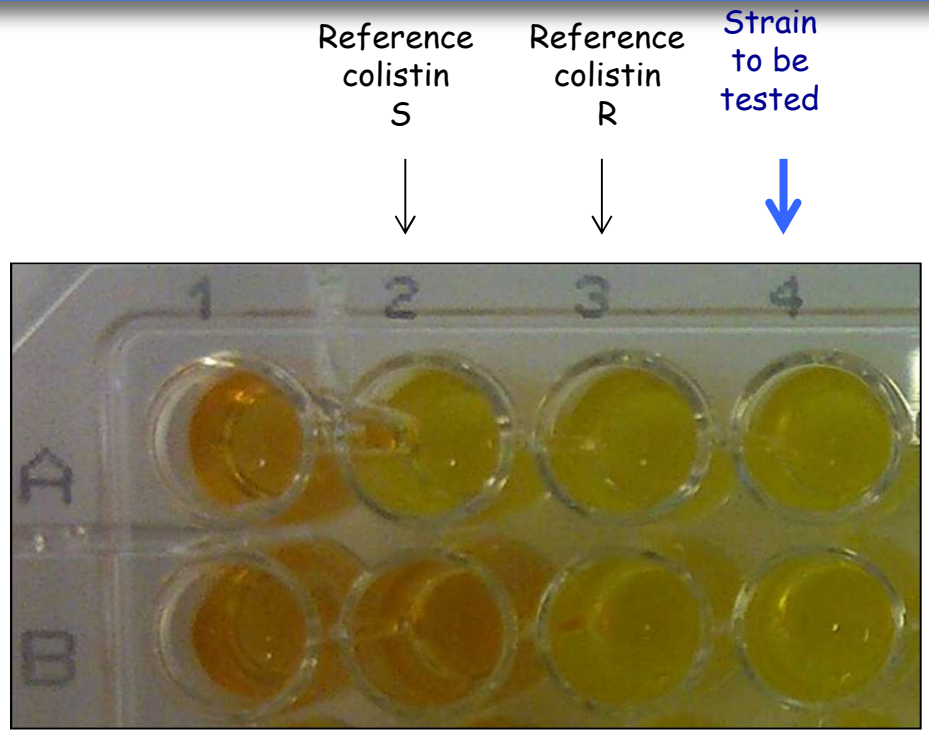


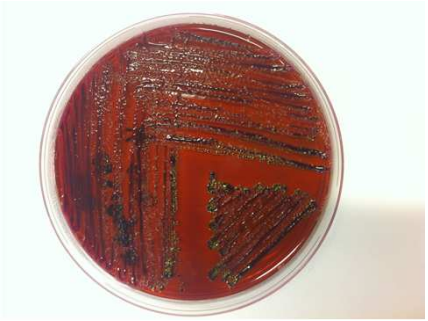
Difficult detection of resistance to colistin; not reliable, time consuming…





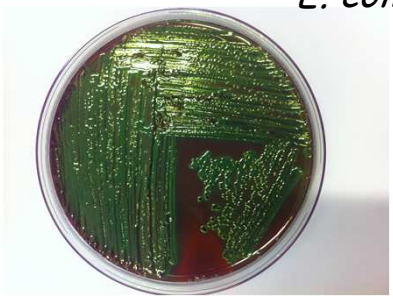
Rapid diagnostic of polymyxin resistance,





Colistin R
K. pneumoniae

Colistin R
E. coli



Rapid Polymyxin NP test
- 1 h -
Sensitivity 99.3%, Specificity 92.3%

SuperPolymyxin screening medium
Sensitivity and Specificity 100%

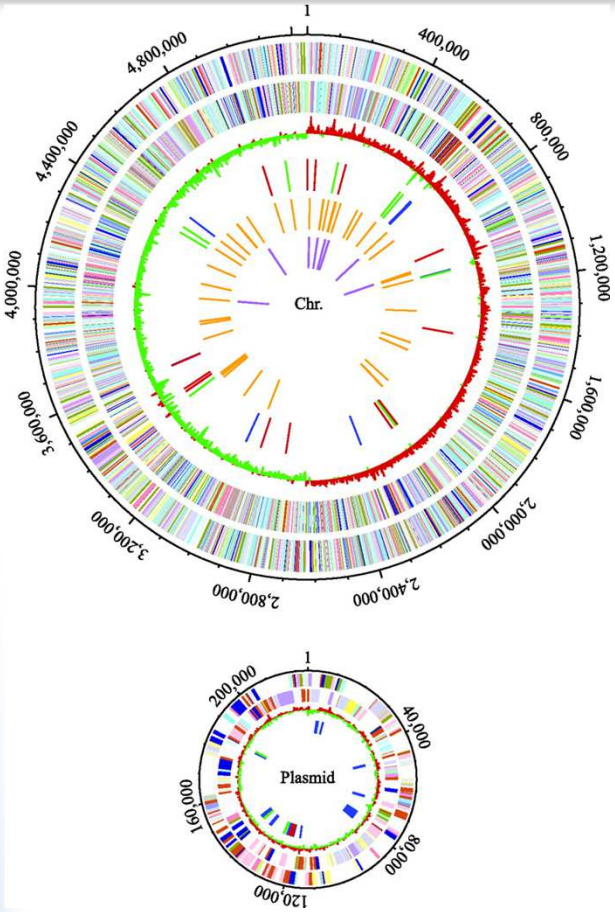
P. Nordmann et al., submitted Nov 2015

The future in diagnosis of emerging resistance

Rapid susceptibility testing: 2-4 h

Accelerate ID/AST

Whole genome sequencin ??



J Antimicrob Chemother 2014; **69**: 1729–1733
doi:10.1093/jac/dku083 Advance Access publication 27 March 2014

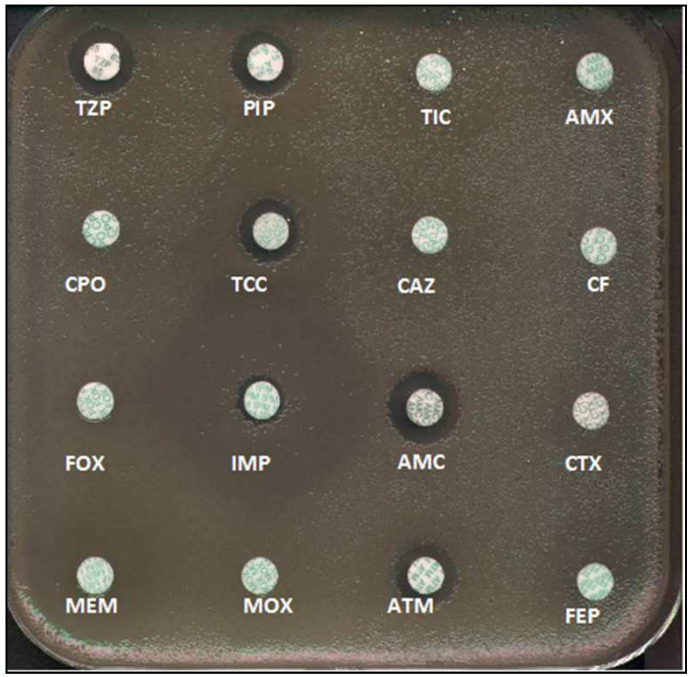
Journal of Antimicrobial Chemotherapy

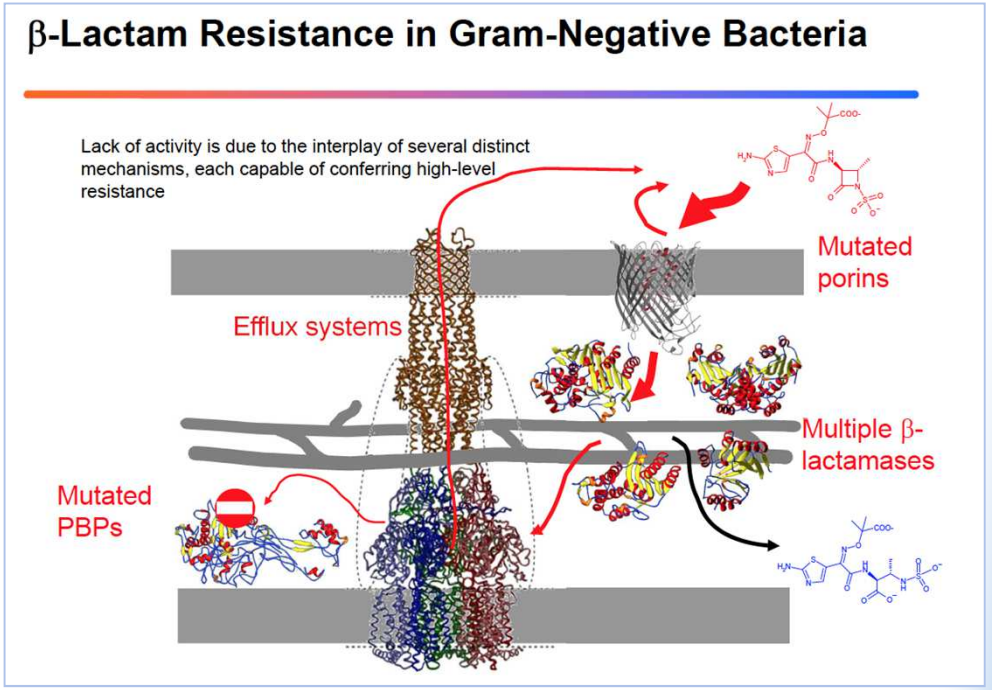
Rapid nucleic acid diagnostics for the detection of antimicrobial resistance in Gram-negative bacteria: is it time for a paradigm shift?

Nina Tuite[1]†, Kate Reddington[1]†, Thomas Barry[1], Alimuddin Zumla[2] and Virve Enne[2]*

[1]Nucleic Acid Diagnostics Research Laboratory (NADRL), Microbiology, School of Natural Sciences, National University of Ireland, Galway, Ireland; [2]Department for Clinical Microbiology, 2nd Floor Royal Free Hospital, University College London, Rowland Hill St., London NW3 2PF, UK

The problem in gram negatives: multiple and combined mechanisms of resistance





‼Surveillance-Outbreaks

Genomically Informed Surveillance for Carbapenem-Resistant Enterobacteriaceae in a Health Care System

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ABSTRACT **Carbapenem-resistant *Enterobacteriaceae* (CRE) are an urgent public health concern. Rapid identification of the resistance genes, their mobilization capacity, and strains carrying them is essential to direct hospital resources to prevent spread and improve patient outcomes. Whole-genome sequencing allows refined tracking of both chromosomal traits and associated mobile genetic elements that harbor resistance genes. To enhance surveillance of CREs, clinical isolates with phenotypic resistance to carbapenem antibiotics underwent whole-genome sequencing. Analysis of 41 isolates of *Klebsiella pneumoniae* and *Enterobacter cloacae*, collected over a 3-year period, identified *K. pneumoniae* carbapenemase (KPC) genes encoding KPC-2, −3, and −4 and OXA-48 carbapenemases. All occurred within transposons, including multiple Tn*4401* transposon isoforms, embedded within more than 10 distinct plasmids representing incompatibility (Inc) groups IncR, -N, -A/C, -H, and -X. Using short-read sequencing, draft maps were generated of new KPC-carrying vectors, several of which were derivatives of the IncN plasmid pBK31551. Two strains also had Tn*4401* chromosomal insertions. Integrated analyses of plasmid profiles and chromosomal single-nucleotide polymorphism (SNP) profiles refined the strain patterns and provided a baseline hospital mobilome to facilitate analysis of new isolates. When incorporated with patient epidemiological data, the findings identified limited outbreaks against a broader 3-year period of sporadic external entry of many different strains and resistance vectors into the hospital. These findings highlight the utility of genomic analyses in internal and external surveillance efforts to stem the transmission of drug-resistant strains within and across health care institutions.**

IMPORTANCE **We demonstrate how detection of resistance genes within mobile elements and resistance-carrying strains furthers active surveillance efforts for drug resistance. Whole-genome sequencing is increasingly available in hospital laboratories and provides a powerful and nuanced means to define the local landscape of drug resistance. In this study, isolates of *Klebsiella pneumoniae* and *Enterobacter cloacae* with resistance to carbapenem antibiotics were sequenced. Multiple carbapenemase genes were identified that resided in distinct transposons and plasmids. This mobilome, or population of mobile elements capable of mobilizing drug resistance, further highlighted the degree of strain heterogeneity while providing a detailed timeline of carbapenemase entry into the hospital over a 3-year period. These surveillance efforts support effective targeting of infection control resources and the development of institution-specific repositories of resistance genes and the mobile elements that carry them.**

Point-of-care technology

Disposable

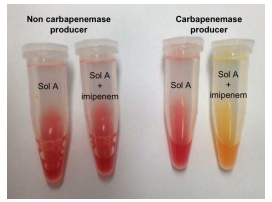
Menu

Expected Test Pipeline	At launch	Mid Term	Long Term
HAI	Cdiff	SA MDR	
STD's		CT/GC	Vaginatis Panel HSV HPV
Critical Infectious Diseases	GBS	GAS FluA	Enteric Panel Respiratory Panel Mtb Malaria
Immuno-compromised			HIV Qt HCV Qt
Genetics			2C19

Instrument

Take home message

1. Rapid evolution towards multidrug resistance=increased complexity of resistance mechanisms
2. Emergence of totally novel genes
3. Personalized medicine; a need for rapid diagnostic techniques for antibiotic choice
4. Cost effective techniques



Complementary approach

1. Antibiogram (rapid)
2. Biochemical detection or immunological detection of resistance proteins
3. Molecular biology