Carbapenemase-producing *Enterobacteriaceae* 2012

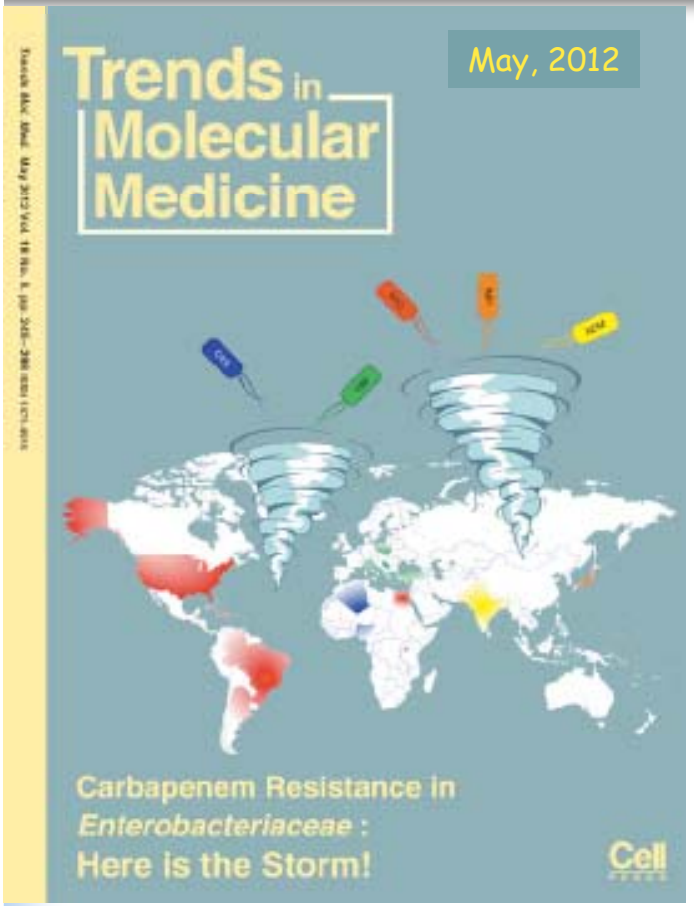
CNR Associé Résistance aux Antibiotiques

Prof. P. Nordmann

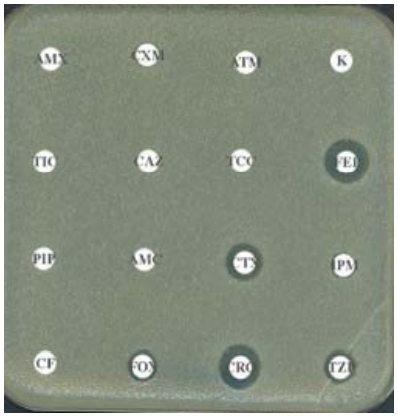
Emerging Antibiotic resistance

Top three WHO priorities (2015-2025): Emerging Antibiotic Resistance, tuberculosis and malaria

Carbapenemases in *Enterobacteriaceae*



P. Nordmann, L. Dortet, L. Poirel

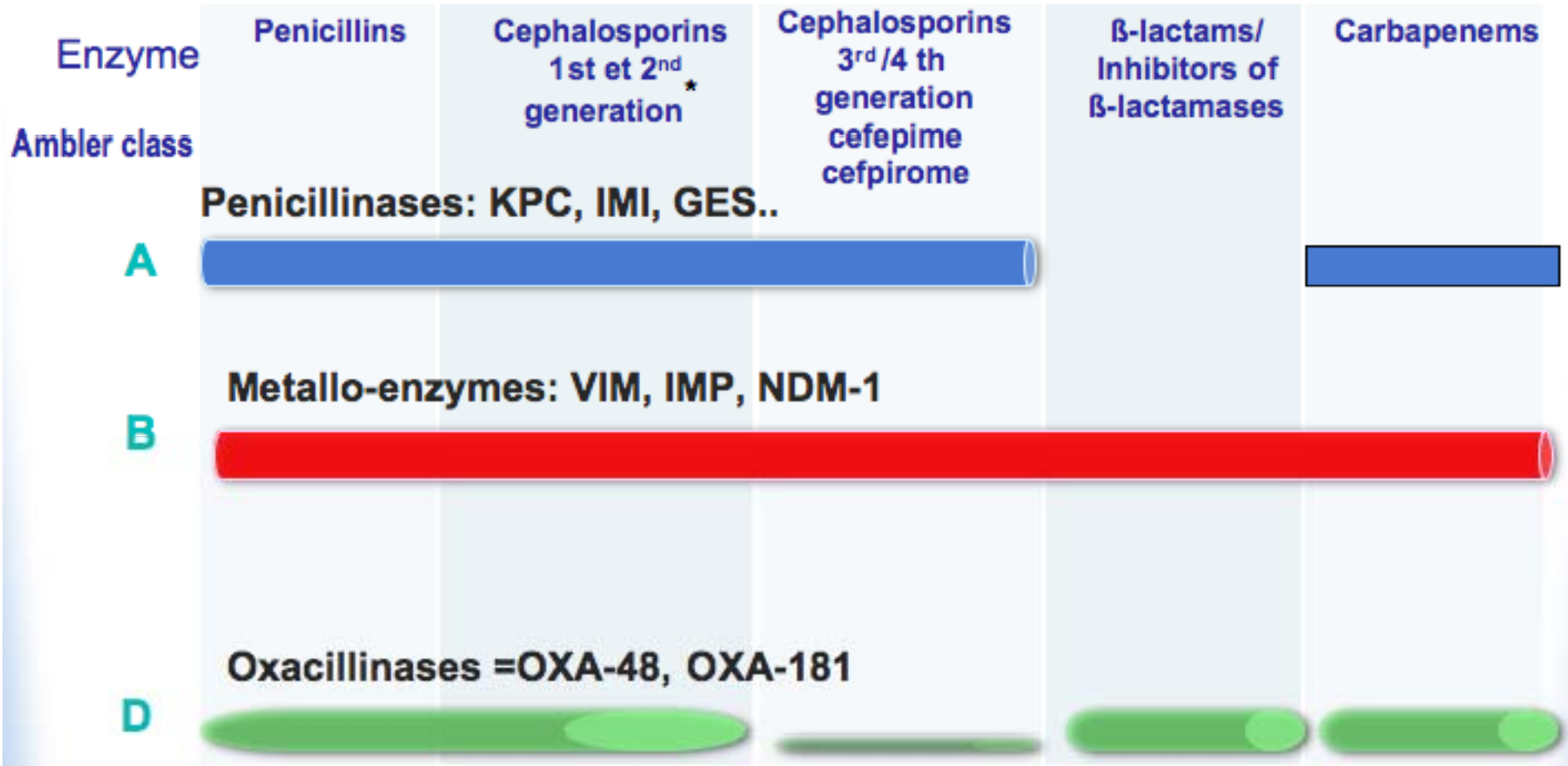


Penicillins Cephalosporins Carbapenems

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

Carbapenemases; VIM.IMP NDM, OXA-48, KPC

The carbapenemases in *Enterobacteriaceae*



* Cephamycins excluded for most class As

KPCs; Klebsiella Pneumoniae Carbapenemase

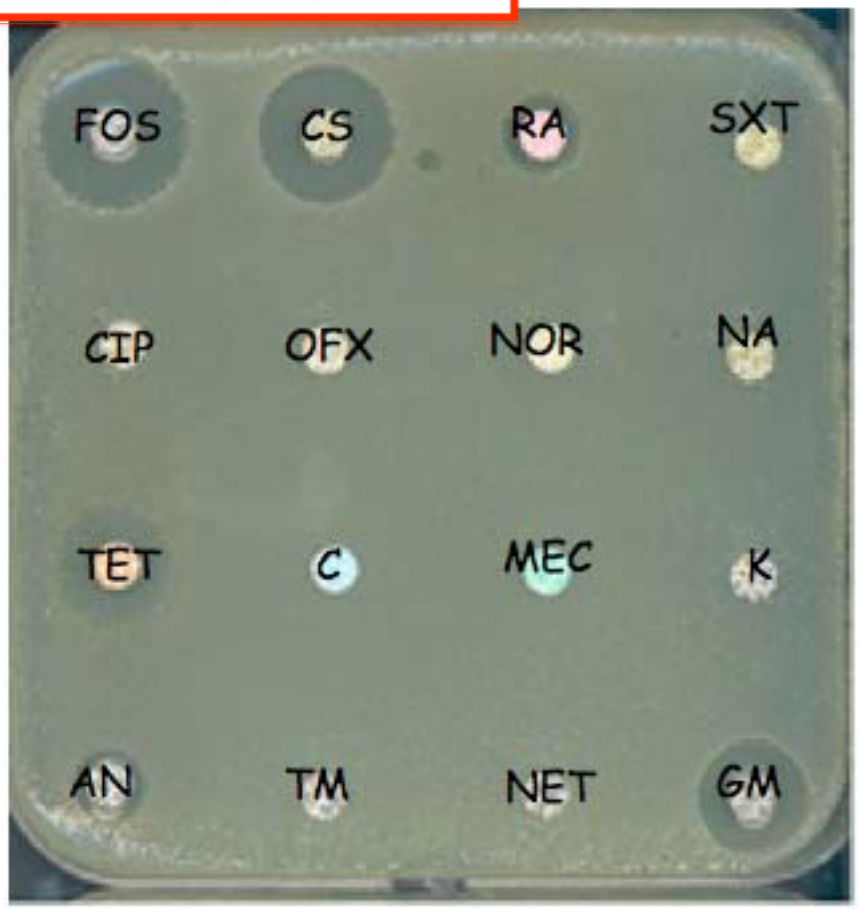
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2001, p. 1151–1161 Vol. 45, No. 4
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.4.1151–1161.2001

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,[4] 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, Andrea Doria, Palma de Mallorca, 07014,[4] and Área de Microbiología, Universidad de las Islas Baleares, Crta. Valldemosa, Palma de Mallorca, 07071,[3] Spain

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



The KPC enzymes

ORIGINAL INVESTIGATION

Rapid Spread of Carbapenem-Resistant *Klebsiella pneumoniae* in New York City

A New Threat to Our Antibiotic Armamentarium

Simona Bratu, MD; David Landman, MD; Robyn Haag, RN; Rose Recco, MD; Antonella Eramo, RN; Maqsood Alam, MD; John Quale, MD

Background: Carbapenem antibiotics are used to treat serious infections caused by extended-spectrum β-lactamase–carrying pathogens. Carbapenem resistance has been unusual in isolates of *Klebsiella pneumoniae*. In this study, the prevalence and molecular epidemiologic characteristics of carbapenem-resistant *K pneumoniae* are analyzed, and the experience involving 2 hospital outbreaks is described.

Methods: A citywide surveillance study was conducted in hospitals in Brooklyn. An observational study involving subsequent outbreaks at 2 hospitals was undertaken. Isolates were genetically fingerprinted by ribotyping and were examined for the presence of KPC-type carbapenem-hydrolyzing β-lactamases.

Results: Of 602 isolates of *K pneumoniae* collected during the citywide surveillance study, 45% had extended-spectrum β-lactamases. Of the extended-spectrum β-lactamase–producing isolates, 3.3% carried the carbapenem-hydrolyzing β-lactamase KPC-2. Several isolates were reported by the clinical microbiology laboratories as being susceptible to imipenem. Although all the isolates were resistant using agar diffusion methods, minimal inhibitory concentrations of imipenem were substantially lower for several isolates using standard broth microdilution tests and were highly dependent on the inoculum used. Two hospitals experienced the rapid spread of carbapenem-resistant isolates involving 58 14-day mortality for bacteremic patie isolates belonged to a single ribotype

Conclusions: Carbapenem-resistant lates are rapidly emerging in New Yor ola strain that possesses a carbapenem tamase has occurred in regional hospi isolates are resistant to virtually all c tibiotics, control of their spread is cru tomated systems used for susceptibili accurately identify all these isolates, w hamper control efforts.

Arch Intern Med. 2005;165:1430-1433

K. pneumoniae KPC+: 33%: ST-type 258

Journal of Antimicrobial Chemotherapy (2007) **60**, 78–82
doi:10.1093/jac/dkm129
Advance Access publication 9 May 2007

JAC

Evolution of antimicrobial resistance among *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in Brooklyn, NY

David Landman[1], Simona Bratu[1], Sandeep Kochar[1], Monica Panwar[1], Manoj Trehan[1], Mehmet Doymaz[2] and John Quale[1]*

[1]*State University of New York – Downstate, Brooklyn, NY, USA;* [2]*Beth Israel Medical Center, New York, NY, USA*

Received 16 February 2007; returned 22 March 2007; revised 2 April 2007; accepted 5 April 2007

Objectives: To document resistance patterns of three important nosocomial pathogens, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, present in hospitals in Brooklyn, NY.

Methods: Susceptibility profiles of pathogens gathered during a surveillance study in 2006 were analysed and compared with similar surveys performed in 1999 and 2001. MICs were determined according to CLSI standards, and selected isolates were screened by PCR for the presence of VIM, IMP and KPC β-lactamases.

Results: For *P. aeruginosa*, susceptibility to most antimicrobials fell in 2001 and then reached a plateau. However, there was a progressive decrease in the number of patients with *P. aeruginosa* during the three surveys. While the total number of isolates of *A. baumannii* remained steady, there was a progressive decrease in susceptibility to most classes of antimicrobial agents, and approximately one-third had combined resistance to carbapenems, fluoroquinolones and aminoglycosides. There was a noticeable rise in the number of isolates of *K. pneumoniae* over the surveillance periods, suggesting that this has become the predominant pathogen in many medical centres. Over one-third of *K. pneumoniae* collected in 2006 carried the carbapenemase KPC, and 22% were resistant to all three classes of antimicrobial agents.

Conclusions: Hospitals in our region have been beset with antimicrobial-resistant Gram-negative bacteria. *K. pneumoniae* has rapidly emerged as the most common multidrug-resistant pathogen. Improved therapeutic agents and methods of detection are needed to reduce transmission of these bacteria.

ORIGINAL INVESTIGATION

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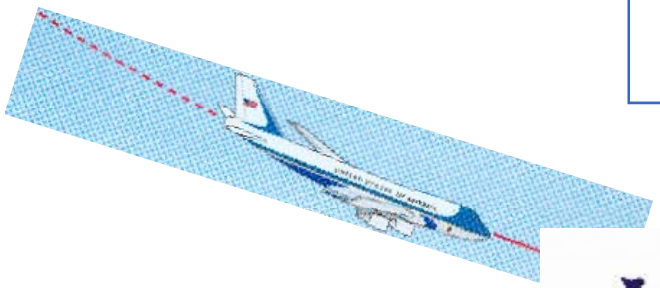
Simor nan, MD; Robin Haag, RN; Rose Recco, MD; Anton Alam, MD; John Quale, MD

Arch Intern Med. 2005;165:1430-1435



Antimicrobial Agents and Chemotherapy, Sept. 2006, p. 3098–3101
0066-4804/06/$08.00+0 doi:10.1128/AAC.00438-06

Plasmid-Mediated Imipenem-Hydrolyzing Enzyme KPC-2 among Multiple Carbapenem-Resistant *Escherichia coli* Clones in Israel

Antimicrobial Agents and Chemotherapy, Aug. 2007, p. 3026–3029 Vol. 51, No. 8
0066-4804/07/$08.00+0 doi:10.1128/AAC.00299-07

Emergence of KPC-2 and KPC-3 in Carbapenem-Resistant *Klebsiella pneumoniae* Strains in an Israeli Hospital[▽]

Azita Leavitt, Shiri Navon-Venezia, Inna Chmelnitsky, Mitchell J. Schwaber, and Yehuda Carmeli*
Division of Epidemiology and the Laboratory for Molecular Epidemiology and Antibiotic Research, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Intercontinental travels of patients and dissemination of plasmid-mediated carbape KPC-3 associated with OXA-9 and TEM-1

Laurent Dortet[1], Irina Radu[1], Valérie Gautier[2], François Blot[3], Elisabeth Chachaty[1] and Guillaume Arlet[2,4]*

(JAC, 2009)

***E. Cloacae* KPC-3**

E. coli, et *E. cloacae*
(Petrella, AAC, 2008)

Plasmid-Mediated Carbapenem-Hydrolyzing β-Lactamase KPC in a *Klebsiella pneumoniae* Isolate from France

Naas, Nordmann, Vedel, Poyart
AAC 2005, 49 ; 4423-4

ORIGINAL INVESTIGATION

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Simor nan, MD; Robin Haag, RN; Rose Recco, MD;
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Arch Intern Med. 2005;165:1430-1

E. Cloacae KPC-4 (Ecosse)

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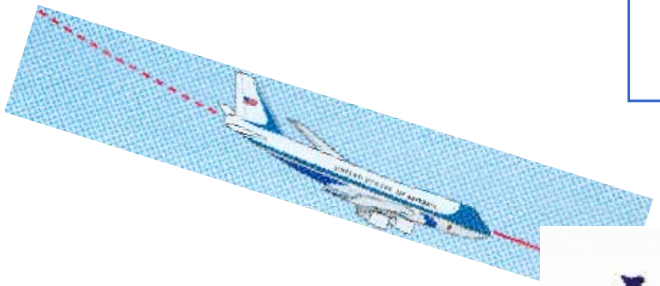

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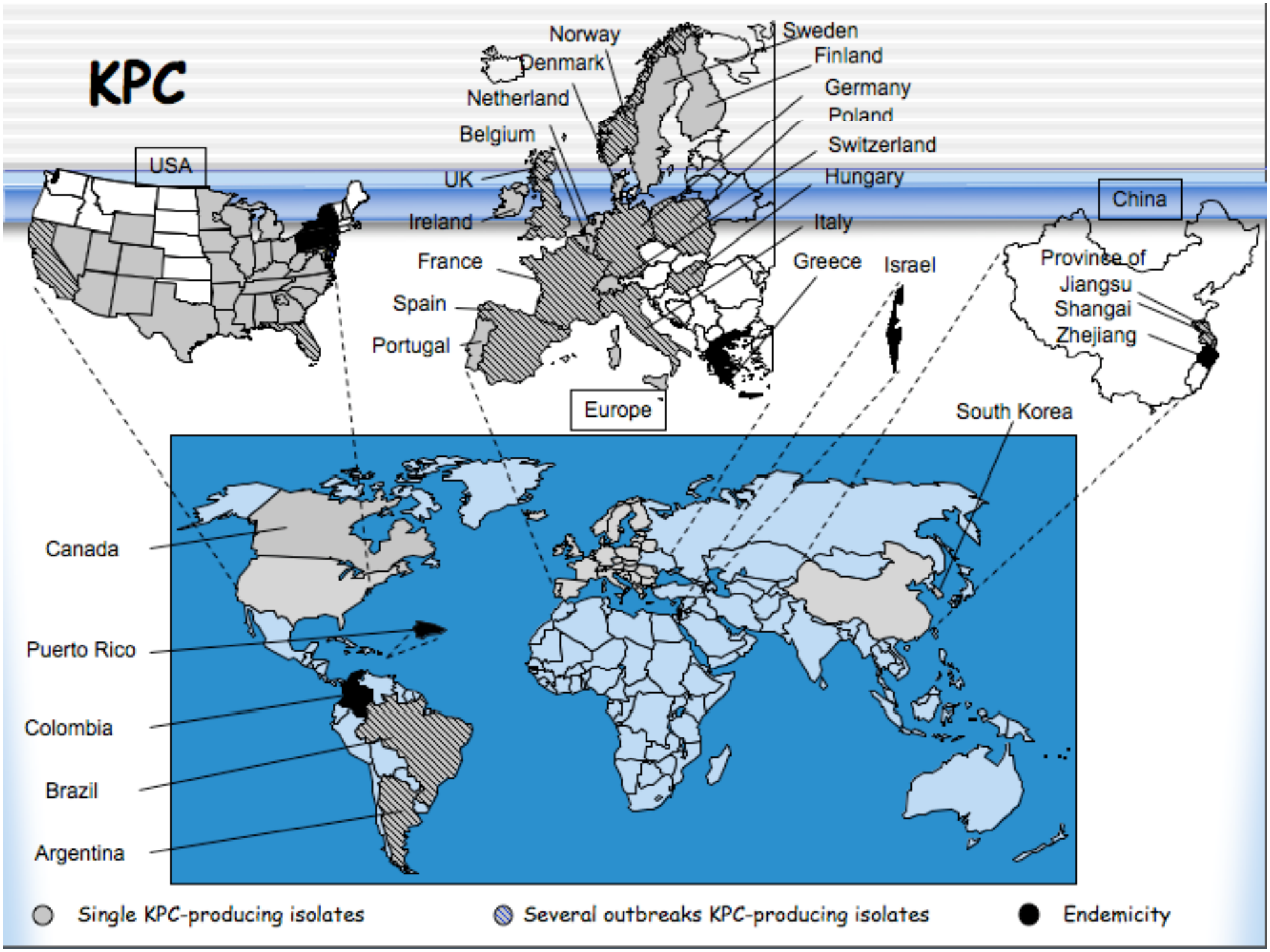
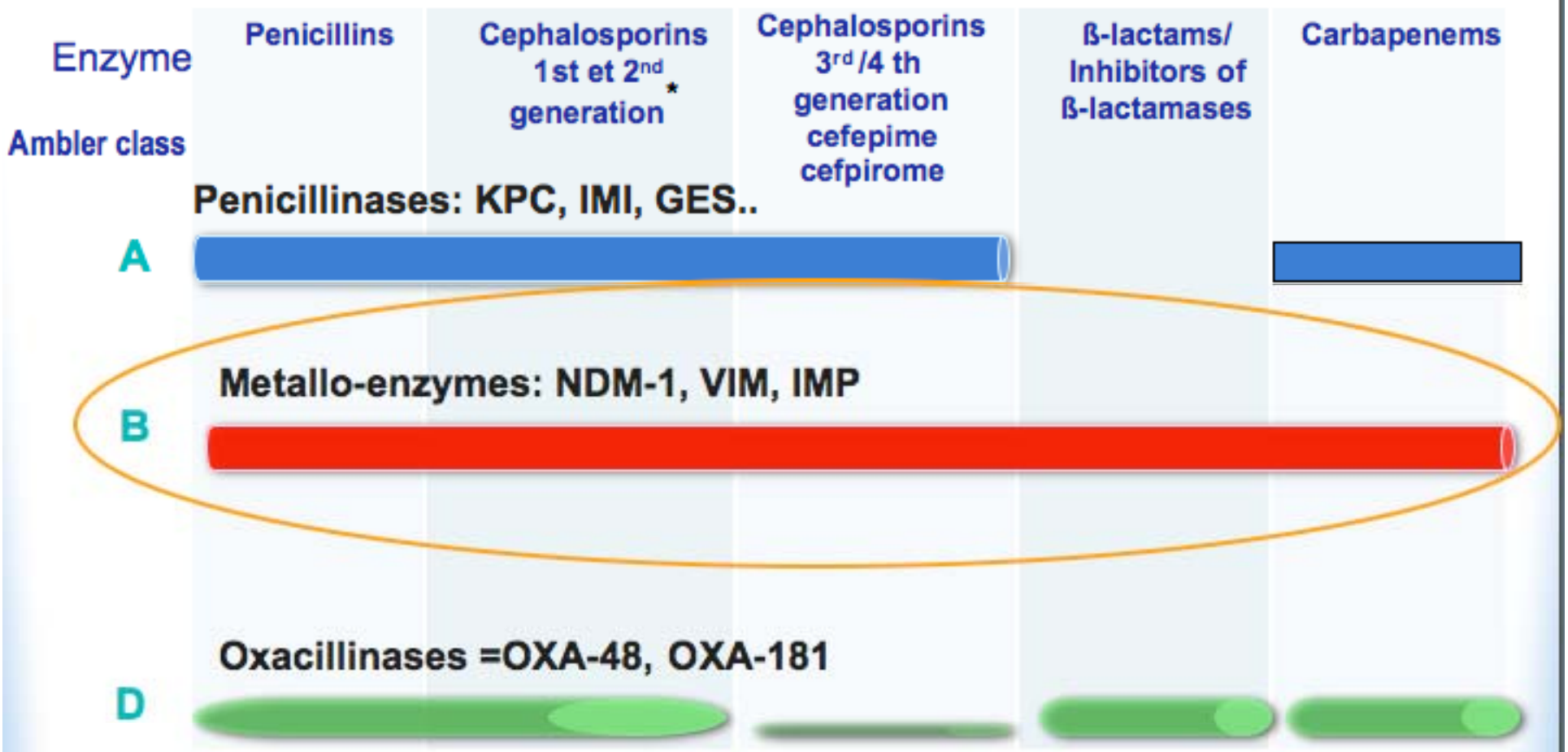
Cuzon, Naas, Demachy, Nordmann
(AAC 2007)

K. pneumoniae KPC-2


KPC
USA
Norway
Denmark
Netherland
Belgium
UK
Ireland
France
Spain
Portugal
Sweden
Finland
Germany
Poland
Switzerland
Hungary
Italy
Greece
Israel
Europe
China
Province of
Jiangsu
Shangai
Zhejiang
South Korea
Canada
Puerto Rico
Colombia
Brazil
Argentina
Single KPC-producing isolates
Several outbreaks KPC-producing isolates
Endemicity

The carbapenemases in *Enterobacteriaceae*



* Cephamycins excluded for most class As

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[▽]

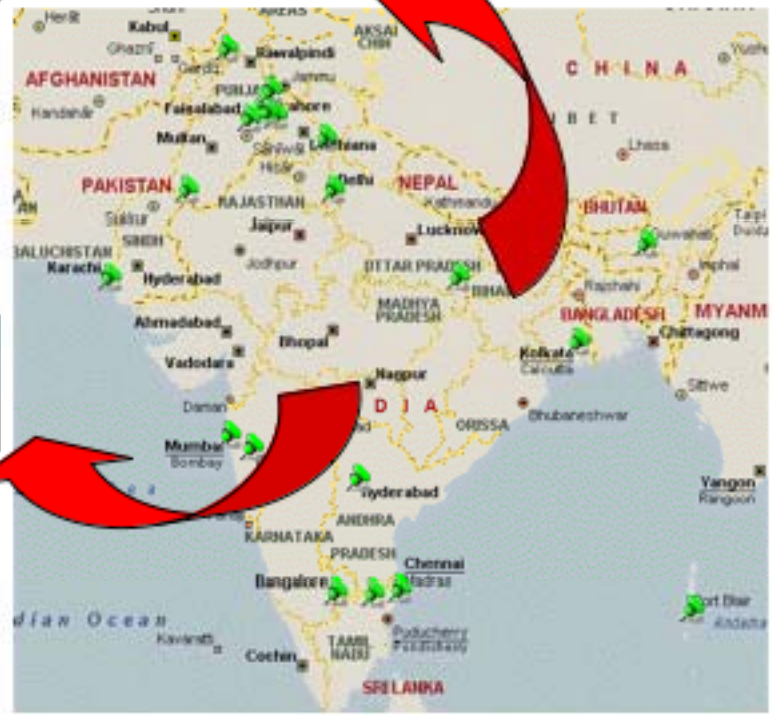
Dongeun Yong,[1,2] Mark A. Toleman,[2] Christian G. Giske,[3] Hyun S. Cho,[4] Kristina Sundman,[5] Kyungwon Lee,[1] and Timothy R. Walsh[2]*

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]

Spread of NDM-1 from India/Pakistan to the UK

bla$_{NDM-1}$-positive bacterial clones?

MDR plasmids?



Courtesy N. Woodford

Multidrug resistance patterns of NDM-1 producers

	UK (n=37)		Chennai (n=44)		Haryana (n=26)	
	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*
Imipenem	32; 128	0%	64; 128	0%	32; 128	0%
Meropenem	32; 32	3%	32; >32	3%	>32; >32	3%
Piperacillin-tazobactam	>64; >64	0%	>64; >64	0%	>64; >64	0%
Cefotaxime	>256; >256	0%	>256; >256	0%	>256; >256	0%
Ceftazidime	>256; >256	0%	>256; >256	0%	>256; >256	0%
Cefpirome	>64; >64	0%	>64; >64	0%	>64; >64	0%
Aztreonam	>64; >64	11%	>64; >64	0%	>64; >64	8%
Ciprofloxacin	>8; >8	8%	>8; >8	8%	>8; >8	8%
Gentamicin	>32; >32	3%	>32; >32	3%	>32; >32	3%
Tobramycin	>32; >32	0%	>32; >32	0%	>32; >32	0%
Amikacin	>64; >64	0%	>64; >64	0%	>64; >64	0%
Minocycline	16; >32	0%	32; >32	0%	8; 16	0%
Tigecycline	1; 4	64%	4; 8	56%	1; 2	67%
Colistin	0·5; 8	89%†	1; 32	94%†	1; 2	100%†

MIC=minimum inhibitory concentration. *Susceptibility defined by British Society for Antimicrobial Chemotherapy and European Committee on Antimicrobial Susceptibility Testing breakpoints; doxycycline breakpoints were used for minocycline. †Colistin-resistant UK isolates were one isolate of *Morganella morganii* and one *Providencia* sp (both intrinsically-resistant species), also one *Klebsiella pneumoniae* and one *Enterobacter* sp.

Table: **Antibiotic susceptibilities for NDM-1-positive Enterobacteriaceae isolated in the UK and north (Chennai) and south India (Haryana)**

Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study

Timothy R Walsh, Janis Weeks, David M Livermore, Mark A Toleman

Summary

Background Not all patients infected with NDM-1-positive bacteria have a history of hospital admission in India, and extended-spectrum β-lactamases are known to be circulating in the Indian community. We therefore measured the prevalence of the NDM-1 gene in drinking water and seepage samples in New Delhi.

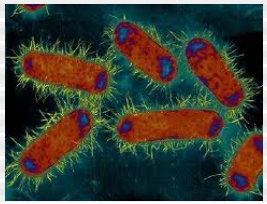
Methods Swabs absorbing about 100 µL of seepage water (ie, water pools in streets or rivulets) and 15 mL samples of public tap water were collected from sites within a 12 km radius of central New Delhi, with each site photographed and documented. Samples were transported to the UK and tested for the presence of the NDM-1 gene, bla_{NDM-1}, by PCR and DNA probing. As a control group, 100 µL sewage effluent samples were taken from the Cardiff Wastewater Treatment Works, Tremorfa, Wales. Bacteria from all samples were recovered and examined for bla_{NDM-1} by PCR and sequencing. We identified NDM-1-positive isolates, undertook susceptibility testing, and, where appropriate, typed the isolates. We undertook Inc typing on bla_{NDM-1}-positive plasmids. Transconjugants were created to assess plasmid transfer frequency and its relation to temperature.

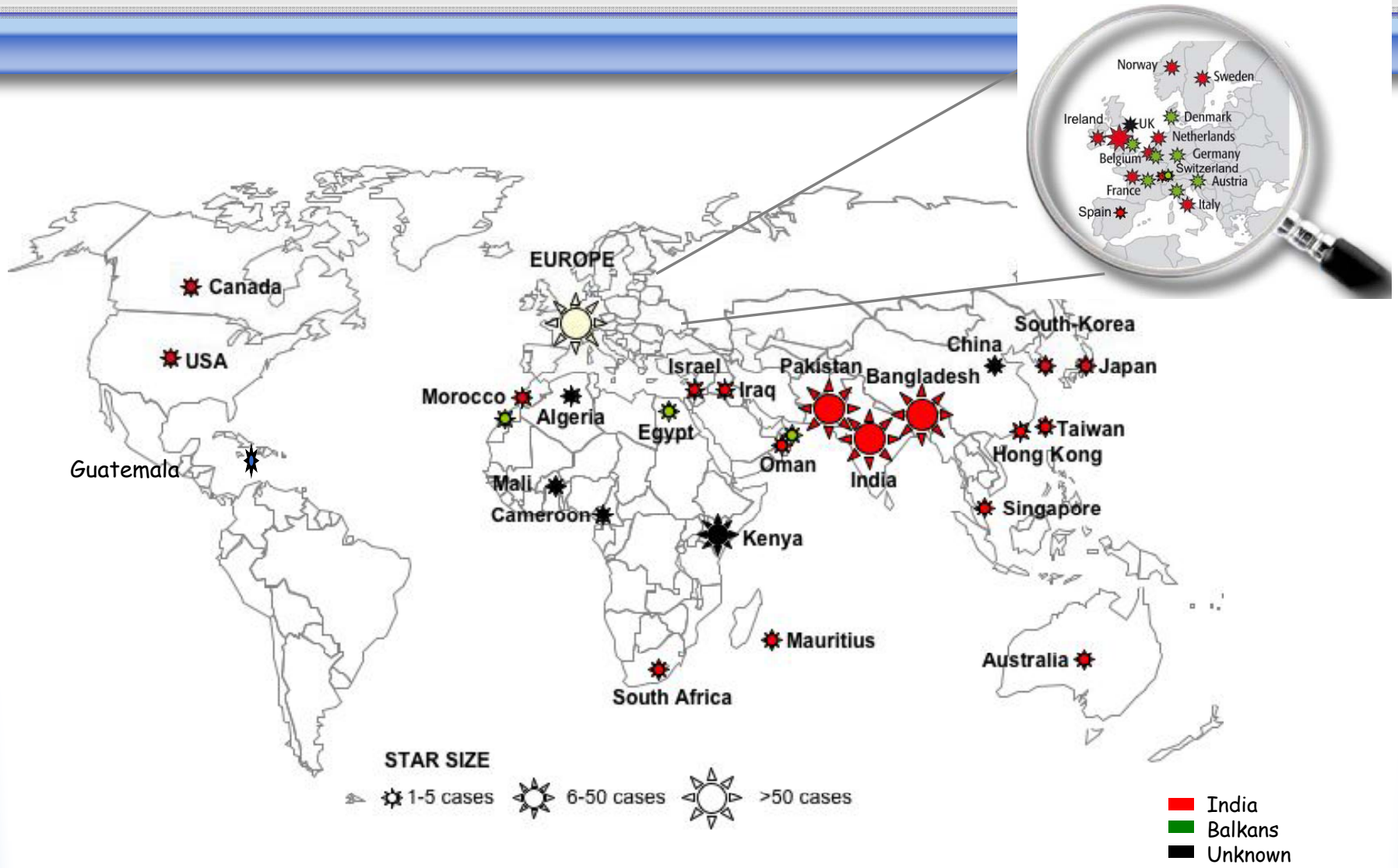
Findings From Sept 26 to Oct 10, 2010, 171 seepage samples and 50 tap water samples from New Delhi and 70 sewage effluent samples from Cardiff Wastewater Treatment Works were collected. We detected bla_{NDM-1} in two of 50 drinking-water samples and 51 of 171 seepage samples from New Delhi; the gene was not found in any sample from Cardiff. Bacteria with bla_{NDM-1} were grown from 12 of 171 seepage samples and two of 50 water samples, and included 11 species in which NDM-1 has not previously been reported, including *Shigella boydii* and *Vibrio cholerae*. Carriage by enterobacteria, aeromonads, and *V cholera* was stable, generally transmissible, and associated with resistance patterns typical for NDM-1; carriage by non-fermenters was unstable in many cases and not associated with typical resistance. 20 strains of bacteria were found in the samples, 12 of which carried bla_{NDM-1} on plasmids, which ranged in size from 140 to 400 kb. Isolates of *Aeromonas caviae* and *V cholerae* carried bla_{NDM-1} on chromosomes. Conjugative transfer was more common at 30°C than at 25°C or 37°C.

Interpretation The presence of NDM-1 β-lactamase-producing bacteria in environmental samples in New Delhi has important implications for people living in the city who are reliant on public water and sanitation facilities. International surveillance of resistance, incorporating environmental sampling as well as examination of clinical isolates, needs to be established as a priority.

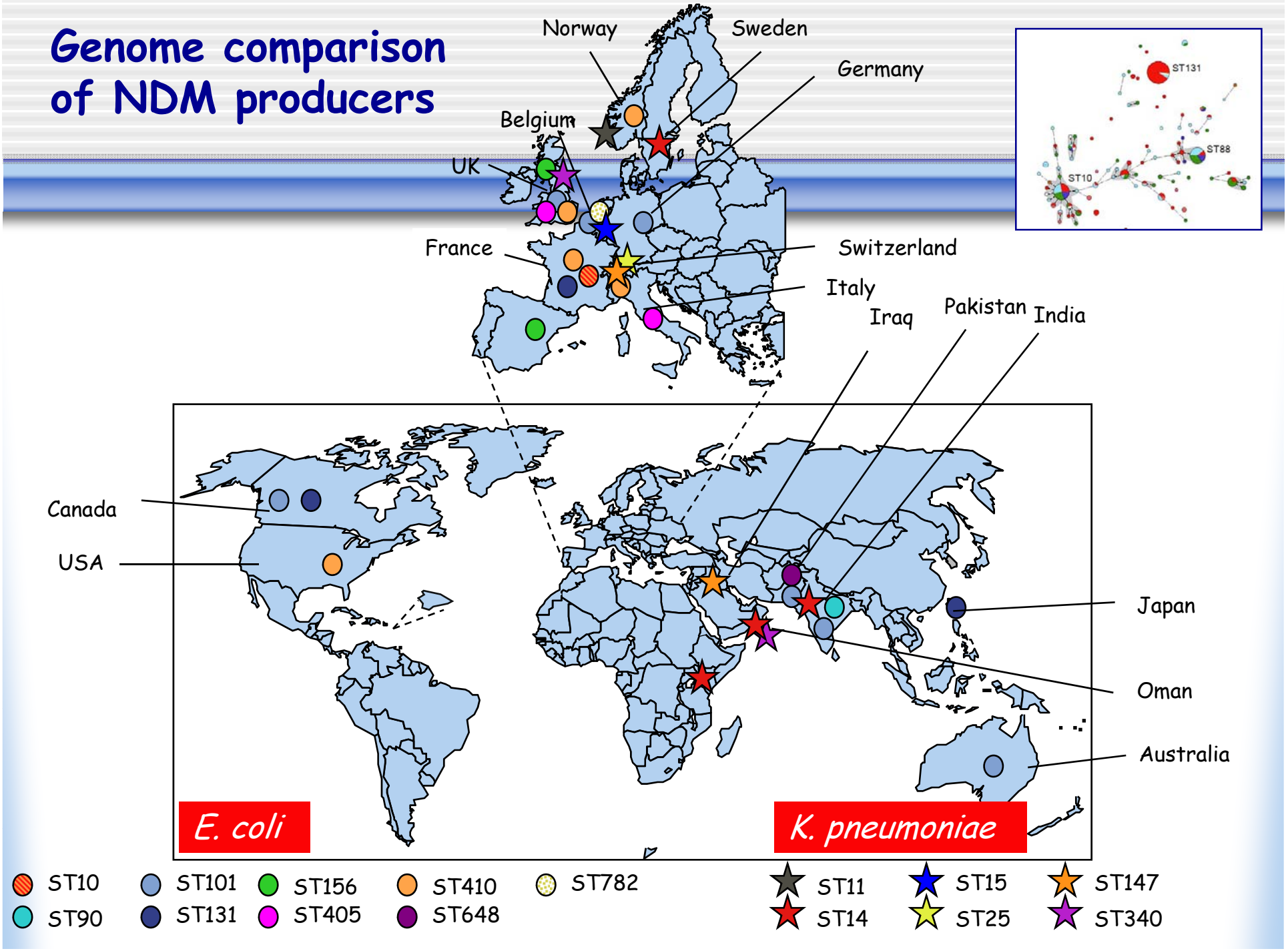
NDM

Wordwide distribution of carbapenemases NDM-2012



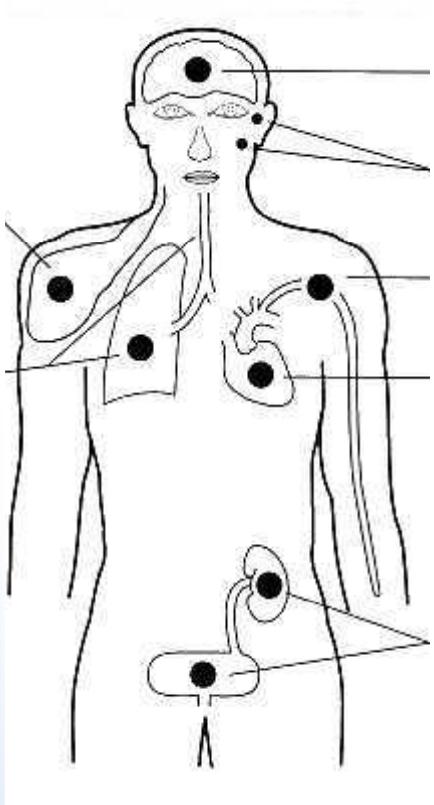


Genome comparison of NDM producers



Infections with NDM producers

***E. coli, Klebsiella**, Enterobacter, Serratia, Citrobacter, Pseudomonas, Acinetobacter*



Asymptomatic colonisation

Wound infection / Diabetic foot

Lower urinary tract infection

Upper urinary tract infection

Nosocomial pneumonia / VAP

Intra-abdominal / pelvic infection

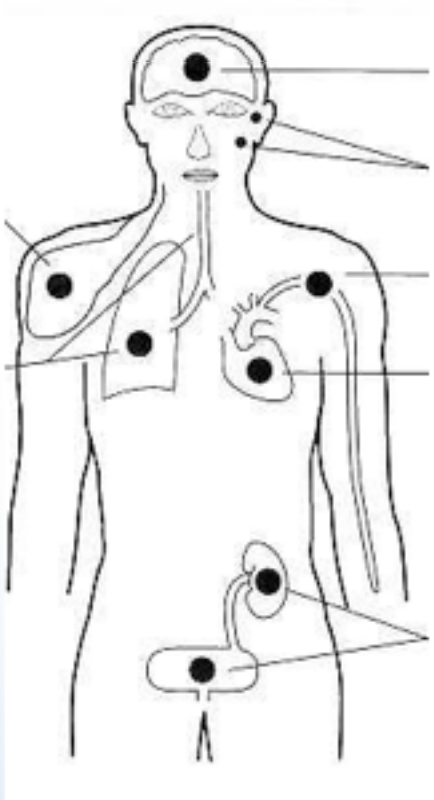
Bacteraemia / septicaemia

Neurosurgical meningitis

SEVERITY

Infections with NDM producers

***E. coli, Klebsiella**, Enterobacter, Serratia, Citrobacter, Pseudomonas, Acinetobacter*



Asymptomatic colonisation

Wound infection / Diabetic foot

Lower urinary tract infection

Upper urinary tract infection

Nosocomial pneumonia / VAP

Intra-abdominal / pelvic infection

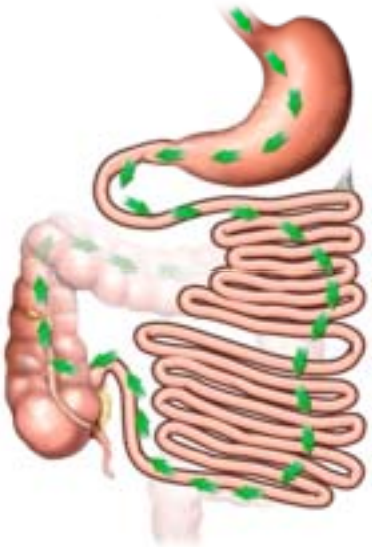
Bacteraemia / septicaemia

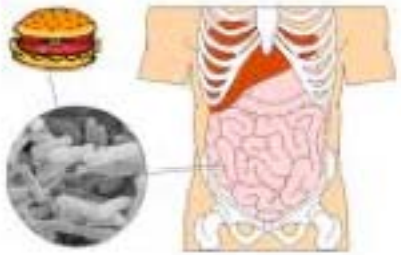
Neurosurgical meningitis

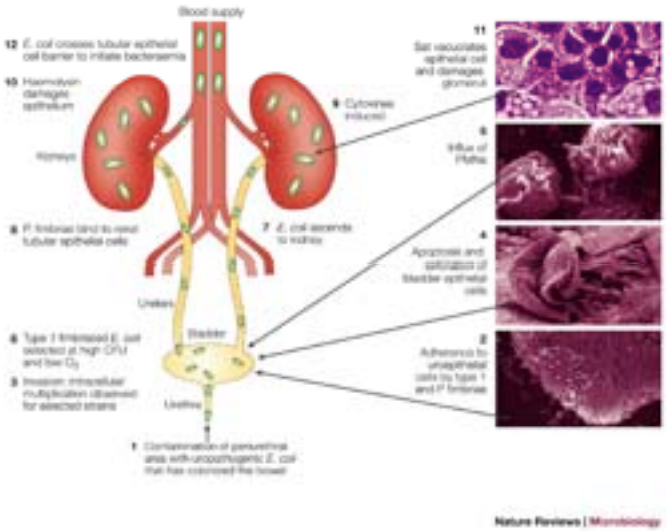
- No difference between NDM and non-NDM producers
- No known virulence factors for NDM producers
- NDM producers will not respond to conventional antibiotics !!!!

Escherichia coli

- 1st human bacterial pathogen
- 1st community-acquired pathogen
- 1st cause of urinary tract infections and diarrhea



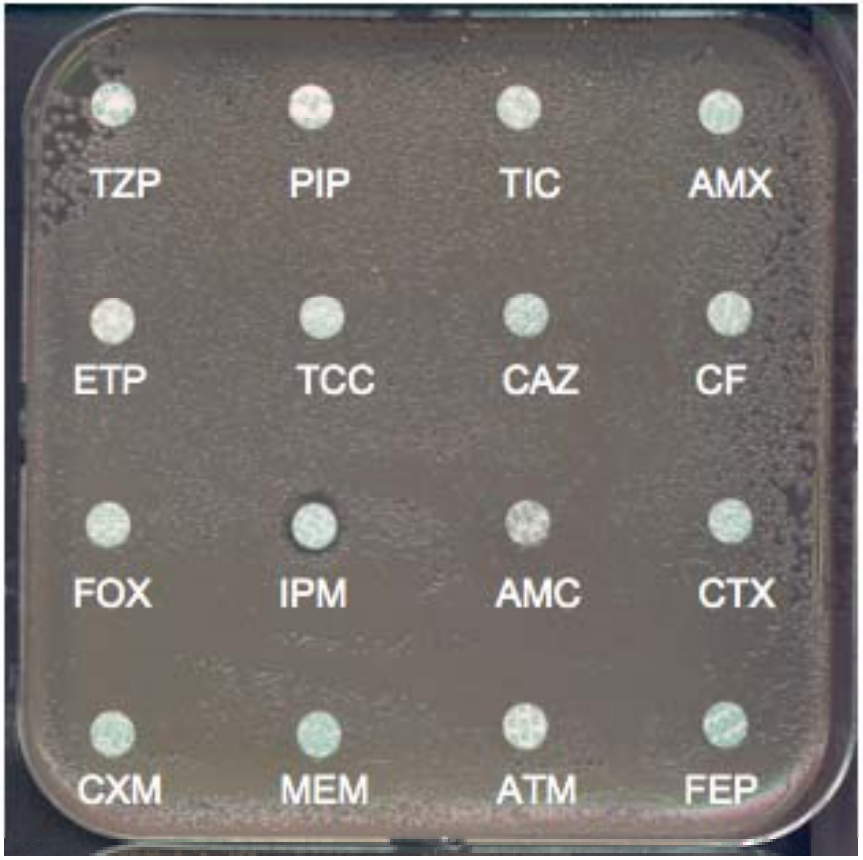




ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4224–4229 Vol. 55, No. 9
0066-4804/11/$12.00 doi:10.1128/AAC.00165-11

Analysis of the Resistome of a Multidrug-Resistant NDM-1-Producing *Escherichia coli* Strain by High-Throughput Genome Sequencing[▽]

Laurent Poirel, Rémy A. Bonnin, and Patrice Nordmann*



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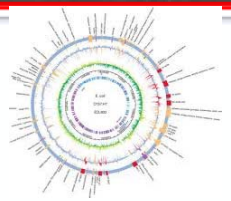
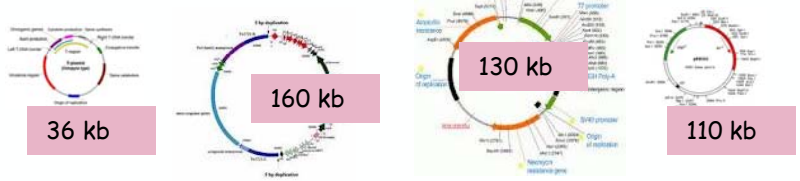


Chromosome

OmpC deficience	Multiresistance
OmpF deficience	Mutliresistance
AmpC cephalosporinase	ß-lactam R
GyrA, ParC	Fluoroquinolone R



Plasmids

NDM-1, CTX-M-15	Broad-spectrum ß-lactam R
TEM-1, OXA-1,OXA-9, OXA-10,	Narrow-spectrum ß-lactam R
ArmA, RmtB, AAC6'	Broad-spectrum aminoglycoside R
AphA, AAC3'	
Acetylase	Chloramphenicol R
Ribosylase	Rifampin R
QepA	Quinolone R
ErmB, mel, mphB	Macrolide R
BleMBL	Bleomycin R
SulI	Sulfamide R
Dhfr1, Dhfr 12	Trimethoprim R
QacE	Quaternary ammonium R
merATPADE	Heavy metals R

A successful story

Hygiena

Diarrhea

Population; overcrowded

Antibiotics; misuse and overuse,
over-the-counter sale

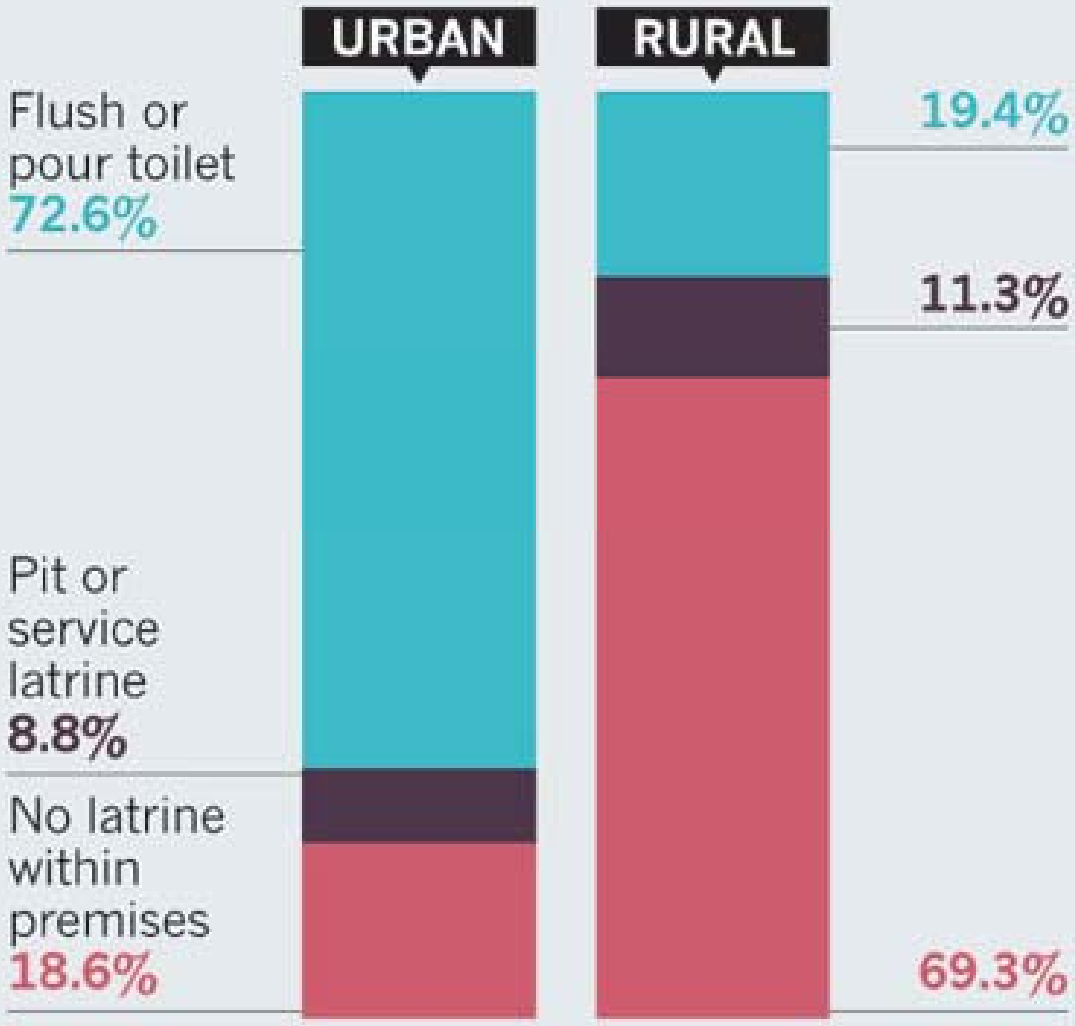
Subtropical continent

Spread of NDM-1 producers in *E. coli*, *K. pneumoniae*...

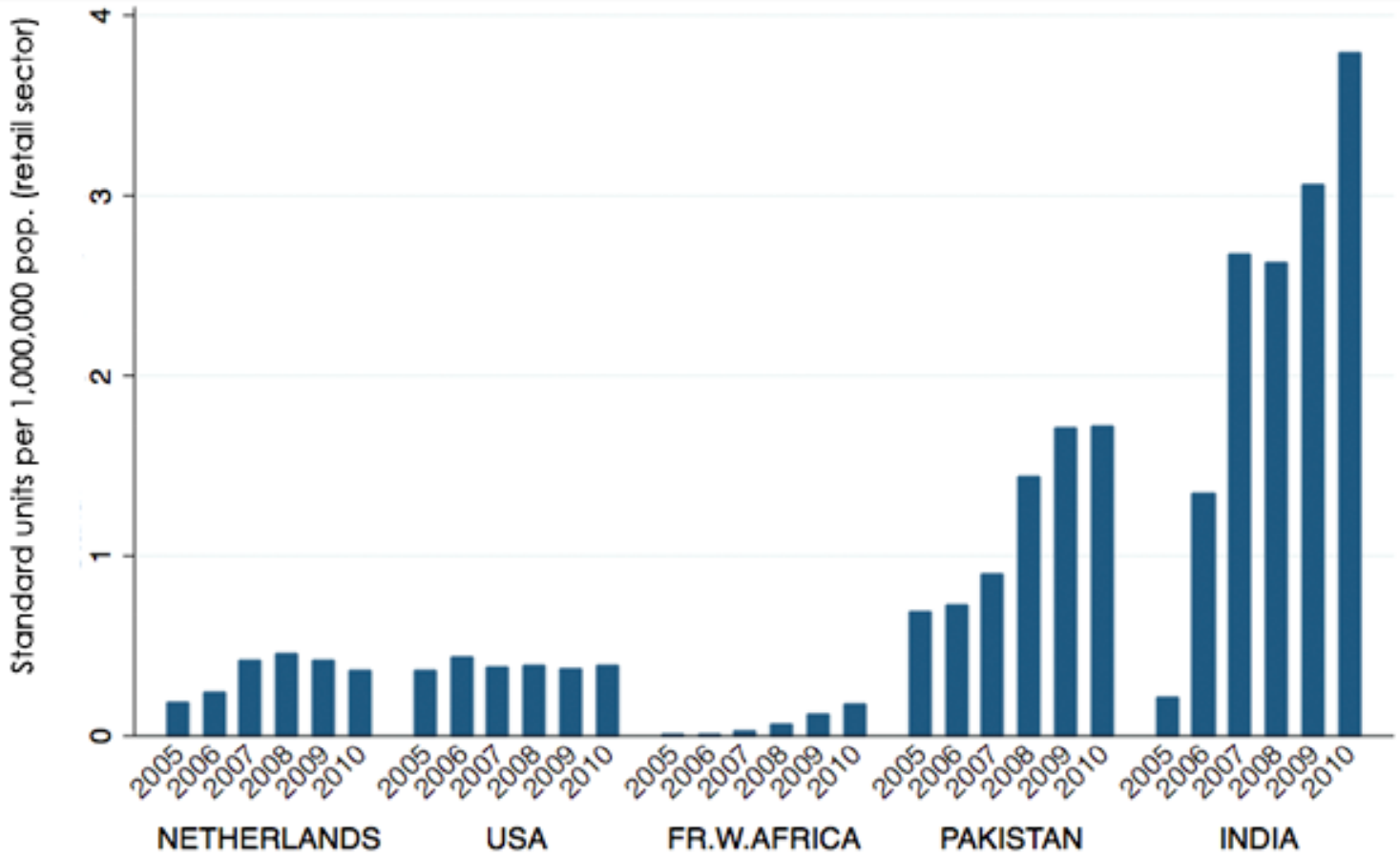
...and then higher mortality rate and length of hospitalization, overuse of broad-spectrum of antibiotics....

INDIA'S SANITATION DIVIDE

City dwellers are more likely than people in rural areas to have flushing toilets, but only one-third of those toilets are connected to underground sewers; the rest go to septic tanks.



Retail sales of carbapenem antibiotics to treat Gram-negative bacteria are increasing rapidly in India and Pakistan



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Journal of Antimicrobial Chemotherapy

Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media

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Objectives: To determine the prevalence and antimicrobial susceptibility of carbapenemase-producing Enterobacteriaceae among hospitalized patients and outpatients attending two military hospitals in Rawalpindi, Pakistan, and to compare the performance of two chromogenic culture media for the isolation of these organisms.

Methods: Stool samples from 200 distinct patients were cultured on MacConkey agar and subsequently on two chromogenic media—Colorex KPC and a prototype chromogenic medium, ID Carba—designed for the isolation of carbapenemase-producing Enterobacteriaceae. All Gram-negative isolates growing on either chromogenic medium were investigated for carbapenemases by phenotypic and molecular methods. Producers were subjected to susceptibility testing with 40 antimicrobials by VITEK 2 or agar dilution.

Results: In total, 64 NDM-1-positive isolates of Enterobacteriaceae, belonging to seven distinct species, were recovered from 37 (18.5%) of the stool samples. No other carbapenemase types were confirmed. Nineteen [illegible] samples among 130 from outpatients (prevalence 13.8%). Fifty-six isolates (87.5%) harbouring the NDM-1 enzyme were recovered on ID Carba compared with 41 isolates (64.1%) on Colorex KPC ($P=0.012$). Multidrug resistance was prevalent, but no pan-resistant isolates were found, with most isolates susceptible *in vitro* to colistin (97%), mecillinam (95%), fosfomycin (94%), tigecycline (89%) and nitrofurantoin (78%).

Conclusions: This study shows a high prevalence of multidrug-resistant Enterobacteriaceae with the NDM-1 enzyme in Rawalpindi. The new chromogenic medium, ID Carba, was more sensitive than Colorex KPC and has potential as a screening medium for isolation of Enterobacteriaceae harbouring the NDM-1 enzyme.

Keywords: β-lactamases, antimicrobial resistance mechanisms, *Escherichia coli*

E. coli NDM-1: community-acquired ! in India

3 Institut Scientifique de la Santé Publique. Guidelines for control of infections in case of cross-border transfer of patients hospitalized in countries with high endemicity of carbapenemase-producing Enterobacteriaceae. Surveillance de germes multi-résistants dans les hôpitaux belges. Sept 21, 2010. http://www.mrb.be/download/MDR/NewDelhi_fiche_alert_VS_FR.pdf (in French) (accessed Nov 8, 2010).

The plasmid-mediated bla_{NDM-1} gene that encodes a powerful carbapenemase was first identified in *Escherichia coli* and in *Klebsiella pneumoniae* in Sweden from a patient who was transferred from India.[1] It was then identified from many patients in the UK, India, and Pakistan in different enterobacterial species.[2] Here we report a woman aged 60 years who was admitted to hospital in April, 2009, for treatment of a breast cancer.

The patient came from Darjeeling, India, where she had lived for several years and had never been hospitalised. Upon her admission in France, bacterial cultures from the surface of her breast tumour were grown. The cultures were of the *E coli* isolate GUE that was resistant to most β-lactams (remaining susceptible to aztreonam) and that had reduced susceptibility to carbapenems (minimum inhibitory concentrations of imipenem 3 µg/mL, ertapenem 3 µg/mL, and meropenem 2 µg/mL).[3] This isolate was also resistant to gentamicin, kanamycin, tobramycin, sulfonamides, tetracycline, and fluoroquinolones, but remained susceptible to amikacin, chloramphenicol, rifampicin, and colistin. PCR and sequencing revealed that *E coli* GUE harboured the bla_{NDM-1} gene. Mating-out assays[4] allowed the bla_{NDM-1} gene to be identified on a 110 kb plasmid, with markers for kanamycin, gentamicin, tobramycin, trimethoprim, and sulfonamide resistance. Multilocus sequence typing[5] identified *E coli* GUE as an ST131-type strain, which corresponds to a genetic background that is also responsible for the worldwide diffusion of another common resistance determinant, CTX-M-15.

This case is the first identification of an NDM-1-producing *E coli* isolate in France, and corresponds again to an imported case from India. This example confirms the recent data suggesting that the Indian subcontinent might represent an important reservoir, and therefore a source, of NDM-producing isolates. The patient had not been hospitalised in India; therefore, the multidrug-resistant isolate had likely been community acquired. Worringly, this resistance gene has been identified here in an *E coli* strain belonging to a genotype that has proved its ability to disseminate widely in the community.

We declare that we have no conflicts of interest. This study was mostly funded by the INSERM (U914), France, and by grants from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, France, and from the European Community (TEMPObed-QC, HEALTH-2009-241742).

*Laurent Poirel, Cécile Hombrouck-Alet, Claire Freneaux, Sandrine Bernabeu, *Patrice Nordmann*
nordmann.patrice@bct.aphp.fr
Hôpital de Bicêtre, Department of Bacteriology-Virology, Le Kremlin Bicêtre, Paris 94275, France

1 Yong D, Toleman MA, Giske CG, et al. 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. Antimicrob Agents Chemother 2009; 53: 5046–54.
2 Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010; 10: 597–602.
3 Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. Jan, 2010. http://www.clsi.org/source/orders/free/m100-s20.pdf (accessed Nov 6, 2010).
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5 Tartof SY, Solberg OD, Manges AR, Riley LW. Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. J Clin Microbiol 2005; 43: 5860–64.

Clinical Infectious Diseases Advance Access published September 29, 2011

0 MONTH

Correspondence

Emergence of an Autochthonous and Community-Acquired NDM-1–Producing *Klebsiella pneumoniae* in Europe

To the Editor—The recently identified carbapenemase New Dehli metallo-β-lactamase (NDM-1) inactivates all β-lactams except aztreonam [1]. The corresponding gene that is usually plasmid-borne has spread mostly in *Escherichia coli* and *Klebsiella pneumoniae* [1, 2]. NDM-1 producers are multidrug resistant or even resistant to all antibiotics [1, 2]. Whereas contamination with NDM-1 producers is mostly hospital associated, rare cases of community acquisition are known and have been traced to the Indian subcontinent [2].

Here, we report a woman aged 83 years who had cystitis due to a multidrug-resistant *K. pneumoniae* in June 2011. She had a history of multiple and recurrent episodes of urinary tract infections caused by diverse Enterobacteriaceae that were always treated with narrow-spectrum antibiotics. Because the patient's symptoms tended to to disappear spontaneously and rapidly, the latest cystitis episode had not been treated.

K. pneumoniae EDU was resistant to all β-lactams, including carbapenems, as detected with a Vitek-2 automated suscep-

Polymerase chain reaction, sequencing, and plasmid analysis, performed as described elsewhere [5], revealed that *K. pneumoniae* EDU harbored the bla_{NDM-1} carbapenemase gene and the $bla_{CTX-M-15}$ extended-spectrum ß-lactamase gene, which were located on 2 different plasmids (both being approximately 150 kb in size). The isolate coexpressed the CMY-2 cephalosporinase gene, which was located on the bla_{NDM-1} plasmid. In addition, it possessed the *qnrB* gene encoding resistance to quinolones and the bla_{OXA-1} gene encoding a restricted-spectrum oxacillinase, both genes being located on the $bla_{CTX-M-15}$ plasmid. Both plasmids were self-transferable by conjugation, and the bla_{NDM-1} plasmid was found to be of the IncA/C broad-host range type [6]. Multilocus sequence typing [7] results showed that *K. pneumoniae* EDU belonged to the sequence type 1, whereas previously reported NDM-1–positive *K. pneumoniae* isolates were of other sequence types (eg, ST14 and ST147) [6].

Neither this patient nor her husband had traveled to any country in the previous 3 years, including countries with a high prevalence of NDM-1 producers (India, Pakistan, Bangladesh, United Kingdom, Balkan states, and Middle Eastern nations) of NDM-1 producers outside its main reservoir (Indian subcontinent). The source of contamination remains unknown but may be difficult to find, because persistence of NDM-1 producers in human flora has been evidenced to be >1 year [9].

This present report may indicate the ongoing spread of NDM producers in the community worldwide. A nightmare perspective could be its spread similar to that reported for extended-spectrum β-lactamases of the CTX-M-type, which are now uncontrolled.

Notes

Financial support. This work was supported by INSERM (U914), France, and by grants from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, France, and from the European Community (TEMPOtest-QC, HEALTH-2009-241742).

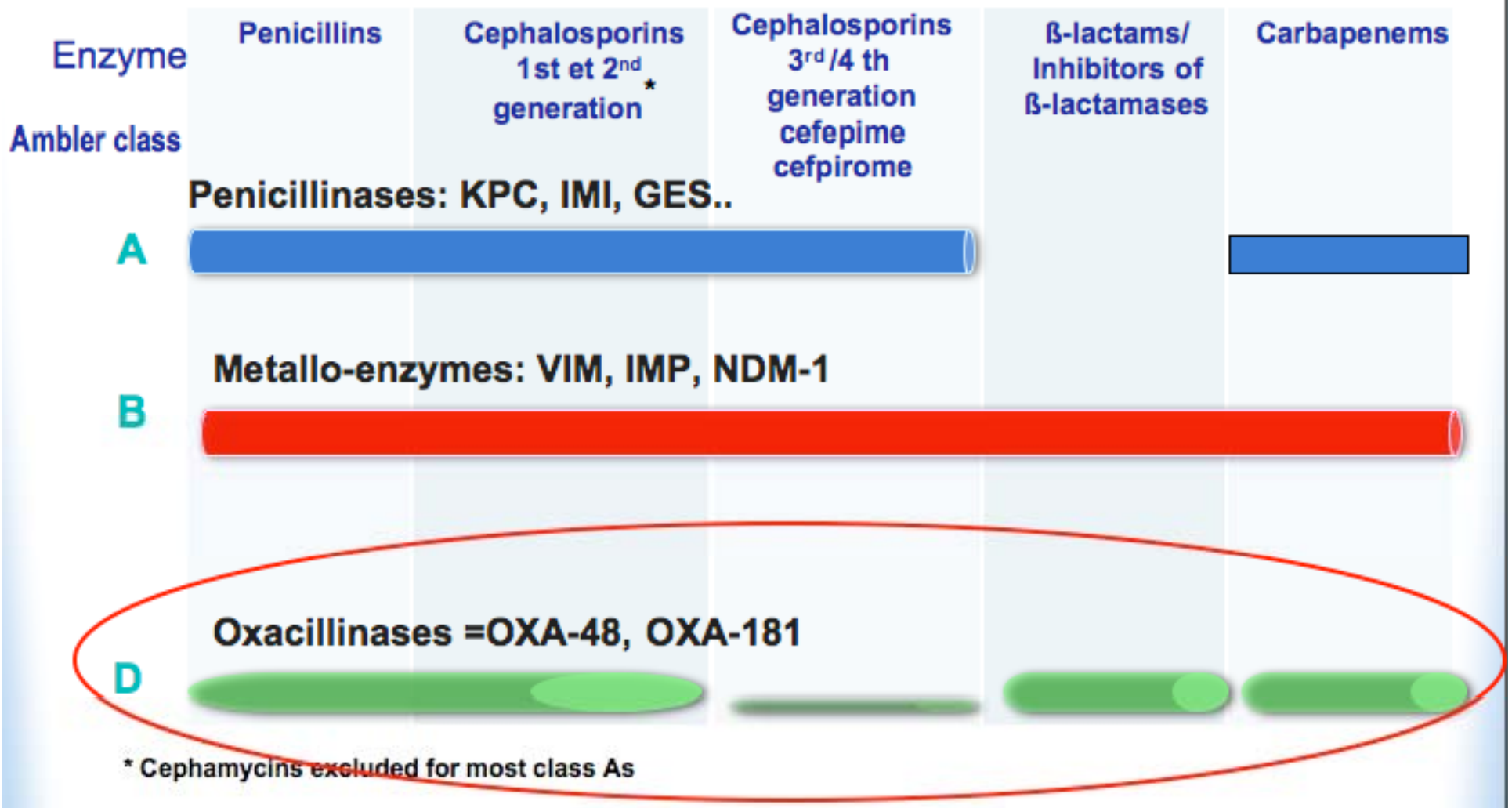
Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

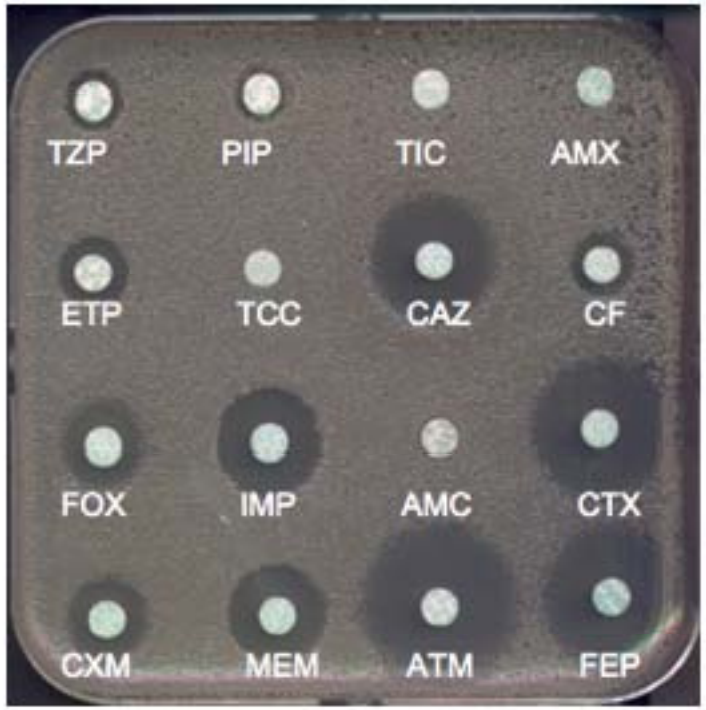
Patrice Nordmann,[1] Jean-Pierre Couard,[2] Dominique Sanzot,[3] and Laurent Poirel[1]

[1]Department of Microbiology, Hôpital de Bicêtre, INSERM U914, Le Kremlin-Bicêtre, [2]Laboratoire Symbiose, Cuers, and [3]Laboratoire de Biologie, Hôpital Fort-Ré, Toulon, France

The carbapenemases in *Enterobacteriaceae*

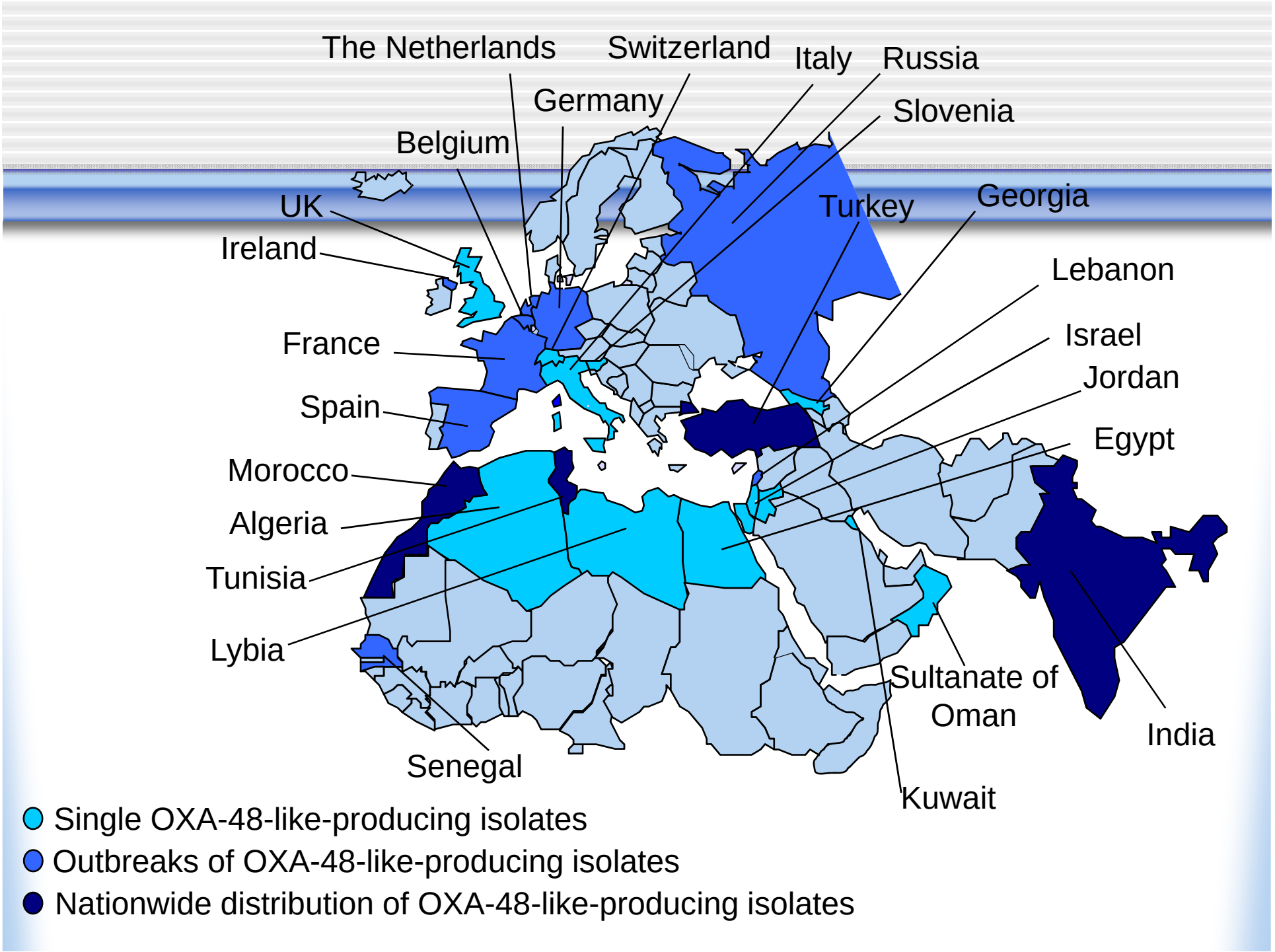


OXA-48



Emergence of oxacillinase-mediated resistance to Imipenem in Klebsiella pneumoniae
Poirel L, Héritier , Nordmann P. Tolün, AAC 2004

OXA-48 + CTX-M-15


The Netherlands
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Ireland
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France
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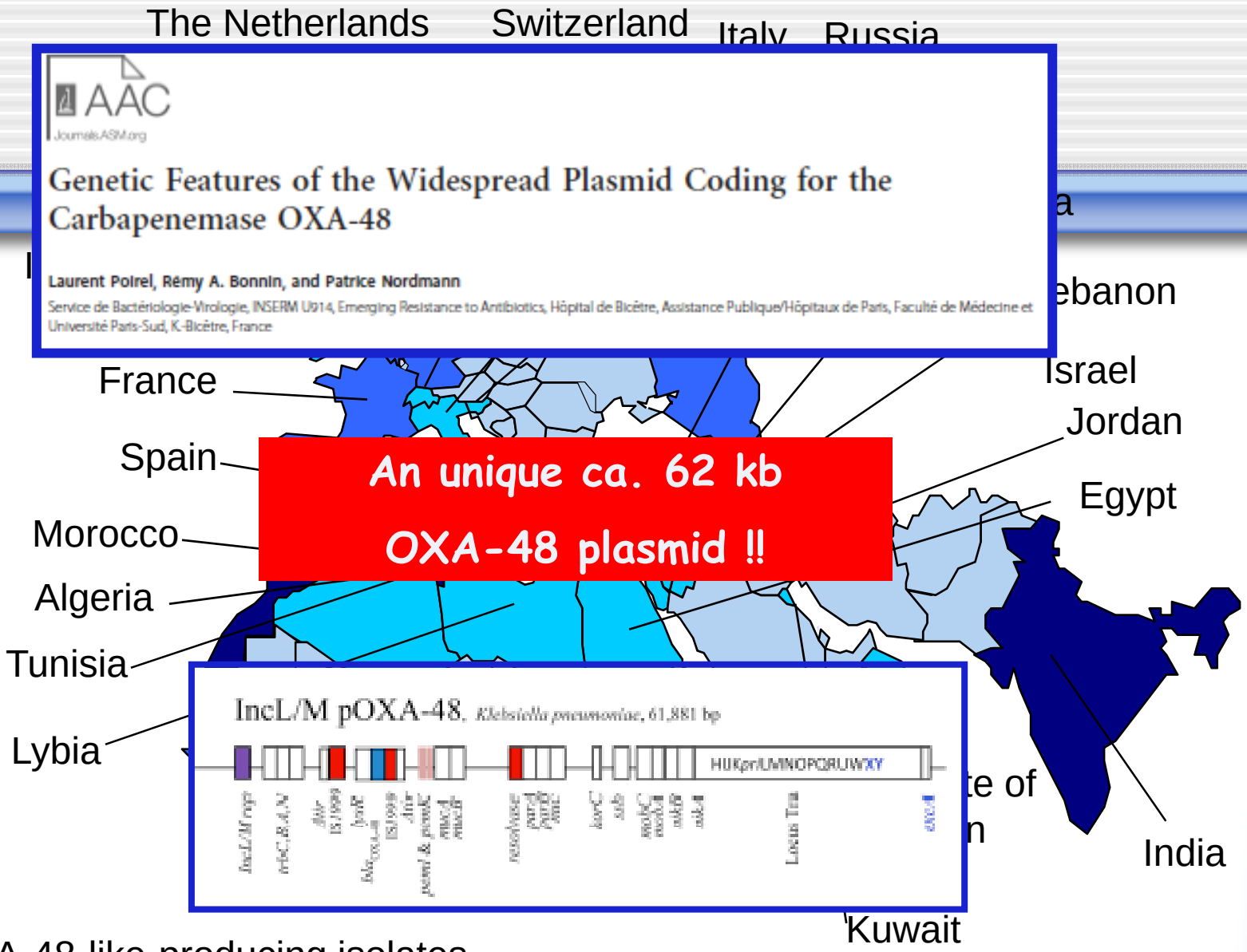
Spain
Egypt
Morocco
Algeria
Tunisia
Lybia
Sultanate of Oman
India
Senegal
Kuwait
Single OXA-48-like-producing isolates
Outbreaks of OXA-48-like-producing isolates
Nationwide distribution of OXA-48-like-producing isolates



- Single OXA-48-like-producing isolates
- Outbreaks of OXA-48-like-producing isolates
- Nationwide distribution of OXA-48-like-producing isolates

European dissemination of a single OXA-48-producing *Klebsiella pneumoniae* clone

A. Potron[1], J. Kalpoe[2], L. Poirel[1] and P. Nordmann[1]

1) *Service de Bactériologie-Virologie, INSERM U914 «Emerging Resistance to Antibiotics», Hôpital de Bicêtre, Assistance Publique/Hôpitaux de*

ulté de Médecine et Université Paris-Sud, K. Bicêt

rtment of Medical Microbiology and Infection Pre

pital, Amsterdam, the Netherlands

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2011, p. 2420–2423 Vol. 55, No. 5
0066-4804/11/$12.00 doi:10.1128/AAC.01452-10

Outbreak of OXA-48-Positive Carbapenem-Resistant *Klebsiella pneumoniae* Isolates in France[▽]

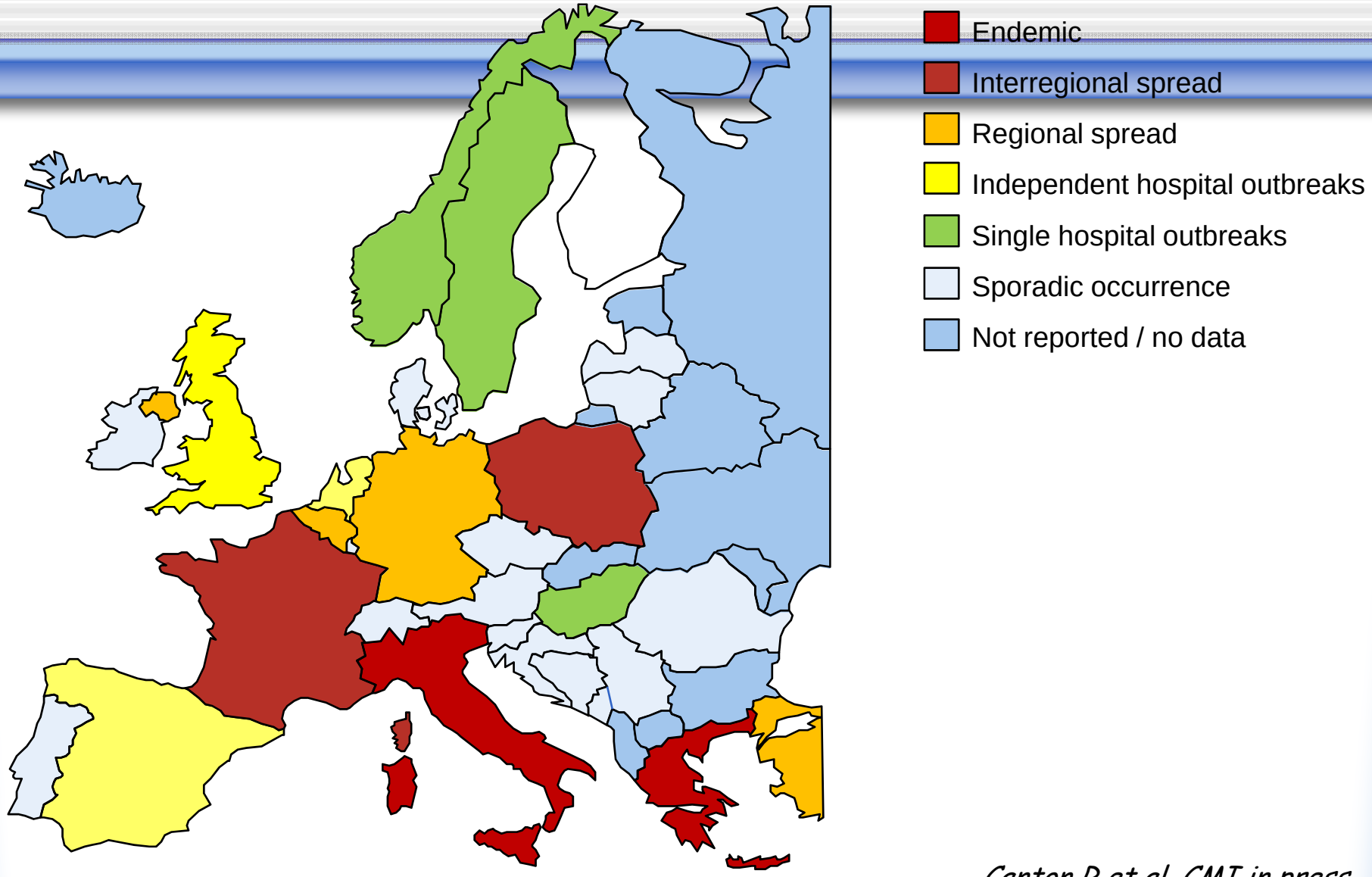
Gaelle Cuzon,[1] Jocelyne Ouanich,[2] Remy Gondret,[3] Thierry Naas,[1] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, INSERM U914: Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, 94275 Le Kremlin-Bicêtre, Faculté de Médecine, Université Paris-Sud, France[1]; Laboratoire de Microbiologie, Centre Hospitalier Intercommunal de Villeneuve-St. Georges, Villeneuve-St. Georges, France[2]; and Service d'Anesthésie-Réanimation, Centre Hospitalier Intercommunal de Villeneuve-St. Georges, Villeneuve-St. Georges, France[3]

Received 20 October 2010/Returned for modification 2 January 2011/Accepted 13 February 2011

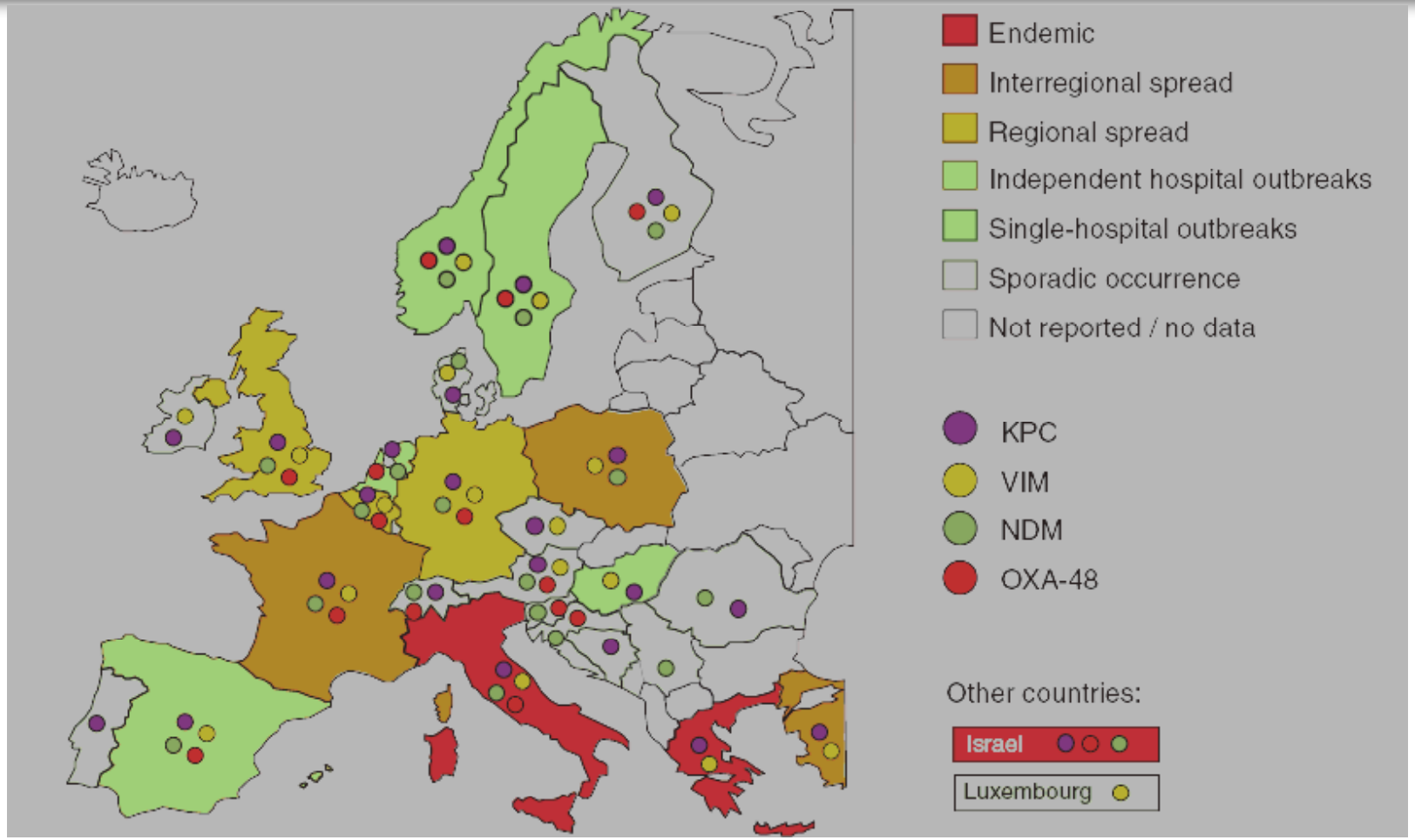
Seventeen *Klebsiella pneumoniae* isolates producing the OXA-48 carbapenemase, obtained from 10 patients hospitalized from April to June 2010, mostly in the medical intensive care unit of the Villeneuve-Saint-Georges Hospital in a suburb of Paris, France, were analyzed. Seven patients were infected, of whom five were treated at least with a carbapenem, and five patients died. Molecular analysis showed that the isolates belonged to a single clone that harbored a 70-kb plasmid carrying the bla_{OXA-48} gene and coproduced CTX-M-15 and TEM-1 β-lactamases. This is the first reported outbreak of OXA-48-producing *K. pneumoniae* isolates in France.

Spread of carbapenemase producers in *Enterobacteriacae* in Europe



Canton R et al. CMI in press

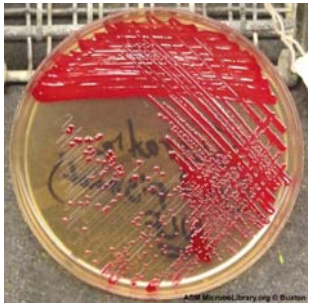
Spread of carbapenemase producers in *Enterobacteriacae* in Europe



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2011, p. 000 Vol. 55, No. 11
0066-4804/11/$12.00 doi:10.1128/AAC.05120-11

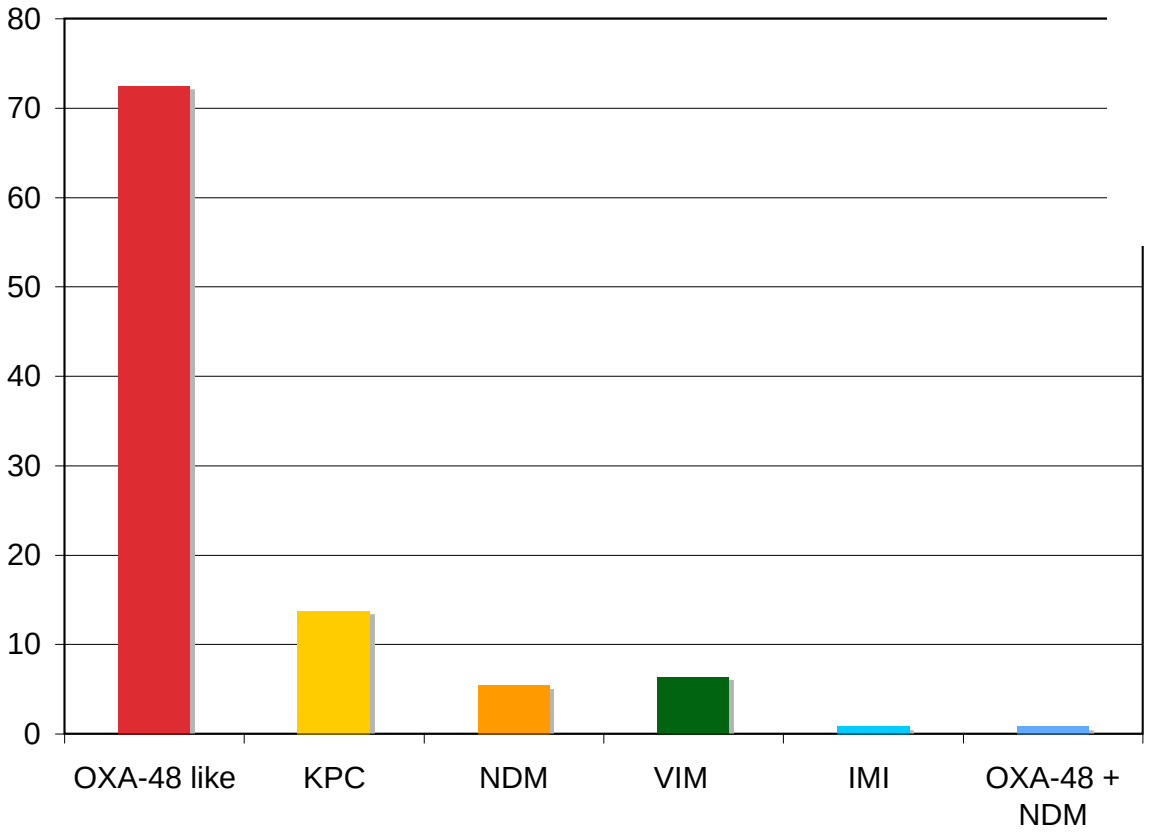
Letter to the Editor

Occurrence of the Carbapenem-Hydrolyzing β-Lactamase Gene bla_{OXA-48} in the Environment in Morocco[▽]



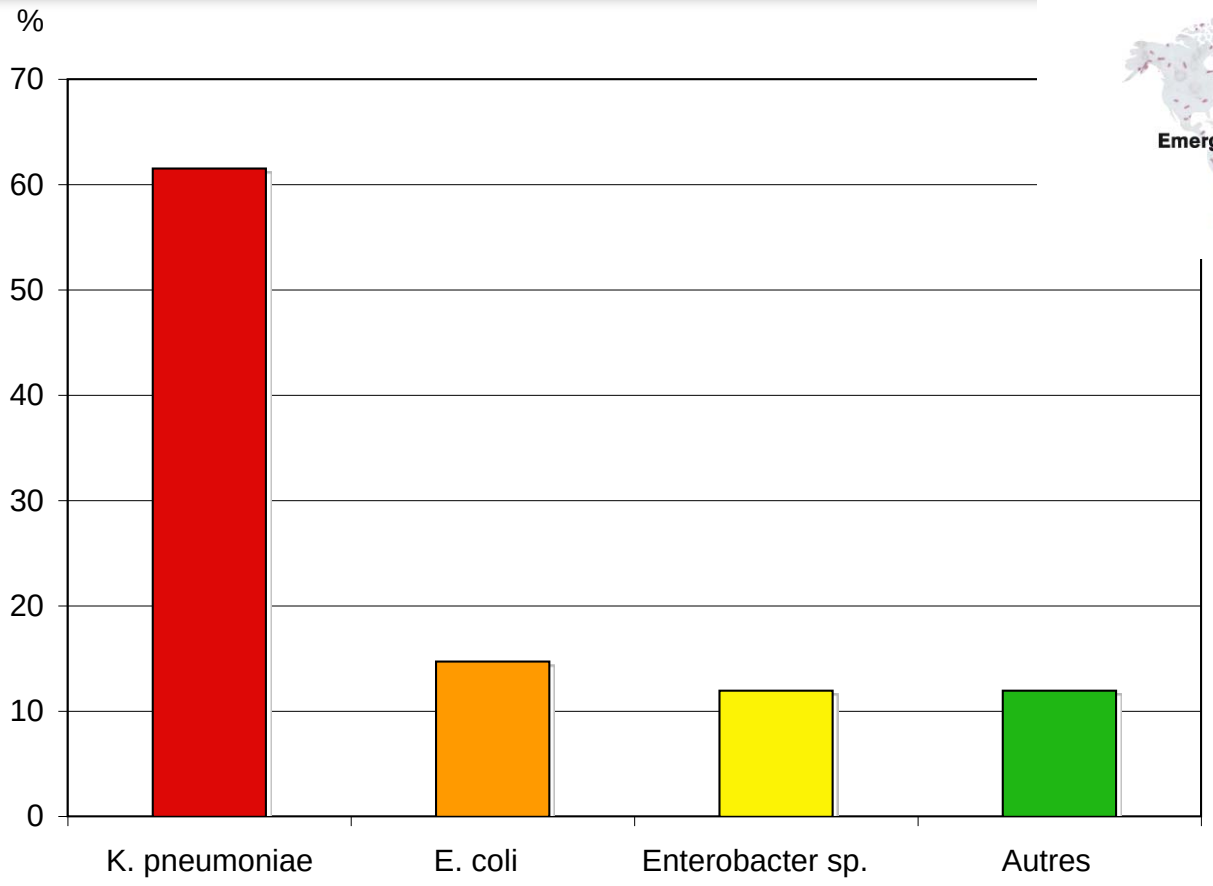
Anaïs Potron
Laurent Poirel
Florence Bussy
Patrice Nordmann*
Service de Bactériologie-Virologie
INSERM U914, Emerging Resistance to Antibiotics
Hôpital de Bicêtre

Répartition (en pourcentage) des souches positives (n=109) CNR Résistance ATB- Janv-Juin 2012, en fonction du type de carbapénèmase identifiée



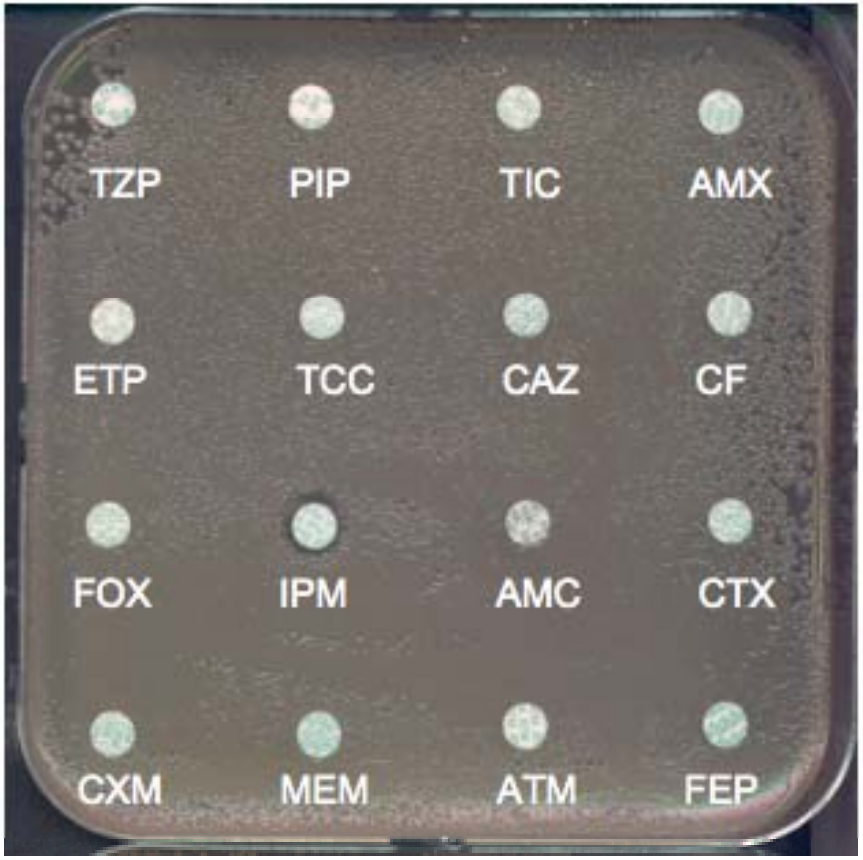
Inserm U914
Résistances Emergentes aux Antibiotiques

Répartition (en pourcentage) des souches positives (n=109) CNR Résistance aux Antibiotiques en fonction du type d'entérobactérie

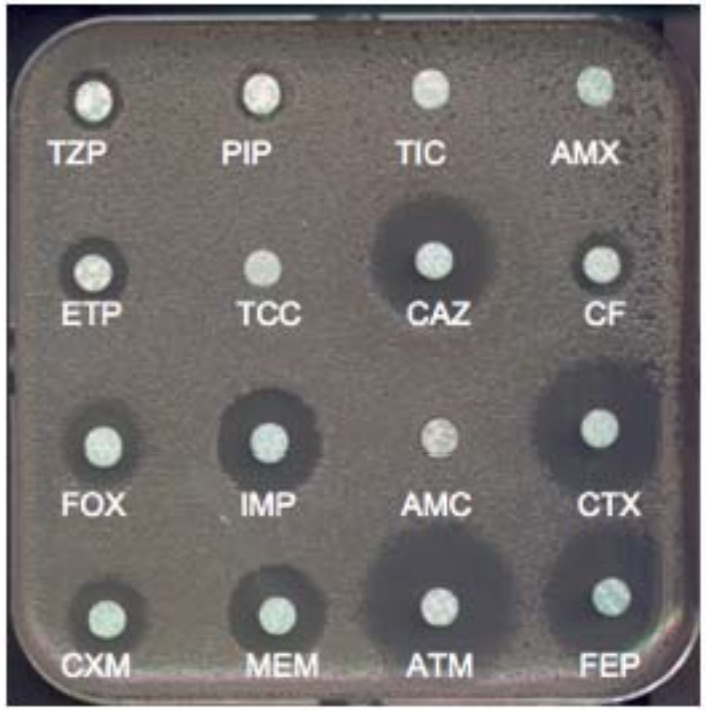


Inserm U914
Résistances Emergentes aux Antibiotiques

When to suspect carbapenemase production ?



OXA-48



Emergence of oxacillinase-mediated resistance to Imipenem in Klebsiella pneumoniae
Poirel L, Héritier , Nordmann P. Tolün, AAC 2004

Carbapenem breakpoints in *Enterobacteriaceae*-2011

	FDA	CLSI		EUCAST	
	S	S	R	S	R
Imipenem	≤4	≤1	≥4	≤2	>8
Meropenem	≤4	≤1	≥4	≤2	>8
Ertapenem	≤2	≤0.25	≥1	≤0.5	>1
Doripenem	≤0.5	≤1	≥4	≤1	>4

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2010, p. 2999–3002 Vol. 48, No. 8
0095-1137/10/$12.00 doi:10.1128/JCM.00341-10

Comparison of BD Phoenix, Vitek 2, and MicroScan Automated Systems for Detection and Inference of Mechanisms Responsible for Carbapenem Resistance in *Enterobacteriaceae*[▽]

Neil Woodford,[1]* Anne T. Eastaway,[2] Michael Ford,[3] Alistair Leanord,[4] Chloe Keane,[4] Reinhard M. Quayle,[5] Jane A. Steer,[5] Jiancheng Zhang,[1] and David M. Livermore[1]

We assessed the ability of three commercial systems to infer carbapenem resistance mechanisms in 39 carbapenemase-producing and 16 other carbapenem-resistant *Enterobacteriaceae*. The sensitivity/specificity values for "flagging" a likely carbapenemase were 100%/0% (BD Phoenix), 82 to 85%/6 to 19% (MicroScan), and 74%/38% (Vitek 2), respectively. OXA-48 producers were poorly detected, but all systems reliably detected isolates with KPC and most with metallo-carbapenemases.

	N°. Isolates							
	Phoenix		Microscan NM36		Microscan NBC39		Vitek 2	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
KPC (*n=8*)	8	0	8	0	8	0	8	0
MBL (IMP, VIM, NDM; *n=20*)	20	0	19	1[a]	20	0	16	4[a]
OXA-48 (*n=11*)	11	0	6	5	4	7	5	6
E. coli/Klebsiella spp., ESBL/porin loss (*n=10*)	10	0	10	0	8	2	8	2
Enterobacter spp., AmpC/ESBL, porin (*n=6*)	6	0	5	1	5	1	2	4

Reasons for such an heterogeneity

- Variability of the strains genetic background
(+/-impermeability and +/- other ß-lactamases)

- Variability of the ß-lactamase variant (+/- strong carbapenemase activity)

- Variability of genetic support:
  chromosomal (1 copy) vs plasmid (+/- high copy number)

- Variability of gene expression:
  +/- strong promoter
  position of the gene cassette if integron-encoded

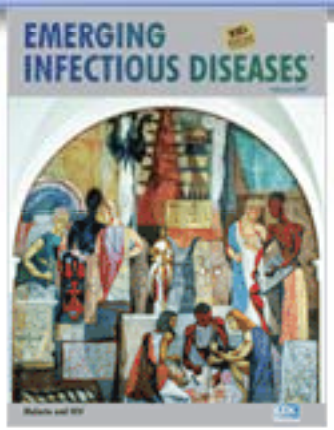
However…

- Carbapenem non-susceptible isolates do not necessary produce carbapenemases

  ⇒ ESBL production + permeability defects may lead to resistance, in particular to ertapenem

  ⇒ AmpC production + permeability defects may lead to resistance, including to imipenem

ESBL + decreased outer membrane permeability

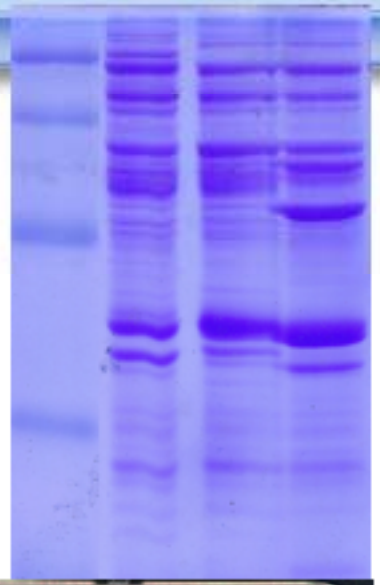


Ertapenem Resistance of *Escherichia coli*

Marie-Frédérique Lartigue,* Laurent Poirel,*
Claire Poyart,† Hélène Réglier-Poupet,†
and Patrice Nordmann*

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 13, No. 2, February 2007

An ertapenem-resistant *Escherichia coli* isolate was recovered from peritoneal fluid in a patient who had been treated with imipenem/cilastatin for 10 days. Ertapenem resistance may be explained by a defect in the outer membrane protein and production of extended-spectrum β-lactamase CTX-M-2.



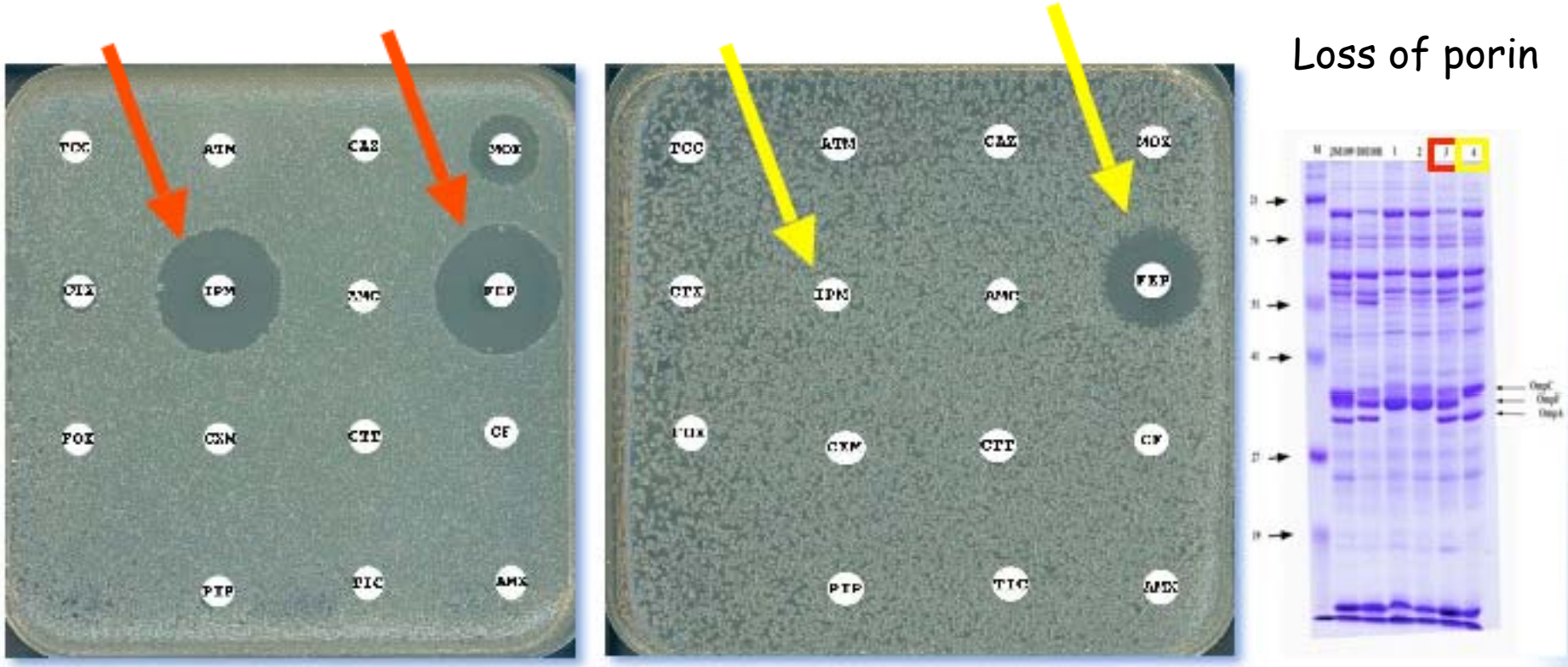
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1997, p. 563–569 Vol. 41, No. 3
0066-4804/97/$04.00+0

Imipenem Resistance in *Klebsiella pneumoniae* Is Associated with the Combination of ACT-1, a Plasmid-Mediated AmpC β-Lactamase, and the Loss of an Outer Membrane Protein

PATRICIA A. BRADFORD,[1]* CARL URBAN,[2,3] NORIEL MARIANO,[2] STEVEN J. PROJAN,[1] JAMES J. RAHAL,[2,4] AND KAREN BUSH[1]†

Wyeth-Ayerst Research, Pearl River, New York[1]; The New York Hospital Medical Center of Queens, Flushing, New York[2]; and Departments of Microbiology[3] and Medicine,[4] Cornell University Medical College, New York, New York

Loss of porin



Cloxacillin-containing plates

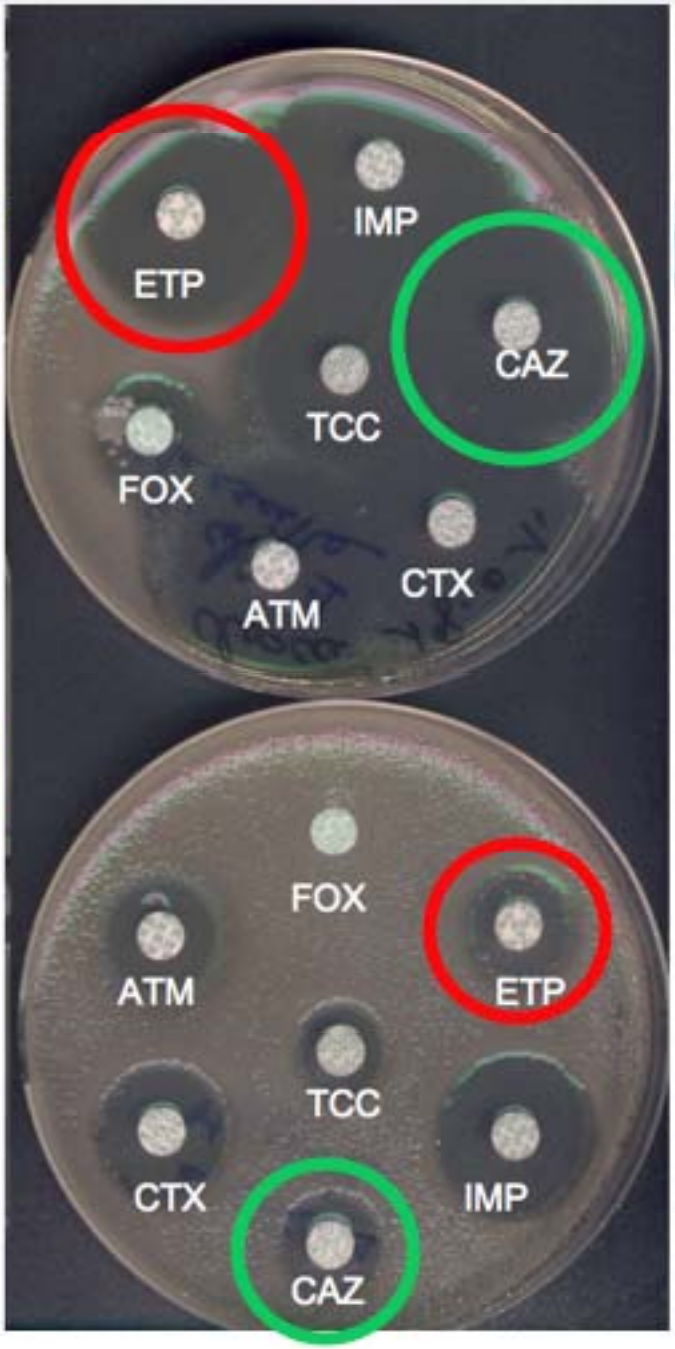
+ Cloxa

-very useful: *Enterobacter Serratia, C. freundii…*

-useful: *K. pneumoniae E. coli…*

E. coli

- Cloxa

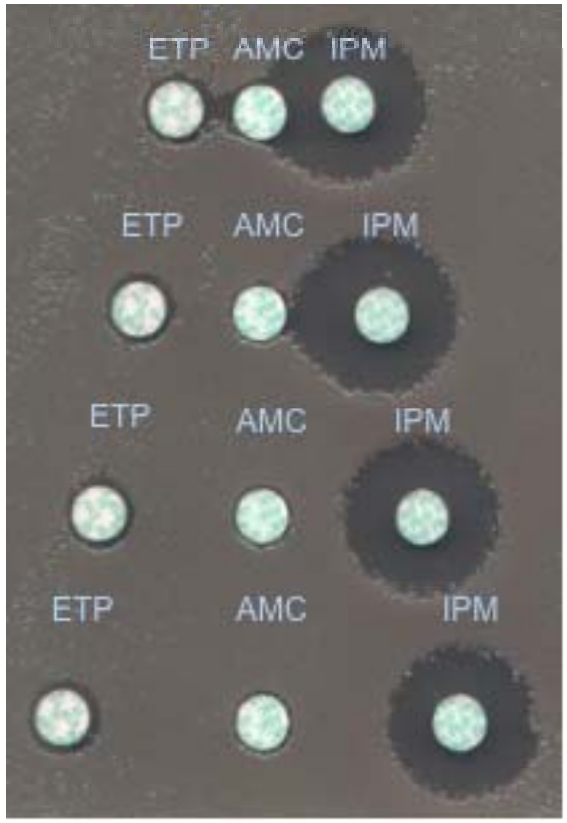


Inhibition by cloxacillin;non-carbapenemase/carbapenemase producers

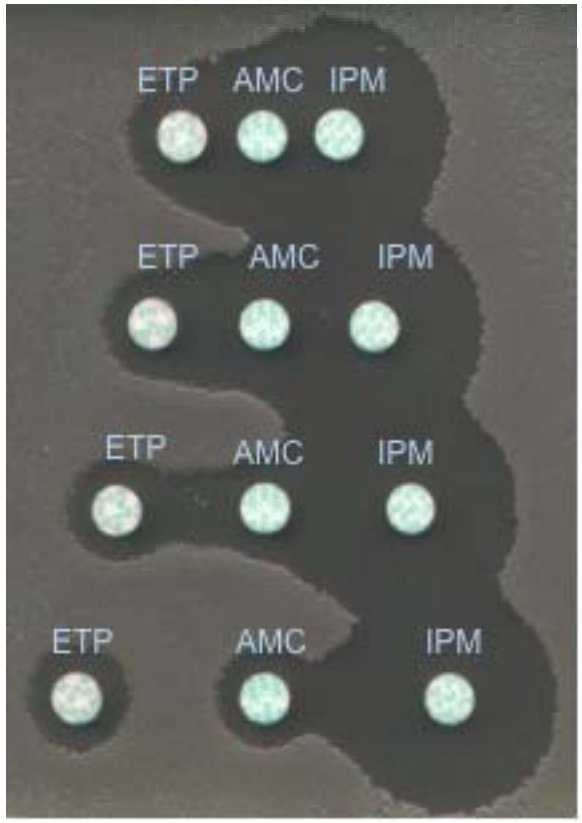
	ERT (mm)	ERT + CLOXA (mm)
E. coli AmpC overproducers n=20	22	31
E. coli carbapenemases n=20	16	16

Inhibition;KPC detection

K. pneumoniae



E. coli



(Nordmann, Cuzon, Naas Lancet Inf. Dis. 2009)

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2009, p. 362–367 Vol. 47, No. 2
0095-1137/09/$08.00+0 doi:10.1128/JCM.01922-08

Evaluation of Boronic Acid Disk Tests for Differentiating KPC-Possessing *Klebsiella pneumoniae* Isolates in the Clinical Laboratory[▽]

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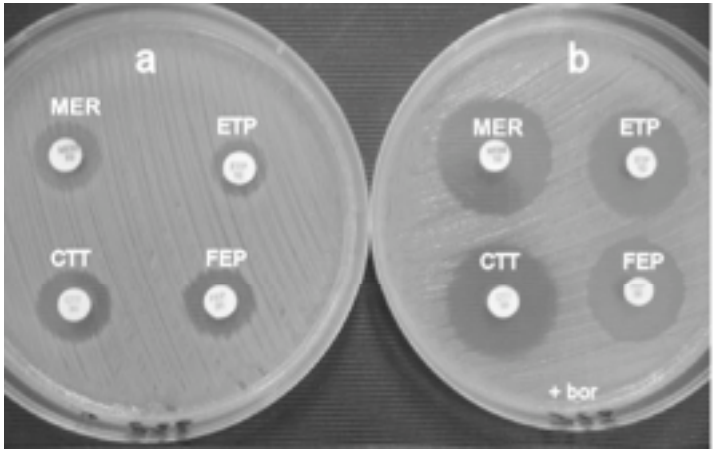
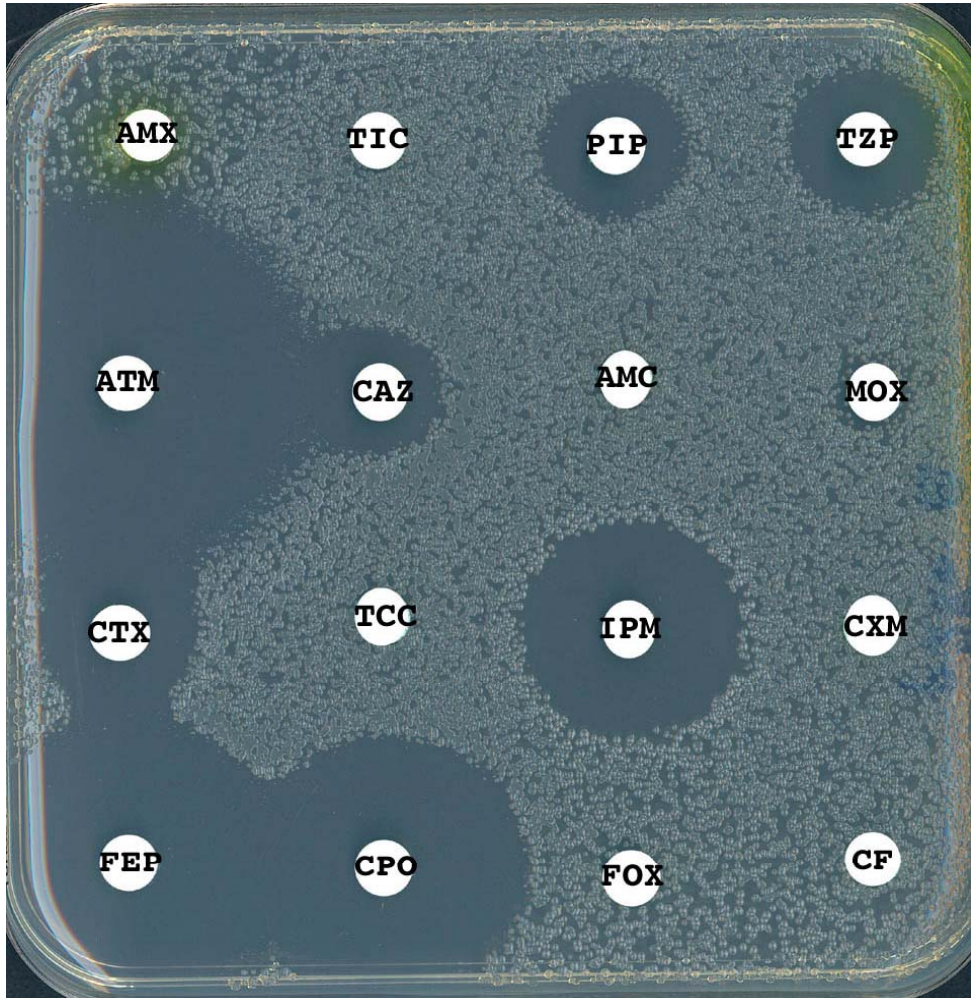
The worldwide increase in the occurrence and dissemination of KPC β-lactamases among gram-negative pathogens makes critical the early detection of these enzymes. Boronic acid disk tests using different antibiotic substrates were evaluated for detection of KPC-possessing *Klebsiella pneumoniae* isolates. A total of 57 genotypically confirmed KPC-possessing *K. pneumoniae* isolates with varying carbapenem MICs were examined. To measure the specificity of the tests, 106 non-KPC-possessing isolates (89 *K. pneumoniae* and 17 *Escherichia coli* isolates) were randomly selected among those exhibiting reduced susceptibility to cefoxitin, expanded-spectrum cephalosporins, or carbapenems. As many as 56, 53, and 40 of the non-KPC-possessing isolates harbored extended-spectrum β-lactamases, metallo-β-lactamases, and plasmid-mediated AmpC β-lactamases, respectively. By use of CLSI methodology and disks containing imipenem, meropenem, or cefepime, either alone or in combination with 400 μg of boronic acid, all 57 KPC producers gave positive results (sensitivity, 100%) whereas all 106 non-KPC producers were negative (specificity, 100%). The meropenem duplicate disk with or without boronic acid demonstrated the largest differences in inhibition zone diameters between KPC producers and non-KPC producers. By use of disks containing ertapenem, all isolates were correctly differentiated except for five AmpC producers that gave false-positive results (sensitivity, 100%; specificity, 95.3%). These practical and simple boronic acid disk tests promise to be very helpful for the accurate differentiation of KPC-possessing *K. pneumoniae* isolates, even in regions where different broad-spectrum β-lactamases are widespread.



Carbapenem-hydrolyzing metallo-ß-lactamases (IMP, VIM, NDM)

- Requires zinc ions to be functional
- Not inhibited by clavulanic acid
- Inhibited in vitro by EDTA and dipicolinic acid
- Hydrolyse penicillins, broad-spectrum cephalosporins, cephamycins, carbapenems, but NOT monobactams

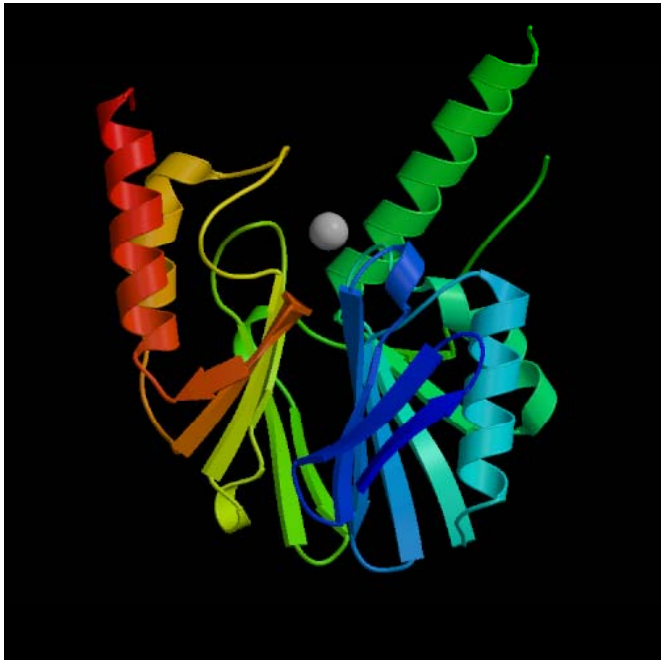
Metallo-ß-lactamase; *E. coli* (NDM-1)



Metallo-ß-lactamase; *K pneumoniae* (NDM-1)

often associated with ESBL, other ß-lactamases and permeability defects

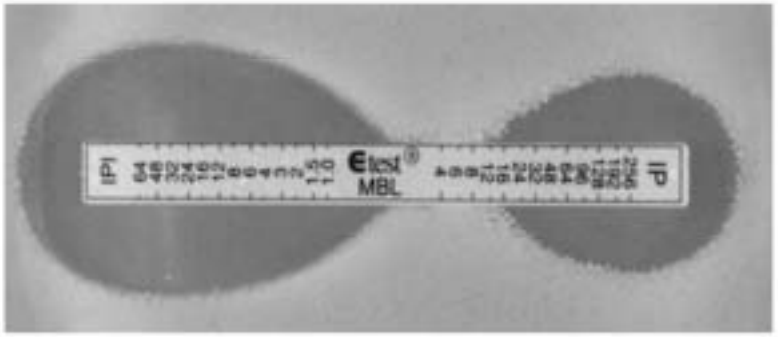
Metallo-carbapenemase; detection



EDTA is a good inhibitor

Walsh *et al.* JCM, 2002, 2755-9

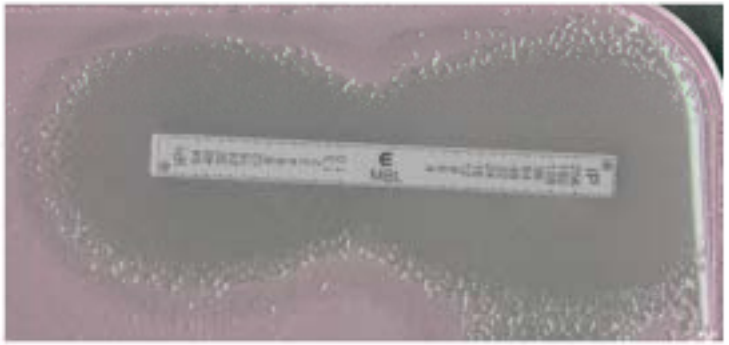
Detection of MBLs



IMP (16 µg/ml)

Test +

IMP + EDTA



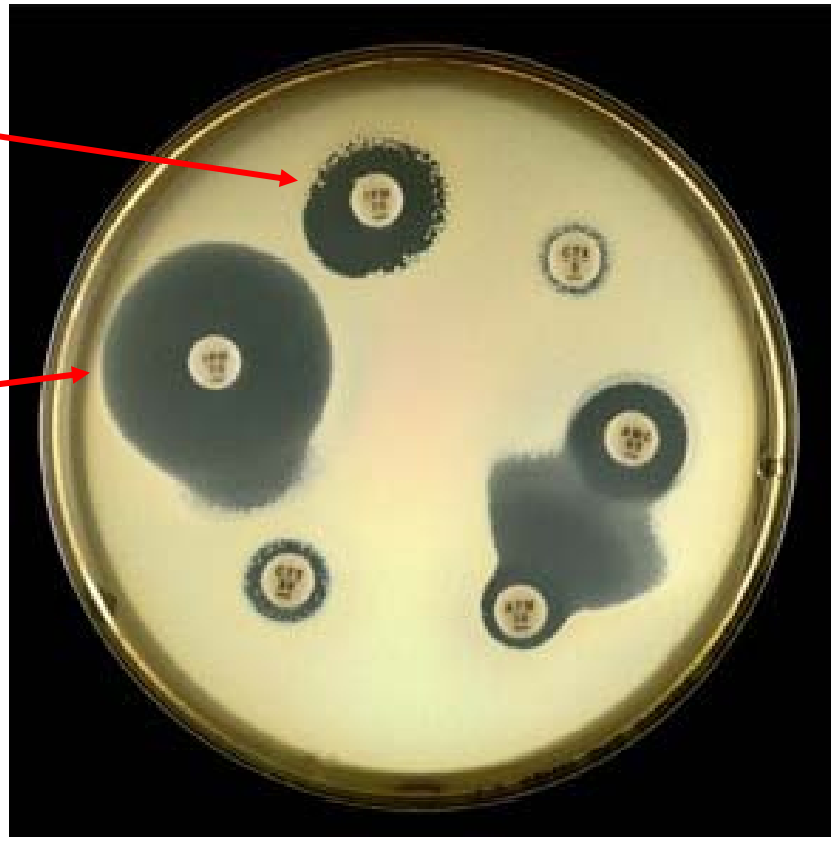
IMP (≤ 4 µg/ml)

Test ?

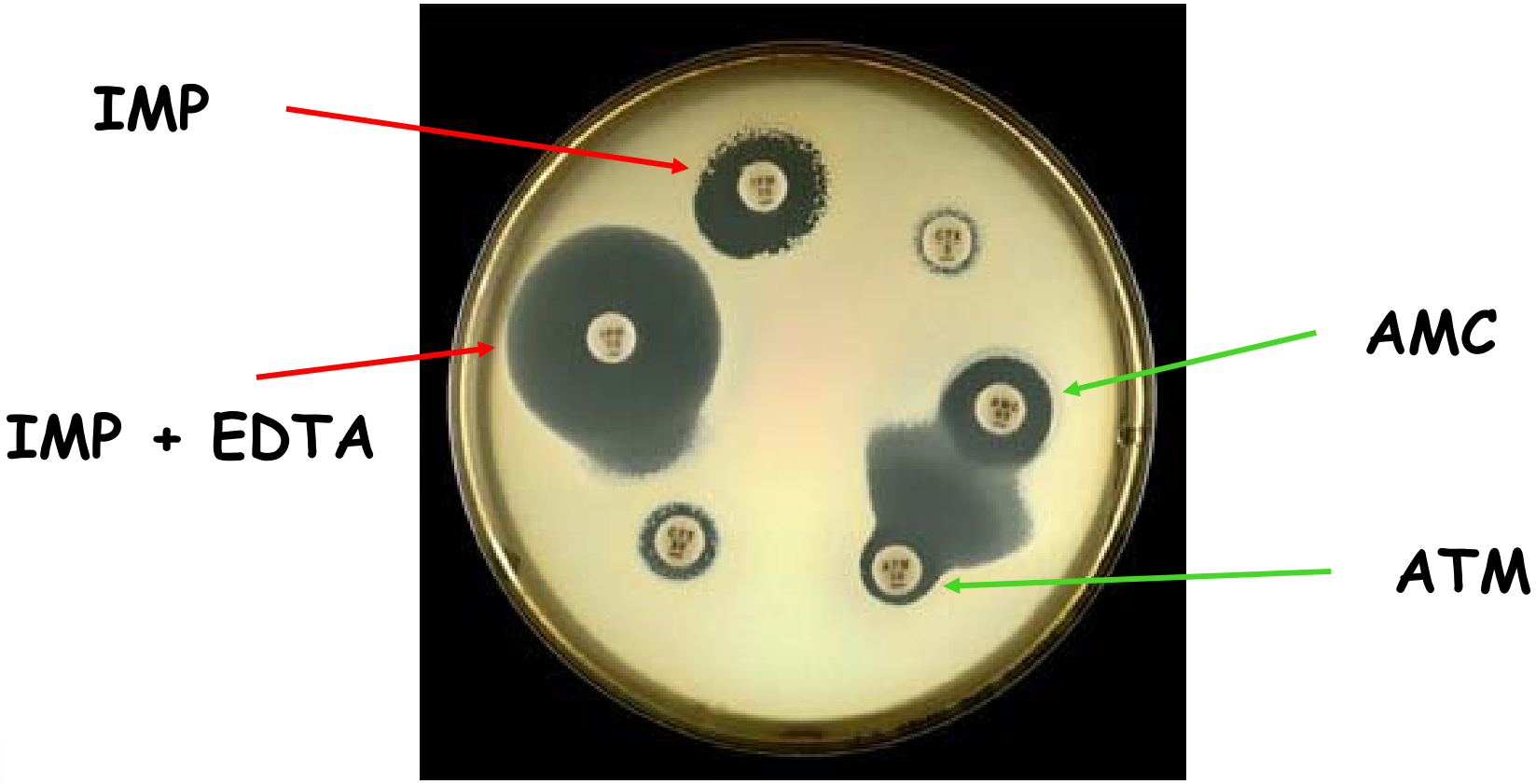
Combined disk tests

IMP

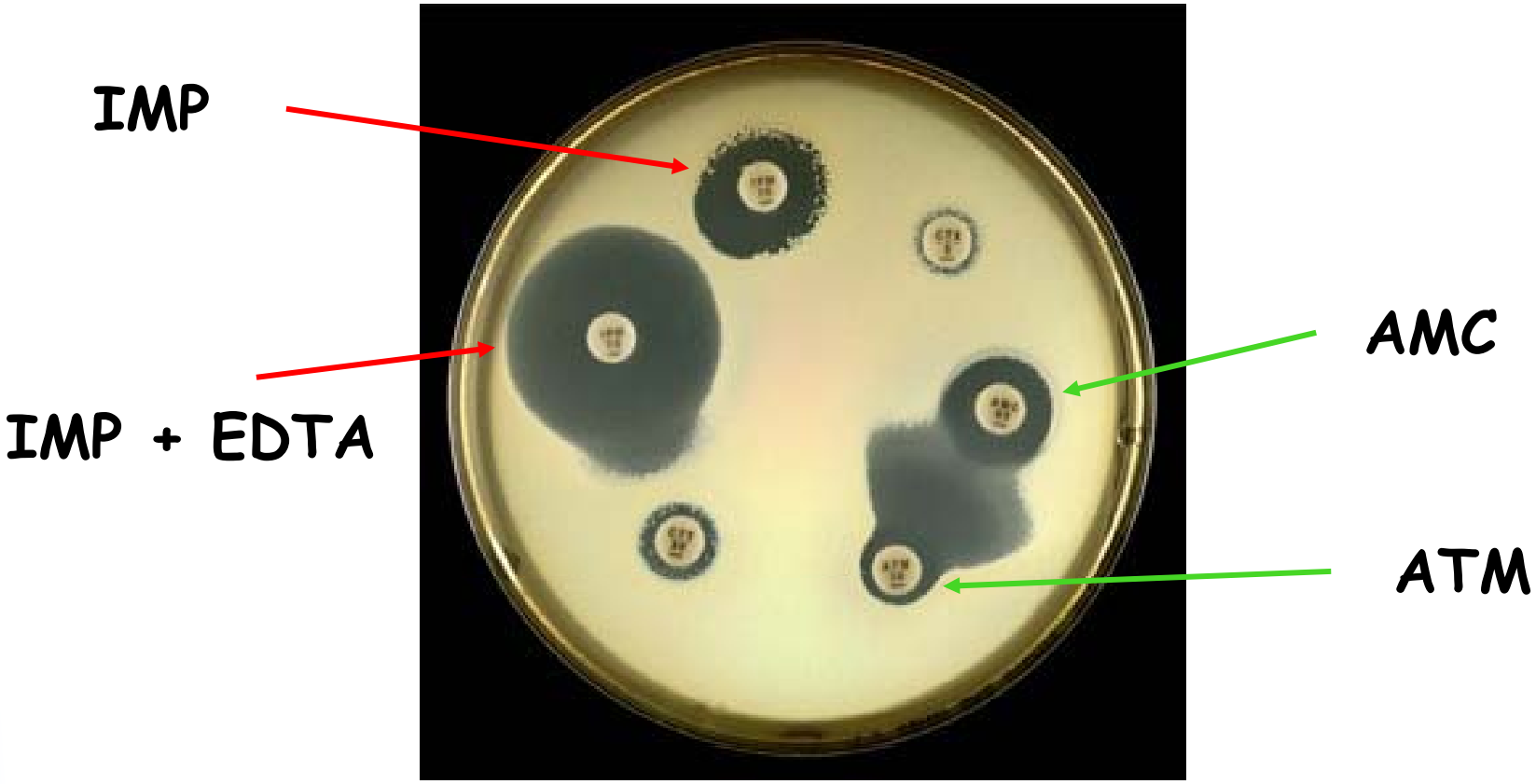
IMP + EDTA



Combined disk tests



Combined disk tests

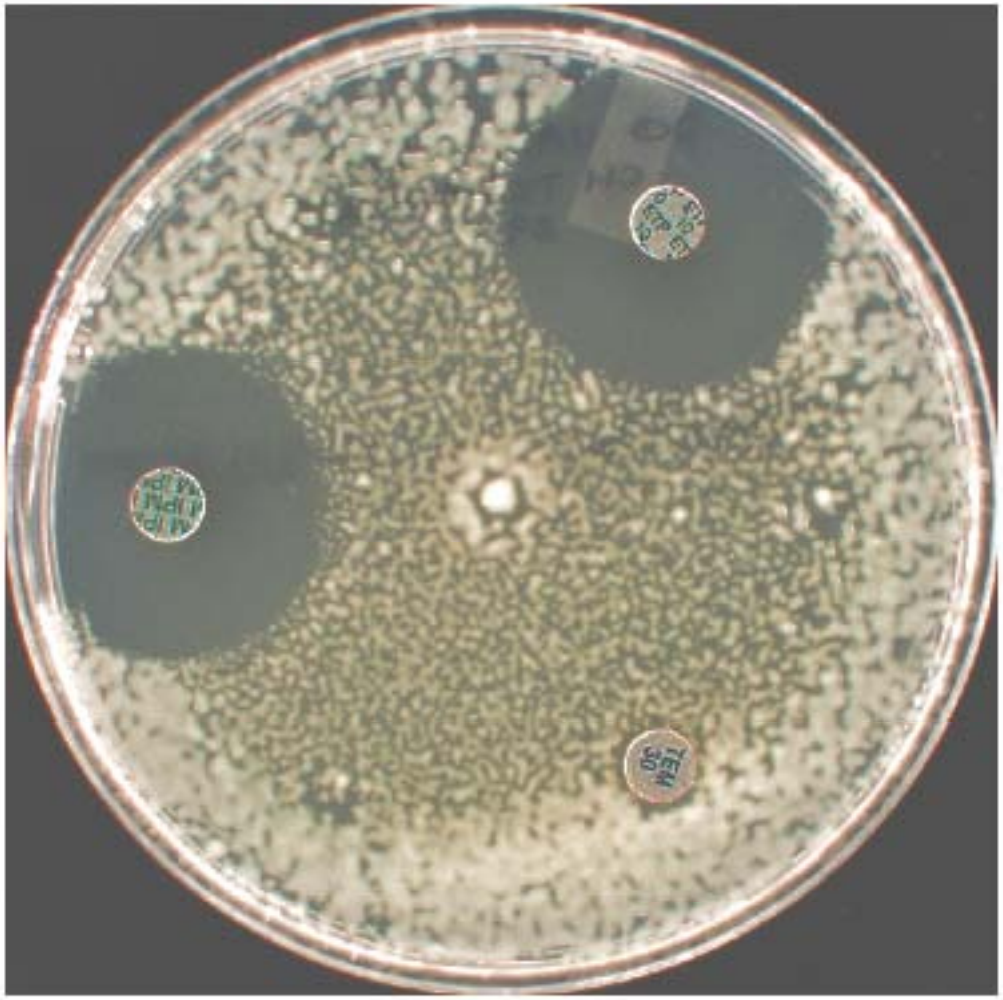


ESBL (SHV-12) and metallo-ß-lactamase (IMP-4) Poirel et al., Pathology, 2004, 36, 366-8

In-vitro spectrum activity of class D ß-lactamases

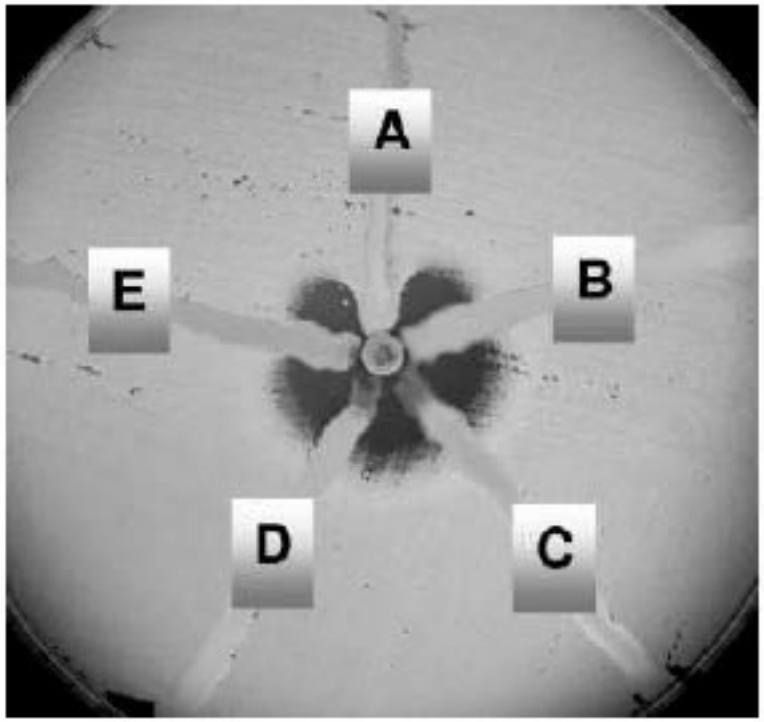
Class	Enzyme	Penicillins	Cephalosporins 1st and 2nd generation	Expanded-spectrum cephalosporins	ß-lactams/ Inhibitors of ß-lactamases	Carbapenems
D	Oxacillinases					

OXA-48 and resistance to temocillin



Phenotypic tests: Hodge test

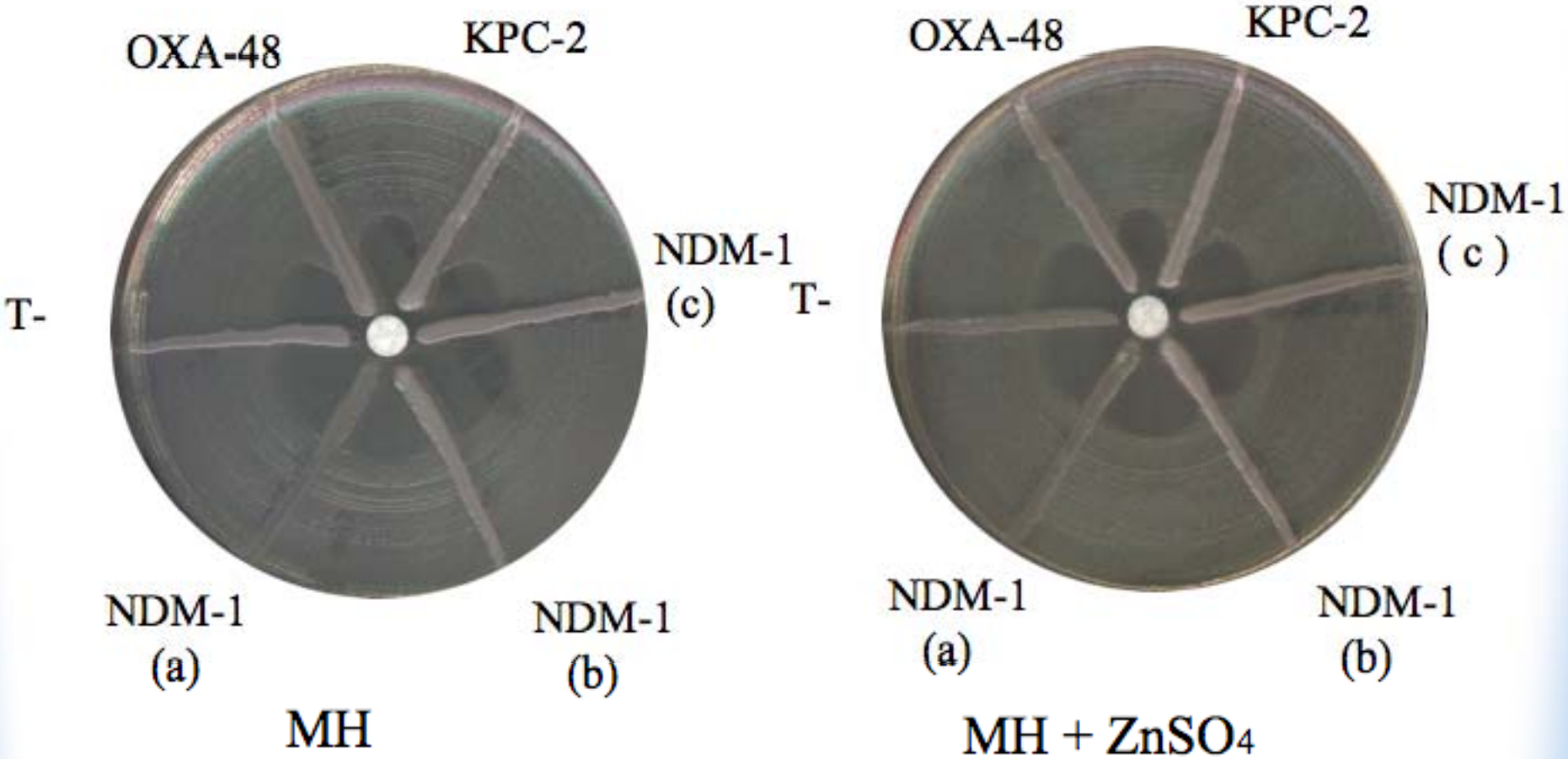
Modified cloverleaf (Hodge) test



- 1:10 dilution of 0.5 McF suspension of E. coli ATCC 25922
- 10 µg imipenem disk in the center
- Each test isolate streaked from the the disk to the edge of the plate
- Isolate A is positive, isolates B-D are negative

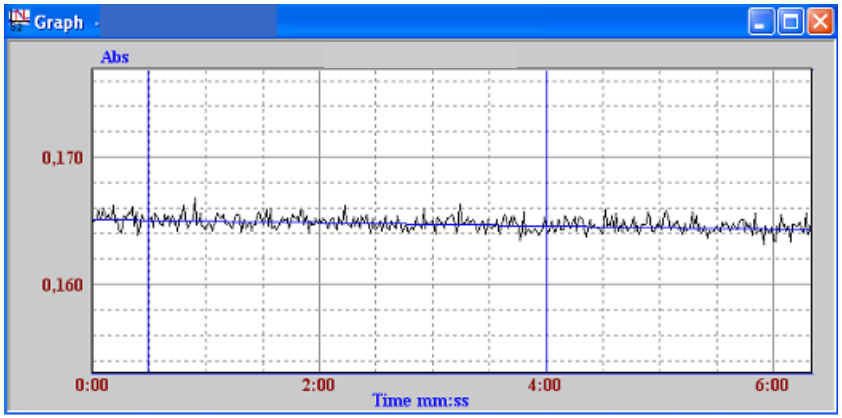
Value of the Modified Hodge test for detection of emerging carbapenemases in *Enterobacteriaceae*.

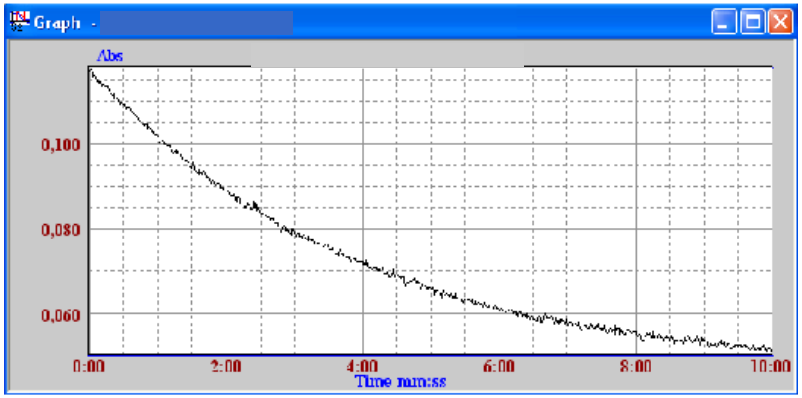
Girlich D, Poirel L, Nordmann P, J Clin Microbiol. 2011 Nov 23. [Epub ahead of print]



A method for expert labs; spectrophotometry detection of carbapenemase activity

- 18 h Culture-protein extraction-measurement of carbapenem hydrolysis by UV spectrophotometry





- 10 µl of bacterial crude extract + 100 µM of imipenem
- wavelength: 297 nm

Bernabeu, Poirel & Nordmann, Diag Microb Infect Dis, 2012 in press

Mass Spectrophotometry

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2011, p. 3321–3324 Vol. 49, No. 9
0095-1137/11/$12.00 doi:10.1128/JCM.00287-11

NOTES

Using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry To Detect Carbapenem Resistance within 1 to 2.5 Hours[▽]

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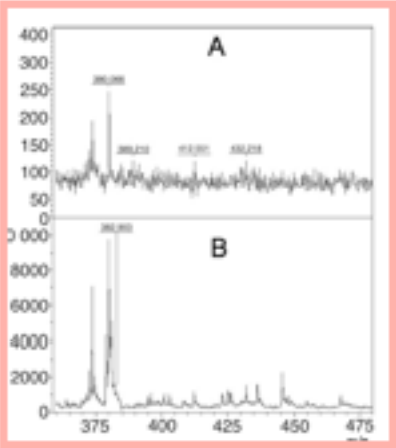
In recent years, the percentage of carbapenem-resistant bacteria has increased a become a major threat for patient survival. Carbapenemase-induced carbapenem res through the detection of carbapenem degradation using matrix-assisted laser deso flight mass spectrometry (MALDI-TOF MS). This method works for strains carrying KPC-2, and different IMP enzymes.



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0095-1137/11/$12.00 doi:10.1128/JCM.00064-11

Carbapenemase Activity Detection by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry[▽]

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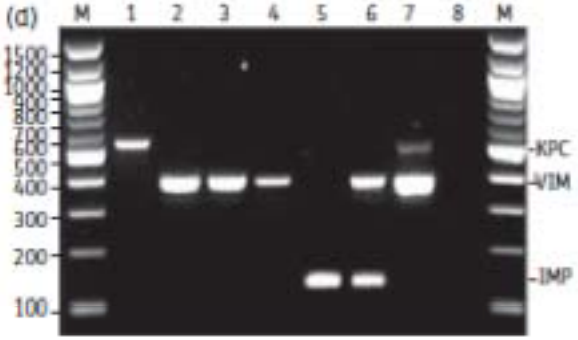
Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry is used for the determination of molecular weights of different chemical compounds. We describe here the use of MALDI-TOF mass spectrometry to detect a carbapenem antibiotic, meropenem, and its degradation products. Buffered meropenem solution (0.1 mM Tris-HCl, pH 6.8) was mixed with an overnight culture of bacteria. After 3-h incubation, the reaction mixture was centrifuged, and the supernatant was analyzed by MALDI-TOF mass spectrometry. The presence or absence of peaks representing meropenem and its sodium salts was crucial. The average turnaround time of this test, considering the use of overnight culture, is 4 h. We validated this method for the detection of resistance to carbapenems in *Enterobacteriaceae* and *Pseudomonas aeruginosa* mediated by carbapenemase production. A total of 124 strains, including 30 carbapenemase-producing strains, were used in the study. The sensitivity of this method is 96.67%, with a specificity of 97.87%. Our results demonstrate the ability of this method to routinely detect carbapenemases in *Enterobacteriaceae* and *Pseudomonas* spp. in laboratories. This assay is comparable with a labor-intensive imipenem-hydrolyzing spectrophotometric assay that is a reference method for the detection of carbapenemase. As demonstrated here, MALDI-TOF mass spectrometry may be used in microbiological laboratories not only for microbial identification but also for other applications, such as studies of mechanisms of antibiotic resistance.

Molecular tests - PCR- Sequencing; Gold Standard



Multiplex PCR for detection of acquired carbapenemase genes

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DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

Updated multiplex polymerase chain reaction for detection of 16S rRNA methylases: high prevalence among NDM-1 producers☆

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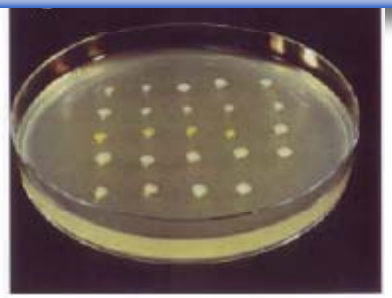
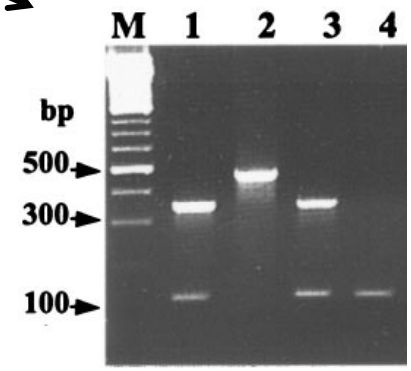
The problems of detection of carbapenemase producers





① Experienced personal
② Time consuming; 24-72 h or more
③ ~~Variable sensitivity and specificity~~
④ Expensive; molecular techniques

Which is the situation ?

Currently available diagnostic tests for carbapenemase producers

- **Susceptibility testing** : imipenem, ertapenem, meropenem:CLSI, EUCAST guidelines
- **Phenotypic detection**

  - Hodge test; modified Hodge test
  - Inhibition; EDTA, clavulanic acid, boronic acid…

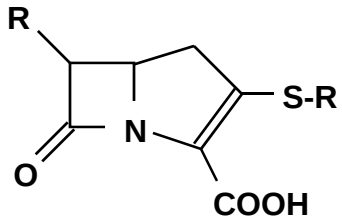
- **Carbapenem hydrolysis** (spectrophotometry, mass spectro)
- **Molecular biology**

  - Specific PCR , muliplex PCR +/- sequencing
  - Real time PCR (Check Point)
  - DNA Microarray (Check Point)

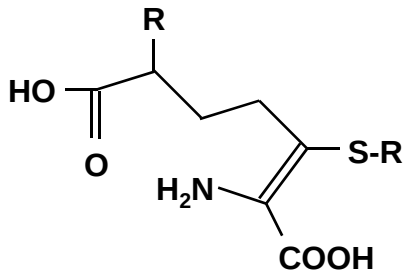
Goal

A rapid, specific, sensitive, cheap test for detection of carbapenemases in *Enterobacteriaceae* worldwide

Principle of the test



Carbapenemase



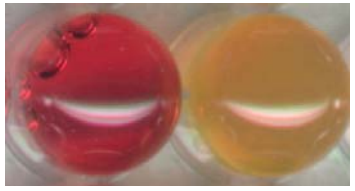
Carbapenems

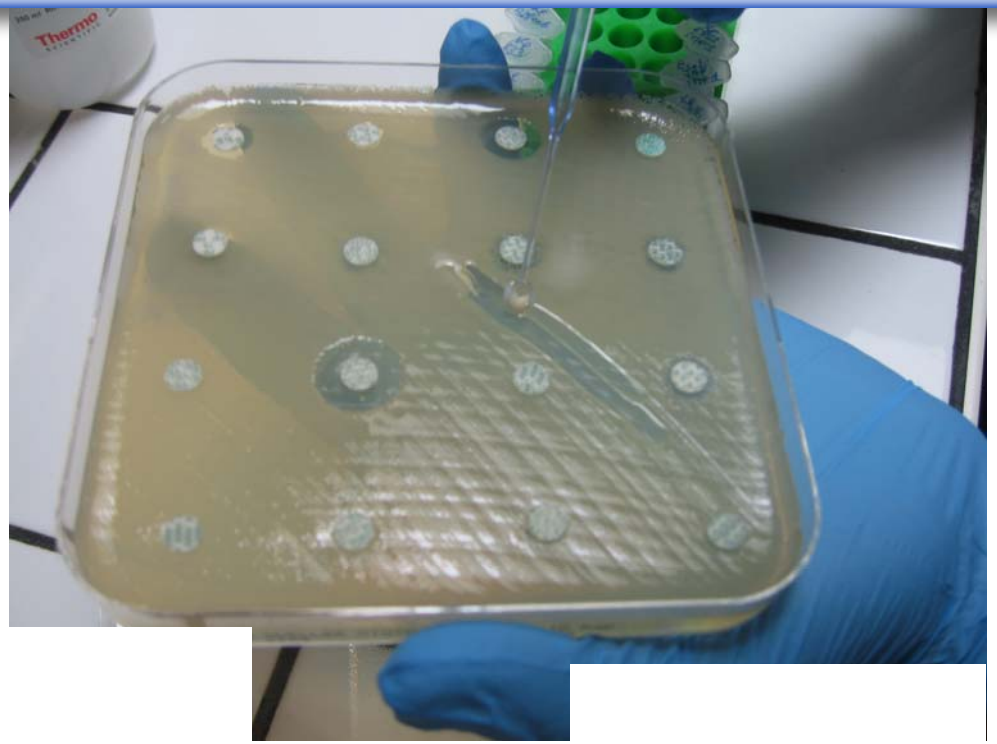
Imipenem
Meropenem
Ertapenem
Doripenem

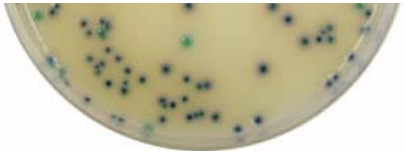
Acid production

Colorimetric detection

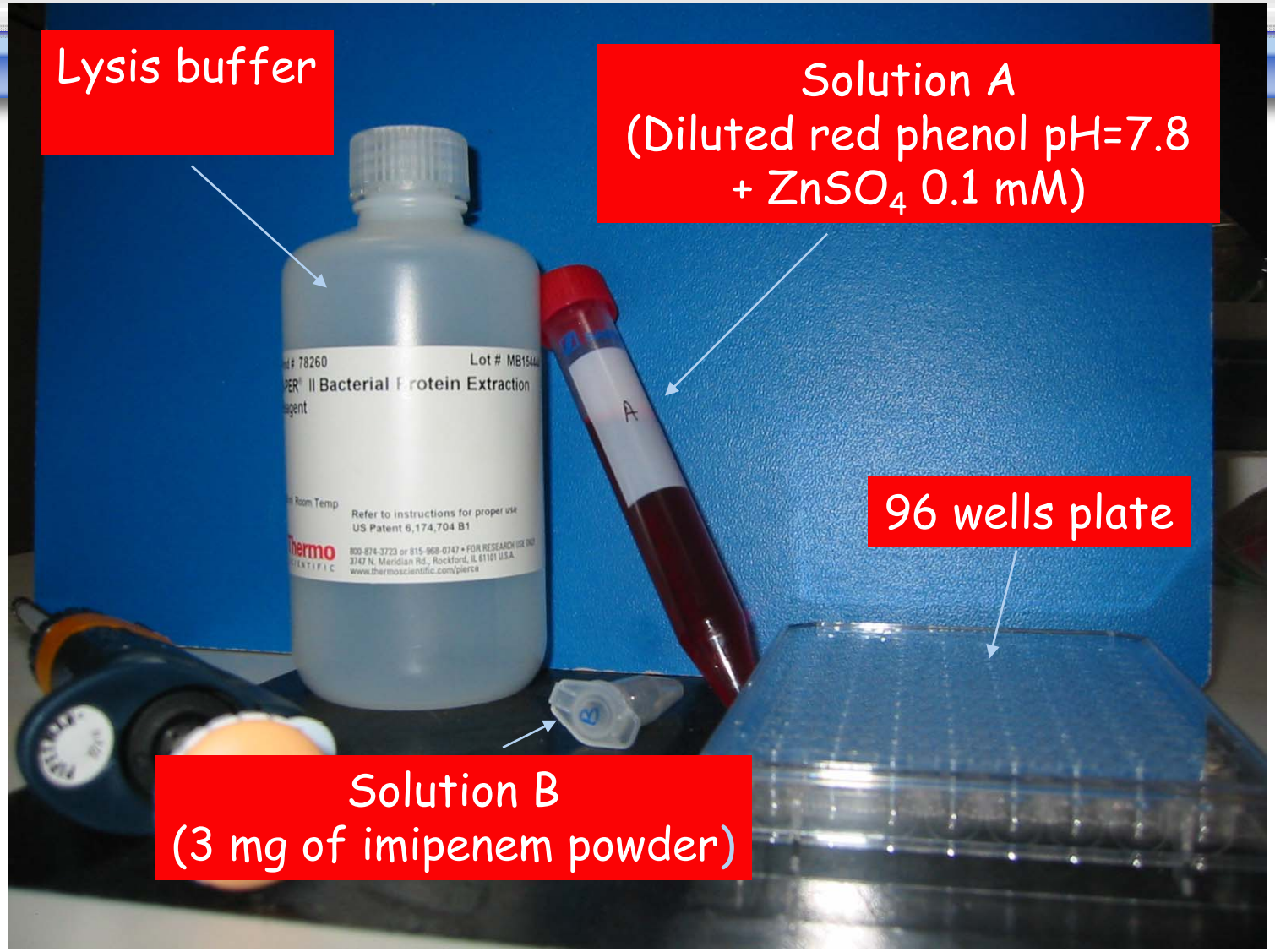
pH




Thermo



The Carba NP test; the kit



Strains collection

- Carbapenemase producers : n=162

– The enterobacterial species; *E .coli* (n= 22), *K. pneumoniae*, (n=81), others (n =59)

- Variability of the level of resistance to carbapenems
- KPC (n=49), VIM (n=26), NDM (n= 28),OXA-48 type (n= 27), others (n= 12)

- Carbapenemase non-producers: n= 46

  – The enterobacterial species; *E.coli* (n=13),*K. pneumoniae* (n= 17), others (n=16)

  - Decrease susceptibility to carbapenems (n=23) including combined mechanisms of resistance (outer membrane permeability defect+ CTX-M, AmpC…)

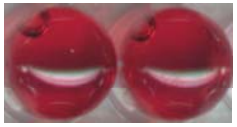
  - Wild-type susceptibility to carbapenems (n=23) including CTX-M or AmpC producers.

Interpretation of the Carba NP test

Imipenem

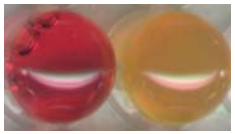
- +

No inoculation



Non-carbapenemase producer

Carbapenemase producer



The Carba NP test

Sensitivity = 100%

Specificity = 100%

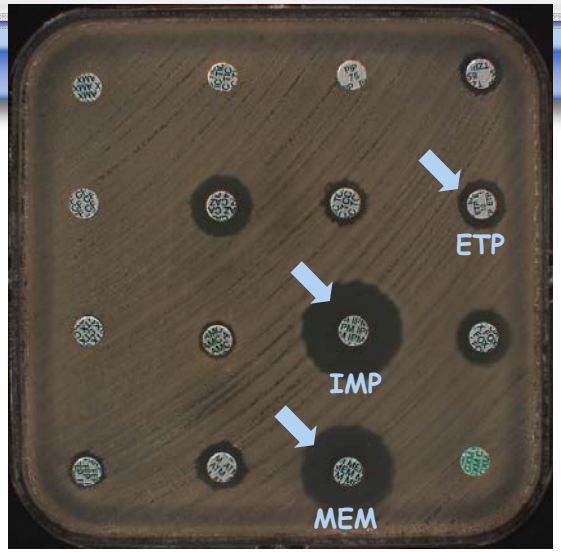
Nordmann, Poirel, Dortet, Emerg Infect Dis, 2012, 18:1503-1507

The Carba NP test

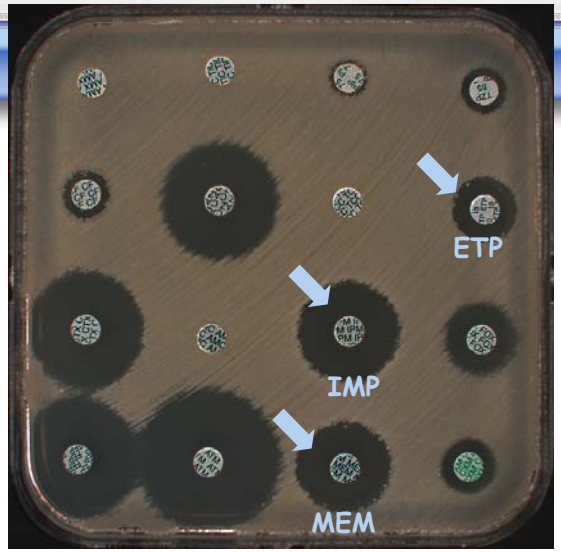
Ambler class	Carbapenemase type	Mean time for positivity
A	KPC	15 min- 1h
A	GES-2, -5	1h-1h30
B	NDM	20-50 min
B	VIM	20-50 min
B	IMP	5-30 min
D	OXA-48	30-40 min

Question ; any carbapenemase here ?

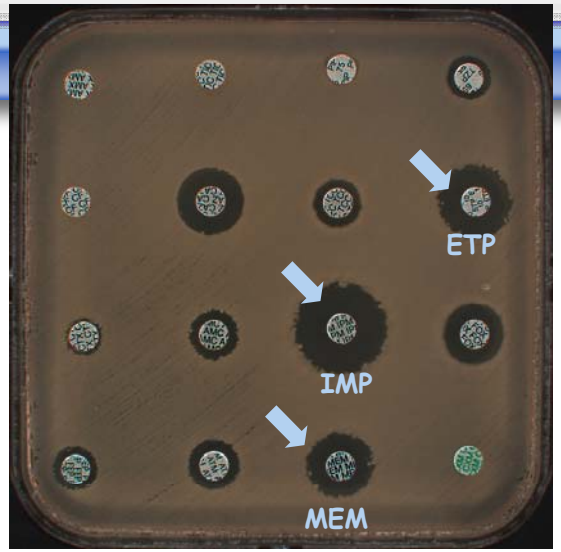
K. pneumoniae



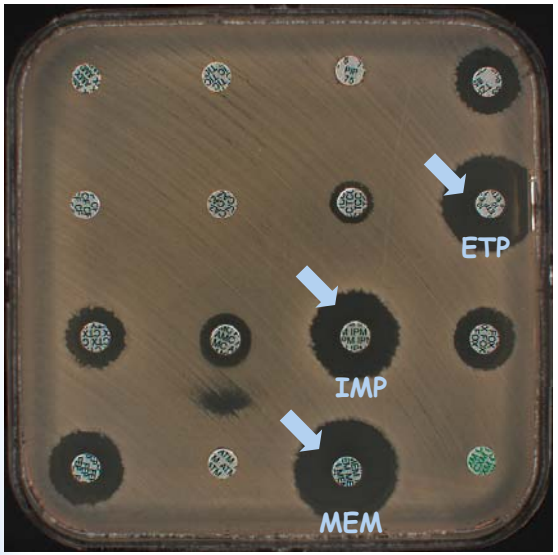
K. pneumoniae



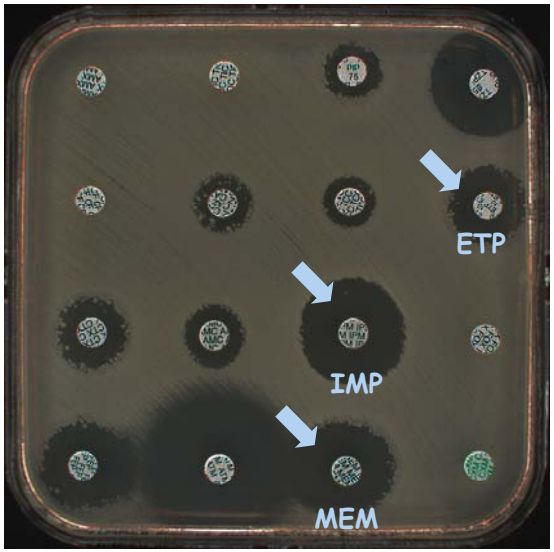
K. pneumoniae



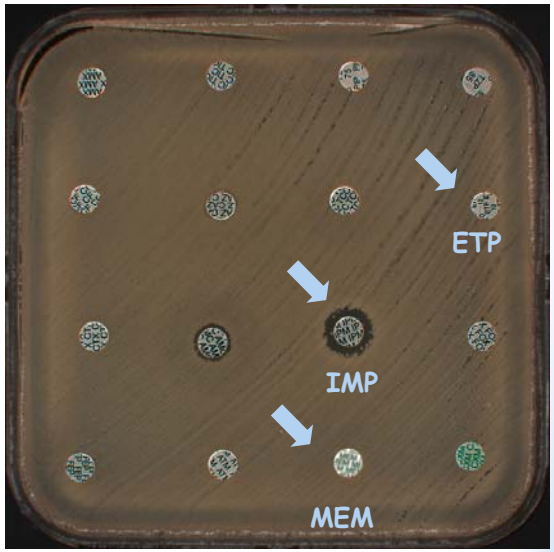
E. coli



E. coli

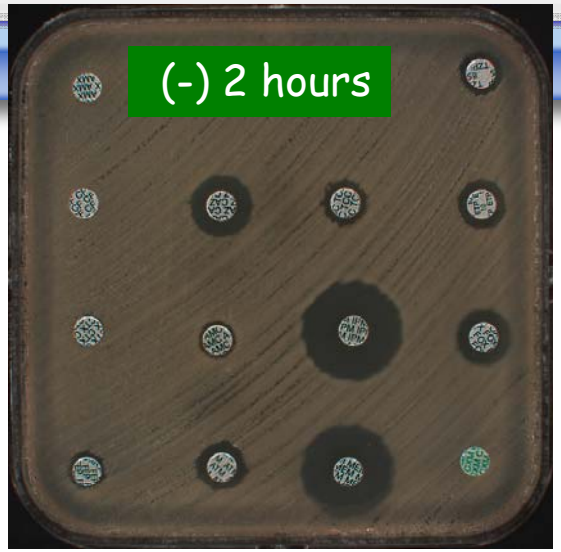


E. coli

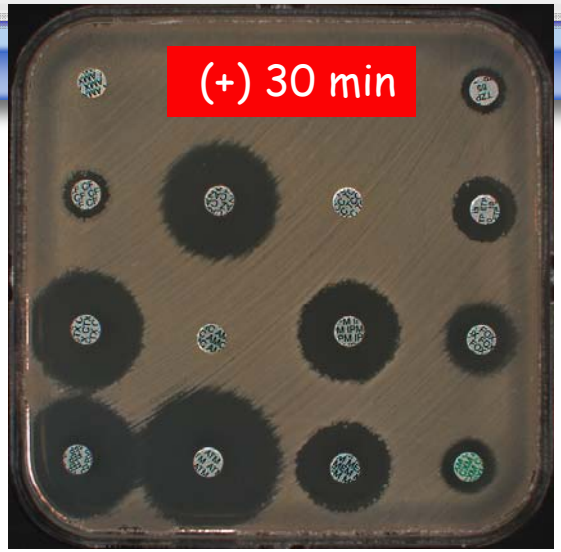


Question : any carbapenemase here ?

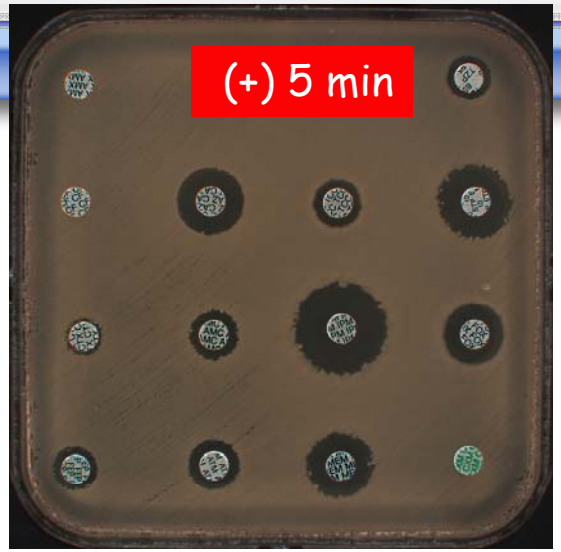
K. pneumoniae
CTX-M15 + impermeability



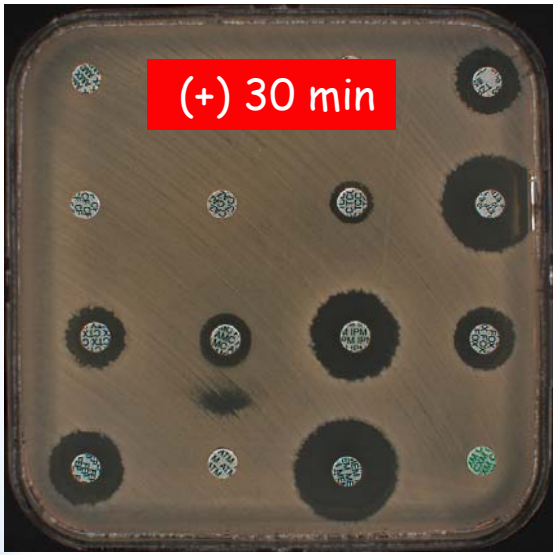
K. pneumoniae OXA-48



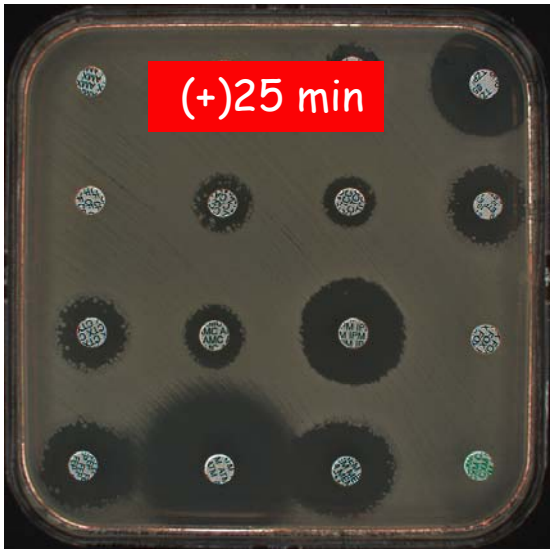
K. pneumoniae KPC-2



E. coli VIM-1



E. coli IMP-1



E. coli NDM-1

The Carba NP test

① Rapid; less than 2 h

② Sensitive; 100%

③ Specific: 100%

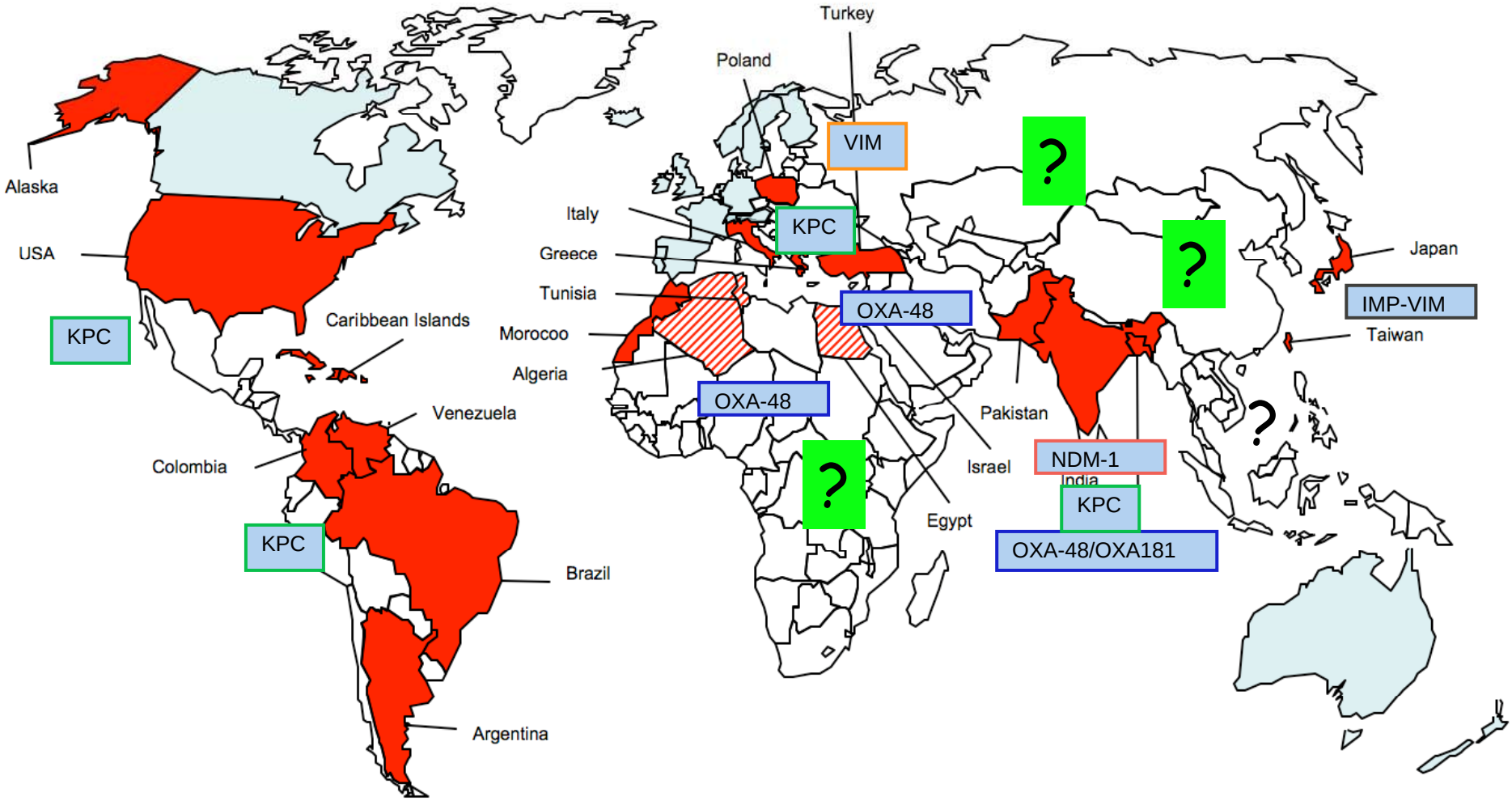
④ Detection of any type carbapenemase activity

⑤ Cheap : 0.5 USD

⑥ Easy-to-handle

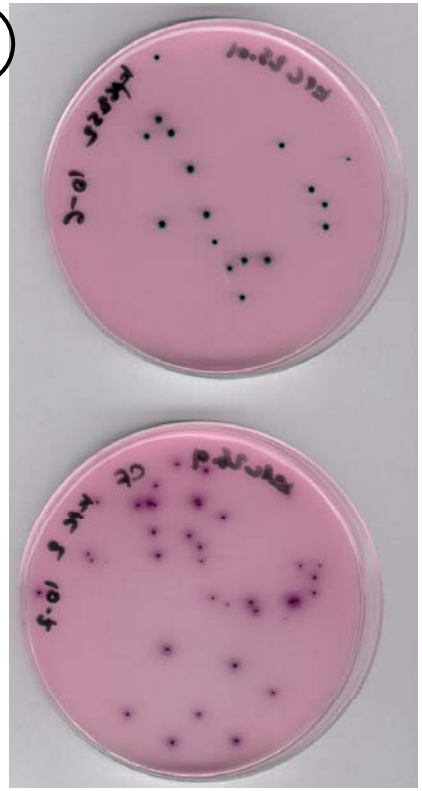
⑦ Implementable worldwide

Carbapenemases- Enterobactericeae reservoirs



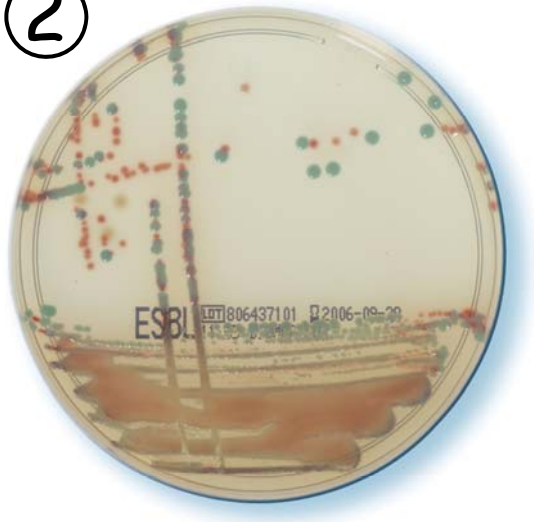
Detection of carbapenemase producers
as colonizers of the intestinal flora

①



CHROMagar KPC
(carbapenemase producers)
contains a carbapenem

②



ESBL ID bioMérieux
(Carbapenemase and ESBL producers)
contains a cephalosporin

③

Oxoid Brillance CRE agar

(Thermo Fisher)
contains a carbapenem

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2011, p. 718–721 Vol. 49, No. 2
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How To Detect NDM-1 Producers[▽]

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

TABLE 1. Sensitivity of detection of 27 NDM-1 producers by ChromID ESBL and CHROMagar KPC media, results of the combined disk test[a] and the Etest MBL, and MICs of several β-lactams

Isolate	Country of isolation	MIC (μg/ml)[b]						Etest MBL	IMP/IMP + EDTA	Lower limit of detection (CFU/ml)	
		CTX	CAZ	IMP	ETP	MER	DOR			ChromID ESBL	CHROMagar KPC
E. coli											
A	Australia	>32	>256	6	4	4	>32	+	+	2×10^1	3×10^1
B	France	>32	>256	3	3	2	2	+	+	2×10^1	3×10^5
C	India	>32	>256	2	>32	24	6	+	+	3×10^1	3×10^1
D	India	>32	>256	16	>32	16	3	+	+	1×10^1	1×10^1
E	India	>32	>256	32	>32	>32	24	+	+	2×10^2	1×10^1
F	India	>32	>256	>32	>32	16	16	+	+	1×10^1	3×10^1
G	India	>32	>256	4	>32	8	3	+	+	3×10^1	4×10^1
H	India	>32	>256	1.5	8	2	1	+	+	1×10^1	2×10^5
I	India	>32	>256	3	>32	6	2	+	+	1×10^1	1×10^1
J	India	>32	>256	2	4	2	1.5	+	+	8×10^0	1×10^4
E. cloacae											
A	India	>32	>256	8	6	6	4	+	+	1×10^1	5×10^1
B	India	>32	>256	>32	12	8	12	+	+	1×10^1	1×10^1
C	India	>32	>256	2	16	2	1	+	+	3×10^2	4×10^4
D	India	>32	>256	0.75	3	1.5	1	—[c]	+	1×10^1	1×10^2
K. pneumoniae											
A	Kenya	>32	>256	>32	>32	>32	>32	+	+	2×10^1	1×10^1
B	Sultanate of Oman	>32	>256	>32	>32	>32	>32	+	+	1×10^1	2×10^1
C	Sultanate of Oman	>32	>256	1	6	2	2	+	+	1×10^1	3×10^2
D	India	>32	>256	>32	>32	>32	>32	+	+	2×10^1	3×10^1
E	India	>32	>256	0.75	8	2	1.5	—	+	1×10^1	3×10^2
F	India	>32	>256	>32	>32	>32	>32	+	+	1×10^1	8×10^1
G	India	>32	>256	2	6	2	1.5	+	+	1×10^1	1×10^2
H	India	>32	>256	>32	>32	>32	>32	+	+	1×10^1	5×10^2
I	India	>32	>256	>32	>32	>32	>32	+	+	1×10^1	5×10^2
J	India	>32	>256	6	>32	16	12	+	+	1×10^1	1×10^1
K. oxytoca A	India	>32	>256	2	4	3	3	+	+	1×10^1	1×10^4
C. freundii A	France	>32	>256	>32	>32	>32	>32	+	+	1×10^1	1×10^1
P. rettgeri A	India	>32	>256	3	0.5	1.5	0.75	+	+	5×10^2	5×10^5

[a] IMP, IMP plus EDTA.
[b] CTX, cefotaxime; CAZ; ceftazidime; IMP, imipenem; ETP, ertapenem; MER, meropenem; DOR, doripenem.
[c] —, not interpretable.

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Isolate	Country of isolation	MIC (μg/ml)[b]						Etest MBL	IMP/IMP + EDTA	Lower limit of detection (CFU/ml)	
		CTX	CAZ	IMP	ETP	MER	DOR			ChromID ESBL	CHROMagar KPC
E. coli											
A	Australia	>32	>256	6	4	4	>32	+	+	2×10^1	3×10^1
B	France	>32	>256	3	3	2	2	+	+	2×10^1	3×10^5
C	India	>32	>256	2	>32	24	6	+	+	3×10^1	3×10^1
D	India	>32	>256	16	>32	16	3	+	+	1×10^1	1×10^1
E	India	>32	>256	32	>32	>32	24	+	+	2×10^2	1×10^1
F	India	>32	>256	>32	>32	16	16	+	+	1×10^1	3×10^1
G	India	>32	>256	4	>32	8	3	+	+	3×10^1	4×10^1
H	India	>32	>256	1.5	8	2	1	+	+	1×10^1	2×10^5
I	India	>32	>256	3	>32	6	2	+	+	1×10^1	1×10^1
J	India	>32	>256	2	4	2	1.5	+	+	8×10^0	1×10^4
E. cloacae											
A	India	>32	>256	8	6	6	4	+	+	1×10^1	5×10^1
B	India	>32	>256	>32	12	8	12	+	+	1×10^1	1×10^1
C	India	>32	>256	2	16	2	1	+	+	3×10^2	4×10^4
D	India	>32	>256	0.75	3	1.5	1	—[c]	+	1×10^1	1×10^2
K. pneumoniae											
A	Kenya	>32	>256	>32	>32	>32	>32	+	+	2×10^1	1×10^1
B	Sultanate of Oman	>32	>256	>32	>32	>32	>32	+	+	1×10^1	2×10^1
C	Sultanate of Oman	>32	>256	1	6	2	2	+	+	1×10^1	3×10^2
D	India	>32	>256	>32	>32	>32	>32	+	+	2×10^1	3×10^1
E	India	>32	>256	0.75	8	2	1.5	—	+	1×10^1	3×10^2
F	India	>32	>256	>32	>32	>32	>32	+	+	1×10^1	8×10^1
G	India	>32	>256	2	6	2	1.5	+	+	1×10^1	1×10^2
H	India	>32	>256	>32	>32	>32	>32	+	+	1×10^1	5×10^2
I	India	>32	>256	>32	>32	>32	>32	+	+	1×10^1	5×10^2
J	India	>32	>256	6	>32	16	12	+	+	1×10^1	1×10^1
K. oxytoca A	India	>32	>256	2	4	3	3	+	+	1×10^1	1×10^4
C. freundii A	France	>32	>256	>32	>32	>32	>32	+	+	1×10^1	1×10^1
P. rettgeri A	India	>32	>256	3	0.5	1.5	0.75	+	+	5×10^2	5×10^5

[a] IMP, IMP plus EDTA.
[b] CTX, cefotaxime; CAZ; ceftazidime; IMP, imipenem; ETP, ertapenem; MER, meropenem; DOR, doripenem.
[c] —, not interpretable.

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0095-1137/10/$12.00 doi:10.1128/JCM.02277-09

Use of ChromID Extended-Spectrum β-Lactamase Medium for Detecting Carbapenemase-Producing *Enterobacteriaceae*[▽]

Amélie Carrër, Nicolas Fortineau, and Patrice Nordmann*

TABLE 1. Sensitivity of detection of ChromID ESBL medium and CHROMagar KPC medium for 28 carbapenemase- and/or ESBL-producing enterobacterial isolates

Isolate	Carbapenemase	ESBL	MIC (μg/ml) of drug[a]:					Lowest limit of detection (CFU/ml)	
			CTX	CAZ	IMP	ETP	MP	ChromID ESBL	CHROMagar KPC
Citrobacter freundii	KPC-2		16	32	4	2	3	1×10^1	4.4×10^1
Serratia marcescens	KPC-2		>256	>256	>32	>32	>32	1×10^1	1.4×10^1
Enterobacter cloacae	KPC-2		>256	>256	4	6	2	1×10^1	2.5×10^1
Enterobacter cloacae HPTU	KPC-2		6	4	1	1.5	1	1×10^1	1×10^4
Escherichia coli	KPC-2		6	4	0.5	0.5	0.5	1×10^1	1×10^4
Klebsiella pneumoniae A28006	KPC-2	CTX-M-2	>256	16	16	24	32	1×10^1	8.3×10^1
Klebsiella pneumoniae 588	KPC-2		16	48	24	32	16	1×10^1	1.4×10^1
Klebsiella pneumoniae 633	KPC-2	CTX-M-12	>256	>256	>32	>32	4	1×10^1	1.6×10^1
Klebsiella pneumoniae 2303	KPC-2		>256	>256	>32	>32	>32	8×10^1	2.5×10^1
Klebsiella pneumoniae GR	KPC-2		>256	>256	12	24	6	1×10^1	1.5×10^1
Escherichia coli	VIM-1	CTX-M-3	>256	>256	1.5	0.5	0.5	1×10^1	2×10^5
Escherichia coli	IMP-1		>256	>256	0.5	3	0.5	1×10^1	2×10^5
Serratia marcescens	IMP-1		>256	6	4	>32	4	1×10^1	7×10^1
Klebsiella pneumoniae 6852	IMP-1		>256	>256	1	2	8	1×10^1	1.7×10^1
Klebsiella pneumoniae	VIM-1	CTX-M-3	>256	>256	1	2	1	3×10^1	2×10^4
Klebsiella pneumoniae 149	IMP-4	SHV-12	>256	>256	2	4	2	2×10^1	1×10^3
Klebsiella pneumoniae	VIM-1	SHV-5	>256	>256	>32	>32	>32	1×10^1	5.7×10^1
Enterobacter cloacae	VIM-1	CTX-M-3	>256	>256	3	4	2	1×10^1	1.5×10^1
Klebsiella pneumoniae BIC	OXA-48		0.5	0.5	0.5	2	0.5	$>2 \times 10^7$	5×10^6
Klebsiella pneumoniae I	OXA-48	CTX-M-15	>256	32	24	24	16	1×10^1	1×10^1
Klebsiella pneumoniae B	OXA-48	CTX-M-15	>256	>256	16	>32	32	2×10^1	5×10^3
Klebsiella pneumonaie BEL	OXA-48		0.5	1	1	4	1	$>2 \times 10^7$	1×10^6
Klebsiella pneumoniae LIB	OXA-48		2	1	16	16	16	$>2 \times 10^7$	5×10^4
Klebsiella pneumoniae EG	OXA-48	CTX-M-15	64	256	2	3	2	2×10^1	1×10^5
Klebsiella pneumoniae 11978	OXA-48	SHV-2a	64	256	>32	>32	>32	2×10^1	1×10^1
Providencia rettgeri	OXA-48	TEM-101	64	256	>32	>32	>32	6×10^1	1×10^2
Citrobacter freundii	OXA-48	VEB-1	64	256	>32	>32	>32	3×10^1	2×10^7
Enterobacter cloacae	OXA-48	SHV-5	>256	>256	0.5	0.5	0.5	2×10^1	1×10^5

[a] CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; ETP, ertapenem; MP, meropenem.

A novel screening medium (made in Bicêtre)

SUPERCARBA medium ©

- Use of a Drigalski medium for selection of Gram negatives
- Supplemented with a carbapenem
- Supplemented with cloxacillin
- Supplemented with $ZnSO_4$

Inserm U914
Résistances Emergentes aux Antibiotiques

Nordmann, Girlich, and Poirel, J. Clin Microbiol. 2012

A novel screening medium (made in Bicêtre hospital)

Combines many advantages

- Excellent sensitivity
  - for OXA-48 producers thanks to a low concentration of the carbapenem
  - for MBL producers (especially NDM) thanks to the addition of zinc ions

- Excellent specificity
  - supplemented with a carbapenem, not a cephalosporin (no growth of an ESBL+ and carbapenem susceptible strain)
  - supplemented with cloxacillin (no growth of an AmpC-mediated carbapenem non-susceptible strain)

Nordmann, Girlich, and Poirel, J. Clin Microbiol. 2012,

A novel screening medium (SUPERCARBA medium©)

	Supercarba	Brilliance CRE	ChromKPC
Sensitivity (%)	96	76	43
Specificity (%)	61	57	68

- Rapid detection of carbapenemase producers in *Enterobacteriaceae* is now possible. To be implemented worldwide
- Carba NP test interesting for antibiotic stewardship and outbreak control
- Hodge test, inhibition techniques…are less likely to be used in the future.. delay for the results
- Molecular techniques are useful mostly for epidemiological purposes
- Screening media are being developed with good sensitivity and specificity