05.12.2017

EQAs in microbiology and standardization of MRSA testing

Accurate Results for Patient Care Workshop 2017
A JCTLM Members' and Stakeholders' meeting
4-5 December 2017
BIPM - Paris - France

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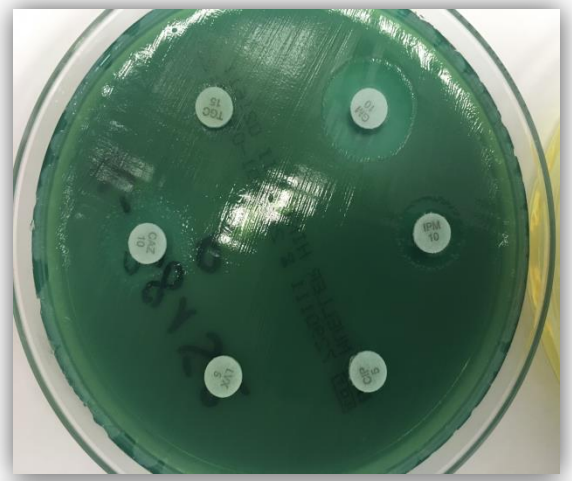
Institut für Med. Labordiagnostik

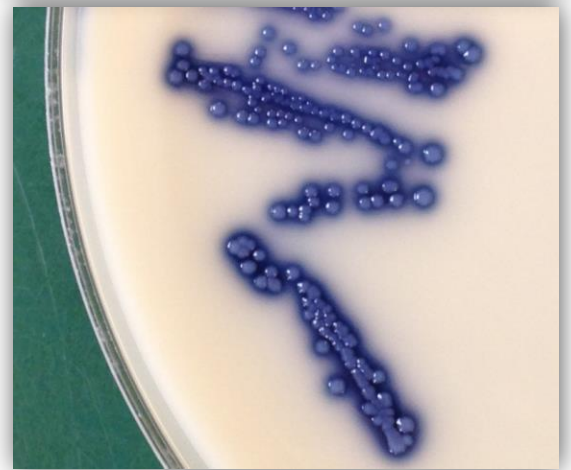
Helios

universität Witten/Herdecke

Outline

- Our Laboratory
- MDR bacteria in today´s health care
- Current technical developments in microbiology
- EQAs in infectious disease
- Approach to generate reference material for MRSA detetion - AntiMicroResist





Institute for Med. Laboratory Diagnostics (IML) at a glance

- 2017: approx. 3 Mio. analysis/year
- Microbiology for >3000 beds / 7 hospitals
- Blood bank
- Accreditated according to DIN ISO 17025 and 15189
- Reference Laboratory for Infectious Diseases of the Reference Institute for Bioanalytics (RfB) of the DGKL e.V.
- Scientific interests
  - QA of complex diagnsotics procedures
  - Mechanism and detection of MDR bacteria
  - Molecular mechanisms of inflammation

The problem

"Antimicrobial resistance has been called one of the world's most pressing public health problems."

Centers for Disease Control and Prevention
Get Smart: know when antibiotics work

http://www.cdc.gov/getsmart/antibiotic-use/fast-facts.html

"Antimicrobial resistance…
has the potential to affect anyone, of any age, in any country."

WHO Press Release Dated 30 April 2014
WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health

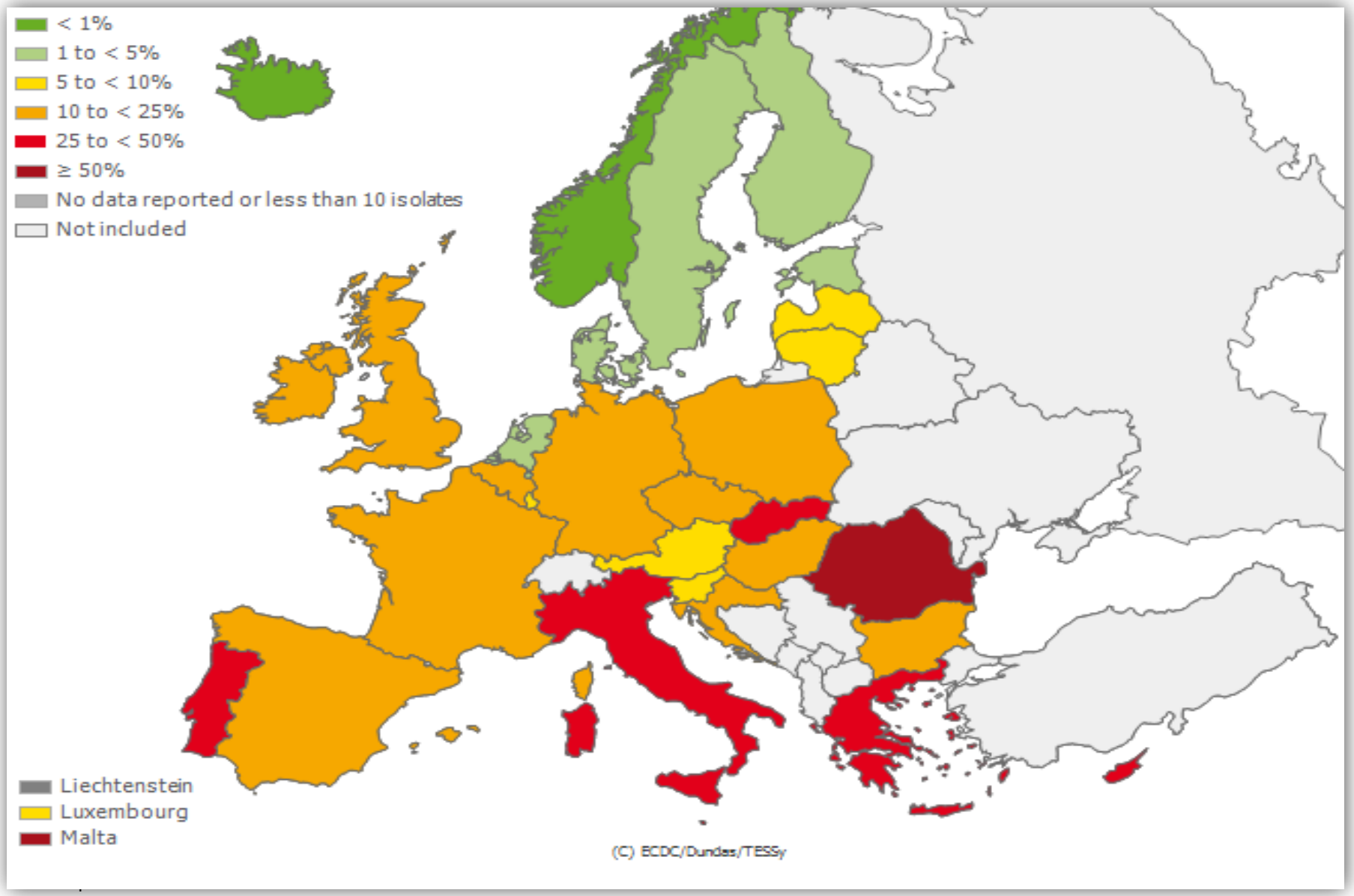
http://www.who.int/mediacentre/news/releases/2014/amr-report/en/

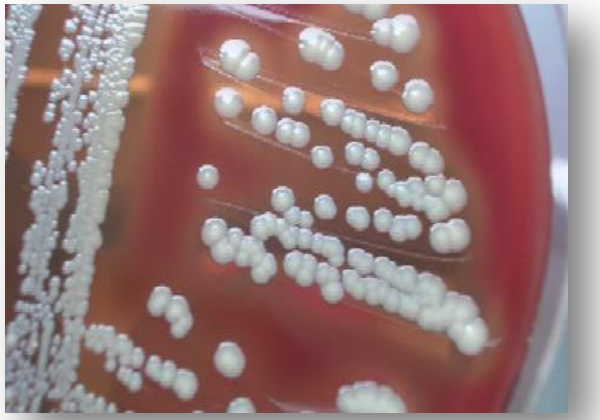
MDR pathogens

- **MRSA (Methicillin-resistant Staphylococcus aureus)**
- **MDR-gram negative enterobacteriaceae / carbapenem resistance**
- **VRE (vancomycin-resistant Enterococcus faecium)**
- **Mycobacterium tuberculosis**

MDR = multidrug resistant

Surveillance of antimicrobial resistance in Europe - 2013

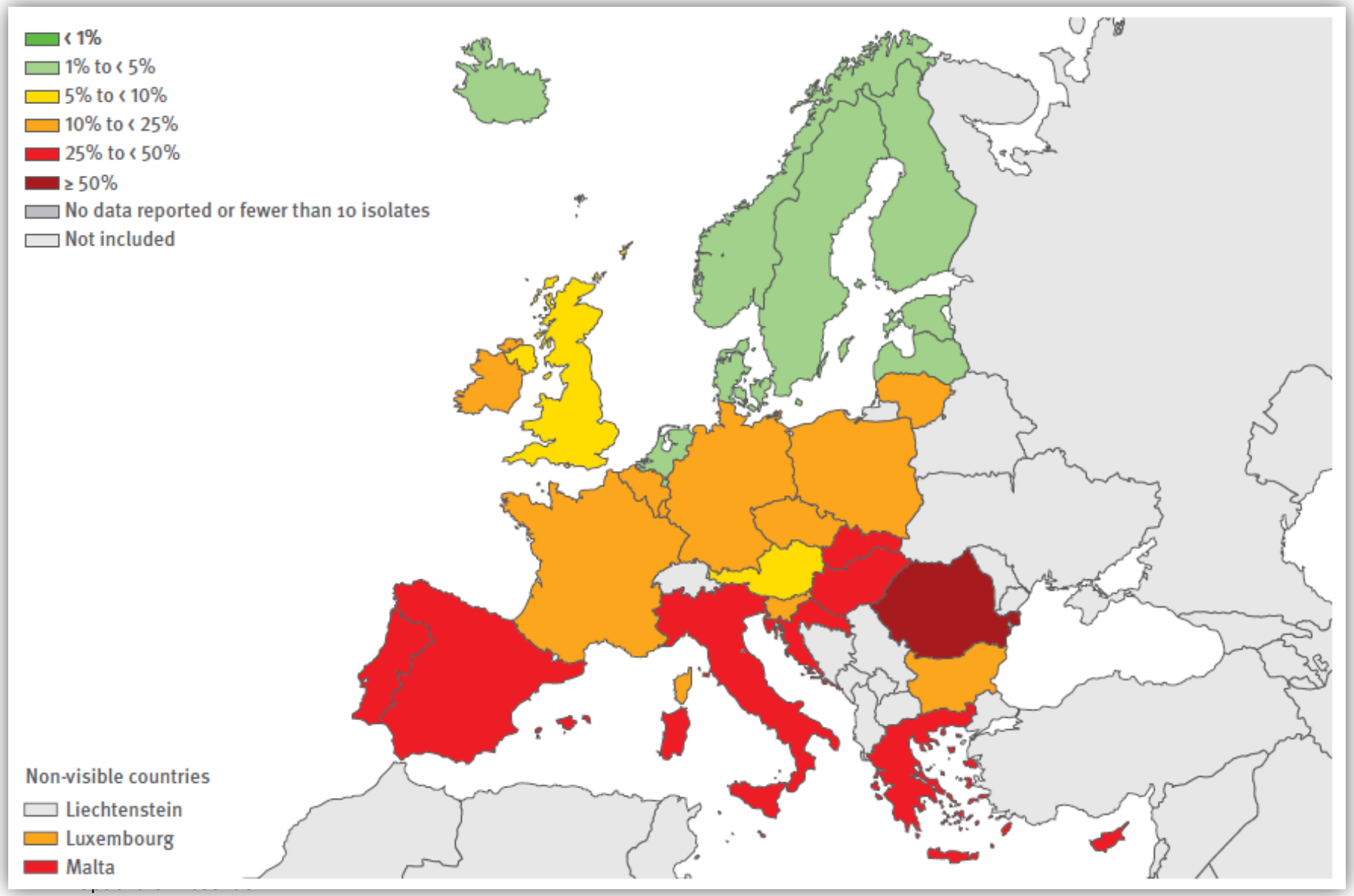




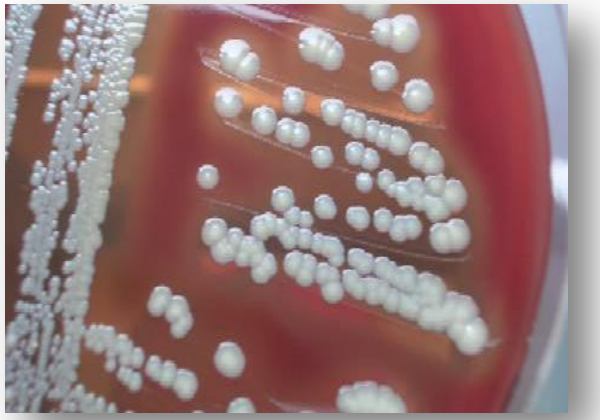
ECDC Data

***Staphylococcus aureus*. Percentage (%) of invasive isolates with resistance to meticillin (MRSA), by country, EU/EEA countries, 2013**

Surveillance of antimicrobial resistance in Europe - 2016



ECDC REPORT



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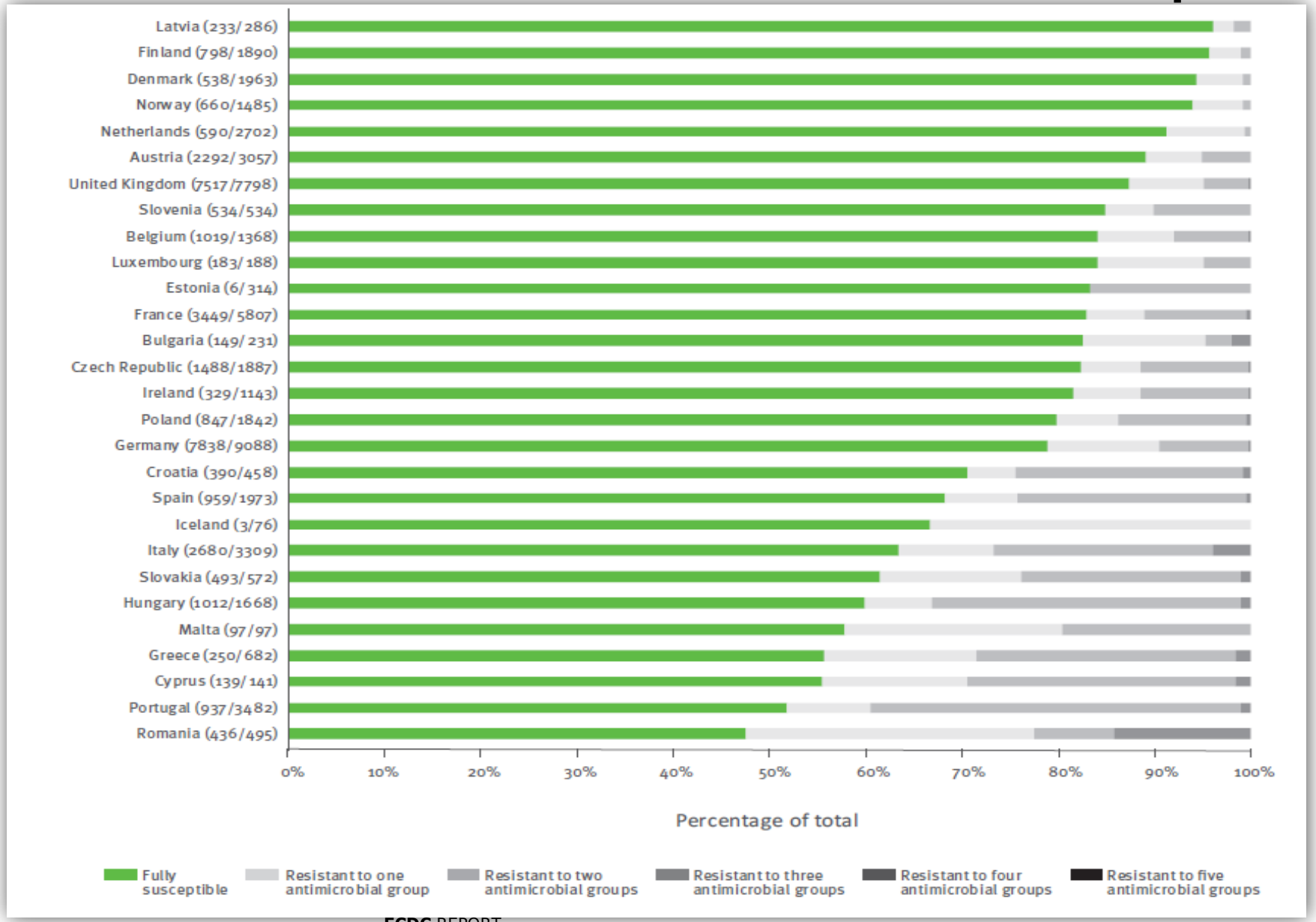
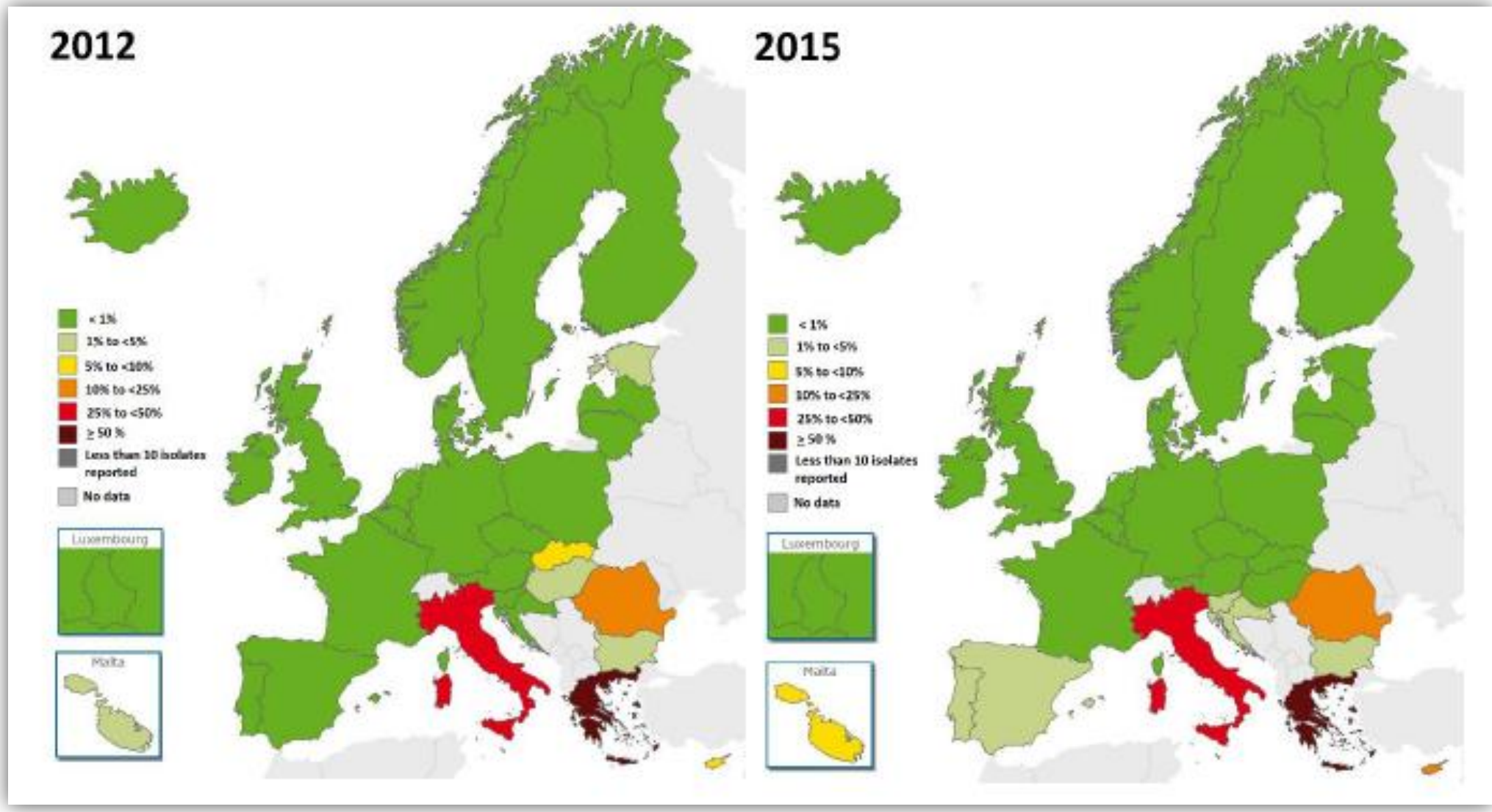


ECDC REPORT

Staphylococcus aureus. **Distribution of isolates: fully susceptible and resistant to one, two and three antimicrobial groups (among isolates tested for meticillin, fluoroquinolones and rifampicin. By country, EU/EEA countries, 2016**

ECDC Data

Klebsiella pneumoniae: percentage of invasive isolates with resistance to carbapenems (%R), 2012-2015

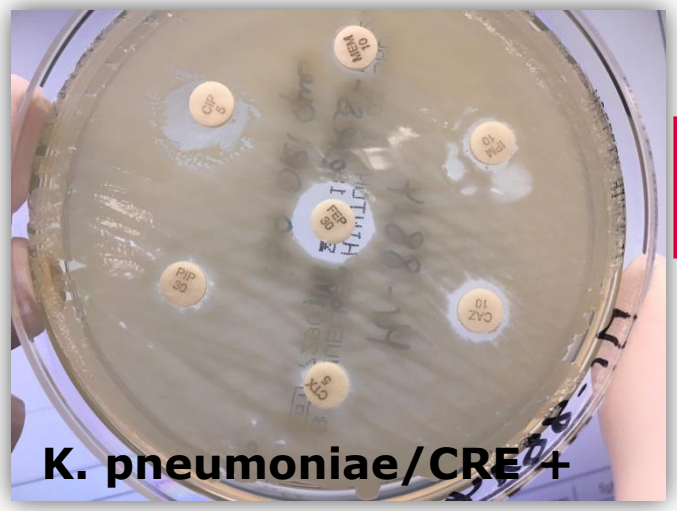


ECDC Data

ECDC Data

Klebsiella pneumoniae:

Total number of invasive isolates tested (N) and percentage with resistance to carbapenems (%R), including 95% confidence intervals (95% CI), EU/EEA countries, 2012-2015



K. pneumoniae/CRE +

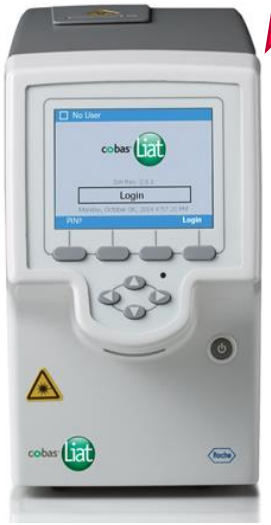
Country	2012			2013			2014			2015			Trend 2012-2015*	
	N	%R	(95% CI)	N	%R	(95% CI)	N	%R	(95% CI)	N	%R	(95% CI)		
Denmark	680	0.3	(0-1)	645	0.2	(0-1)	830	0.2	(0-1)	846	0.0	(0-0)		
Estonia	79	1.3	(0-7)	74	2.7	(0-9)	92	0.0	(0-4)	56	0.0	(0-6)		
Finland	536	0.0	(0-1)	550	0.0	(0-1)	583	0.0	(0-1)	658	0.0	(0-1)		
Iceland	16	0.0	(0-19)	28	0.0	(0-12)	28	0.0	(0-12)	35	0.0	(0-11)		N/A
Latvia	77	0.0	(0-5)	92	0.0	(0-4)	118	1.7	(0-6)	112	0.0	(0-3)		
Lithuania	185	0.0	(0-2)	144	0.0	(0-3)	154	1.3	(0-5)	177	0.0	(0-2)		
Luxembourg	48	0.0	(0-7)	53	1.9	(0-10)	66	1.5	(0-8)	60	0.0	(0-6)		
Sweden	977	0.1	(0-1)	1269	0.0	(0-0)	978	0.0	(0-0)	900	0.0	(0-0)		
Germany	661	0.0	(0-1)	763	0.7	(0-2)	1006	0.7	(0-1)	1520	0.1	(0-0)		
Hungary	481	2.9	(2-5)	531	1.7	(1-3)	621	1.1	(0-2)	687	0.1	(0-1)		<
Netherlands	684	0.1	(0-1)	646	0.2	(0-1)	903	0.2	(0-1)	907	0.1	(0-1)		
Norway	623	0.5	(0-1)	645	0.2	(0-1)	746	0.0	(0-0)	700	0.1	(0-1)		
Czech Republic	1307	0.3	(0-1)	1133	0.5	(0-1)	1148	0.1	(0-0)	1100	0.3	(0-1)		
United Kingdom	888	0.5	(0-1)	1051	0.5	(0-1)	1069	0.8	(0-2)	962	0.4	(0-1)		
Belgium	545	0.7	(0-2)	618	0.3	(0-1)	429	0.5	(0-2)	389	0.5	(0-2)		
France	1627	0.5	(0-1)	1842	0.7	(0-1)	2103	0.5	(0-1)	2244	0.5	(0-1)		
Ireland	338	0.0	(0-1)	317	0.3	(0-2)	353	0.6	(0-2)	389	0.5	(0-2)		
Poland	359	0.8	(0-2)	370	0.8	(0-2)	451	1.3	(0-3)	660	0.5	(0-1)		
Austria	738	0.8	(0-2)	910	1.2	(1-2)	971	0.6	(0-1)	1022	0.8	(0-2)		
Slovakia	331	6.3	(4-10)	342	0.6	(0-2)	456	2.6	(1-5)	436	0.9	(0-2)		<#
Slovenia	254	0.4	(0-2)	245	0.4	(0-2)	233	0.9	(0-3)	237	1.3	(0-4)		
Spain	1152	0.8	(0-1)	1241	1.6	(1-2)	1266	2.3	(2-3)	1483	2.2	(1-3)		>
Croatia	331	0.0	(0-1)	376	0.5	(0-2)	334	0.9	(0-3)	380	2.4	(1-4)		>
Bulgaria	108	1.9	(0-7)	129	0.0	(0-3)	139	7.2	(4-13)	95	3.2	(1-9)		
Portugal	749	0.7	(0-2)	904	1.8	(1-3)	1701	1.8	(1-3)	2085	3.4	(3-4)		>
Malta	57	3.5	(0-12)	69	5.8	(2-14)	101	9.9	(5-17)	92	5.4	(2-12)		
EU/EEA (population-weighted mean)	**16287**	**6.2**	**(6-7)**	**17932**	**8.2**	**(8-9)**	**19619**	**7.1**	**(7-7)**	**21749**	**8.1**	**(8-8)**		**>**
Cyprus	65	9.2	(3-19)	68	5.9	(2-14)	80	5.0	(1-12)	62	12.9	(6-24)		
Romania	102	13.7	(8-22)	215	20.5	(15-26)	257	31.5	(26-38)	271	24.7	(20-30)		>#
Italy	845	29.1	(26-32)	1453	34.3	(32-37)	1315	32.9	(30-36)	1999	33.5	(31-36)		
Greece	1460	60.5	(58-63)	1209	59.4	(57-62)	1088	62.3	(59-65)	1185	61.9	(59-65)		

** The symbols > and < indicate significant increasing and decreasing trends, respectively. The symbol # indicates a significant trend in the overall data, which was not observed when only data from laboratories consistently reporting for all four years were included.*

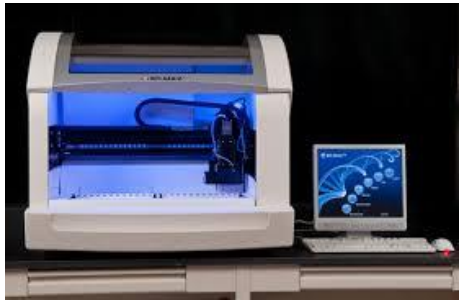
Current technical developments

New technologies in microbiology- microbiology is changing

POCT PCR/walkaway System, DNA prep, ampification & detection e.g.MRSA/Influenza etc…



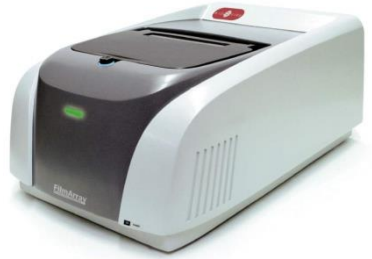
POCT PCR/walkaway System, DNA prep, ampification & detection e.g.MRSA etc….



Isothermal amplification

Multiplex detection, in part nucleic acid based, Detection from blood culture

Identification from blood culture and Antimicrobial susceptibility testing in



New diagnostic techniques

System	Diagnostics from blood culture	MRE Detection	MIC ?	Lab developed test possible ?	Multiplex Assays?
Roche Liat	-	MRSA	-	-	duplex
GenMark DX	+	mecA, mecC, vanA, vanB, CTX-M, IMP, KPC, NDM, OXA, VIM (Blood culture panel)	-	-	+
Biofire Film Array	+	MRSA, carbapenemase Van A & B	-	-	+
Cepheid - GeneXpert	+	+	-	- (miRNA?)	-
BD-Max		MRSA, carbapenemase, Van A & B	-	+	-
Seegene	+	-	-	?	+
Accelerate	+	+	+	-	pheno

Increasing demand
(e.g. ABS or hygiene)

New diagnostic techniques
New assay formats

Clinical benefit?

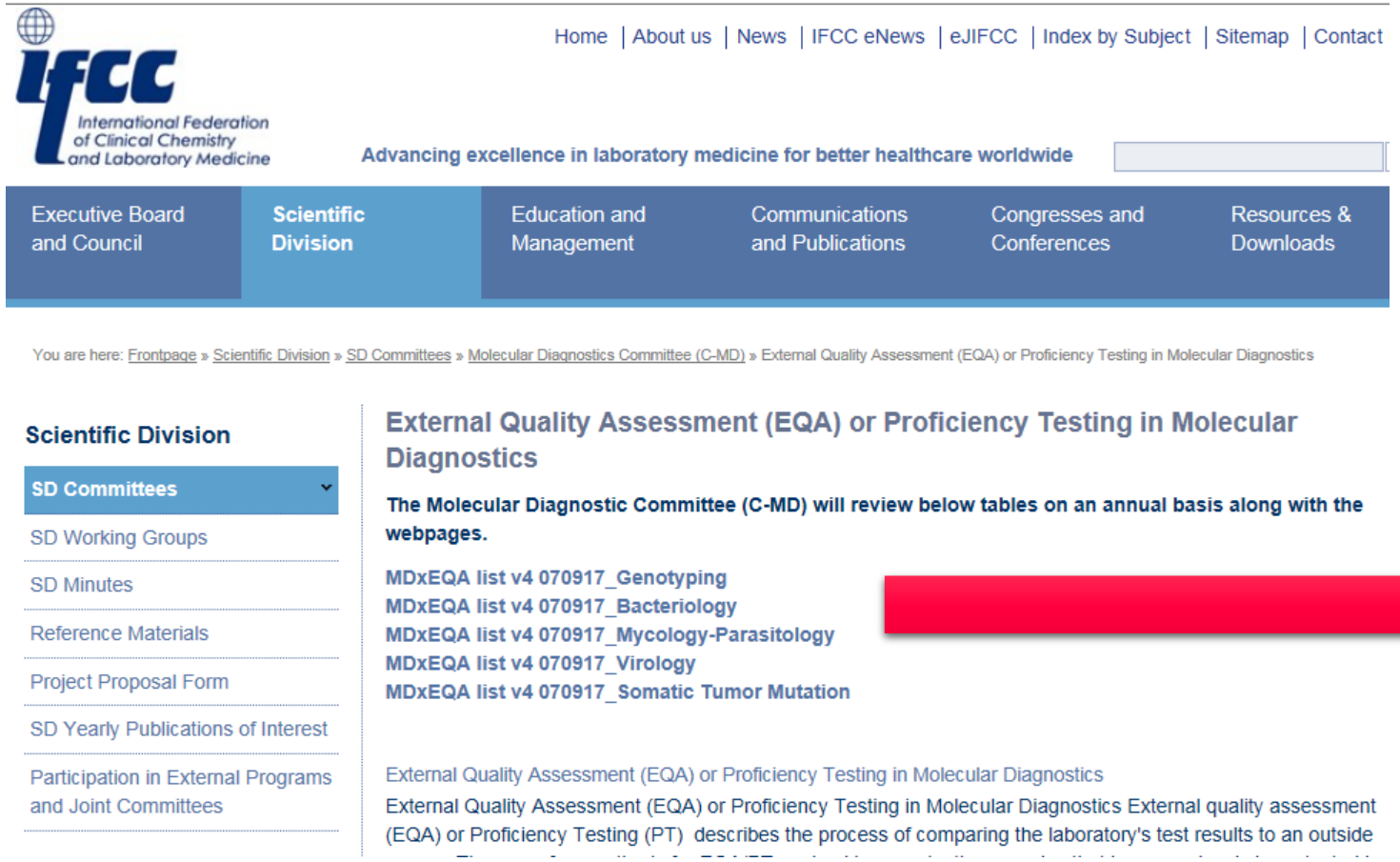
New EQA formats?
New reference materials?

Economic impact in health care?

EQAs in infectious disease

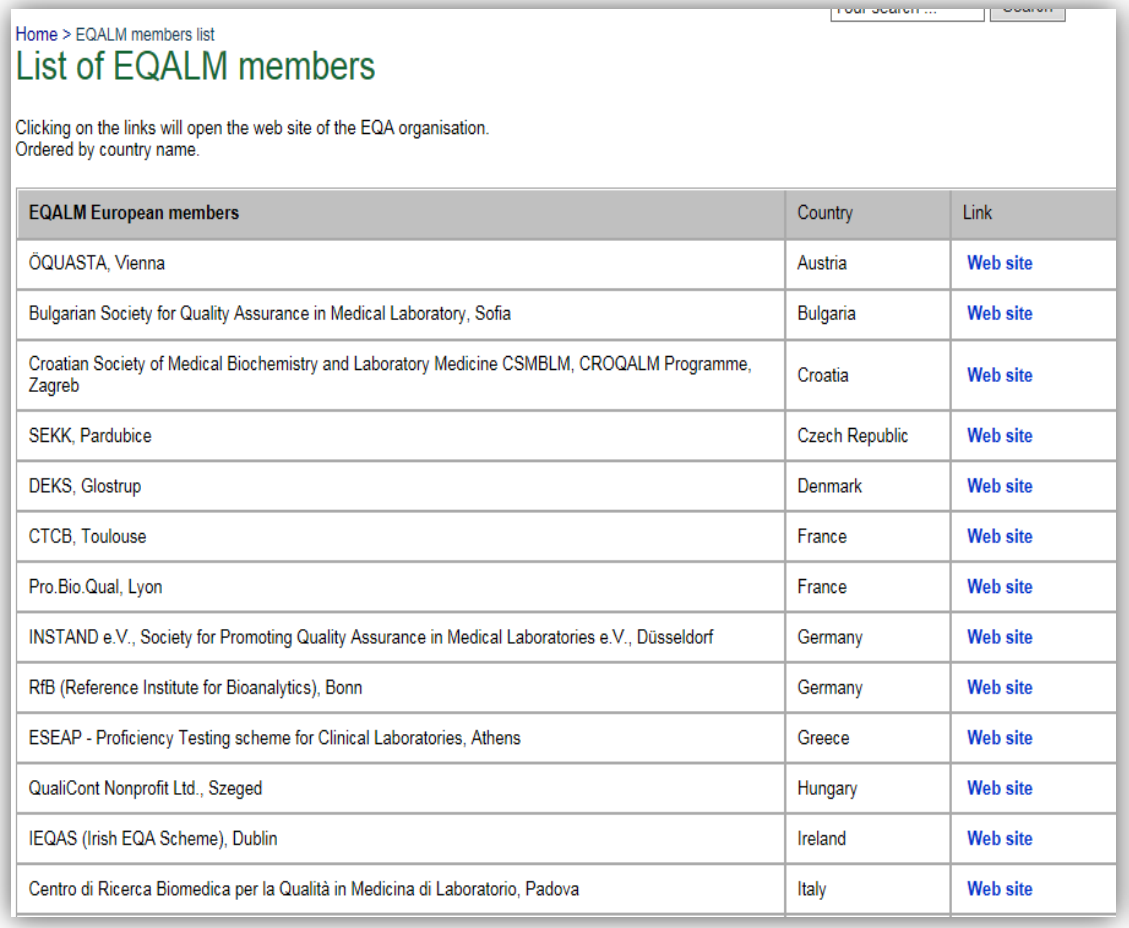
brief summary

C-MD of the IFCC addressing MDX EQA in microbiology



Parameter	EQA Provider
Bacillus anthracis	INSTAND
Bordetella pertussis	CAP INSTAND RfB QCMD
Bordetella parapertussis	CAP QCMD
Borrelia burgdorferi	INSTAND RfB QCMD
Campylobacter	CAP
Carbapenemase genes	INSTAND RfB QCMD
C. pneumoniae	INSTAND QCMD CAP RfB
C. trachomatis	Labquality CTCB Equalis QCMD INSTAND CAP RfB
Clostridium difficile	Labquality QCMD INSTAND CAP RfB
Coxiella burnetii	INSTAND RfB

EQUALM



Home > EQALM members list

List of EQALM members

Clicking on the links will open the web site of the EQA organisation.
Ordered by country name.

EQALM European members	Country	Link
ÖQUASTA, Vienna	Austria	Web site
Bulgarian Society for Quality Assurance in Medical Laboratory, Sofia	Bulgaria	Web site
Croatian Society of Medical Biochemistry and Laboratory Medicine CSMBLM, CROQALM Programme, Zagreb	Croatia	Web site
SEKK, Pardubice	Czech Republic	Web site
DEKS, Glostrup	Denmark	Web site
CTCB, Toulouse	France	Web site
Pro.Bio.Qual, Lyon	France	Web site
INSTAND e.V., Society for Promoting Quality Assurance in Medical Laboratories e.V., Düsseldorf	Germany	Web site
RfB (Reference Institute for Bioanalytics), Bonn	Germany	Web site
ESEAP - Proficiency Testing scheme for Clinical Laboratories, Athens	Greece	Web site
QualiCont Nonprofit Ltd., Szeged	Hungary	Web site
IEQAS (Irish EQA Scheme), Dublin	Ireland	Web site
Centro di Ricerca Biomedica per la Qualità in Medicina di Laboratorio, Padova	Italy	Web site

EQALM

European Organisation For External Quality Assurance Providers in Laboratory Medicine

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Your search ... Search

Home > Working groups >Microbiology

Working group on Microbiology

Convener: Michael Allen Noble Send Email

Current Documents

Clicking each of these links will open a PDF file; Adobe Reader needed.

- Report on the EQALM Microbiology Working Group survey (01.2016)

Former Microbiology Working Group Reports

EQALM symposium 2013:
- Report of the WG (Bucharest, 2013)
- Results of questionnaire on not mainstream EQA (Excel file)
- Externalized internal quality control in Bacteriology : susceptibility of bacteria, S. Albarède, CTCB, France
- Malaria RDT Photo survey (IPH 2013), K. Vernelen, IPH, Belgium

EQALM symposium 2012:
- Report of the Working Group on Microbiology (Herlev, 2012)
- Questionnaire on sample production microbiology 2012
- Sample production microbiology IPH, K. Vernelen, IPH, Belgium
- How CTCB produces samples, S. Albarède, CTCB, France

EQALM symposium 2011:
- Homogeneity 2011
- Overview homogeneity bacteriology 2011
- Overview homogeneity parasitology 2011

EQALM symposium 2010:
- Report of the WG (Lisbon, 2010)
- Production Microbiology Samples of the IPH

EQA formats

Shipment 2-4x/year

Tests for infection diagnostics by molecular genetic testing methods:

- EQA samples are provided as lyophilized DNA of 500 ng

Detection of antigens or antibodies

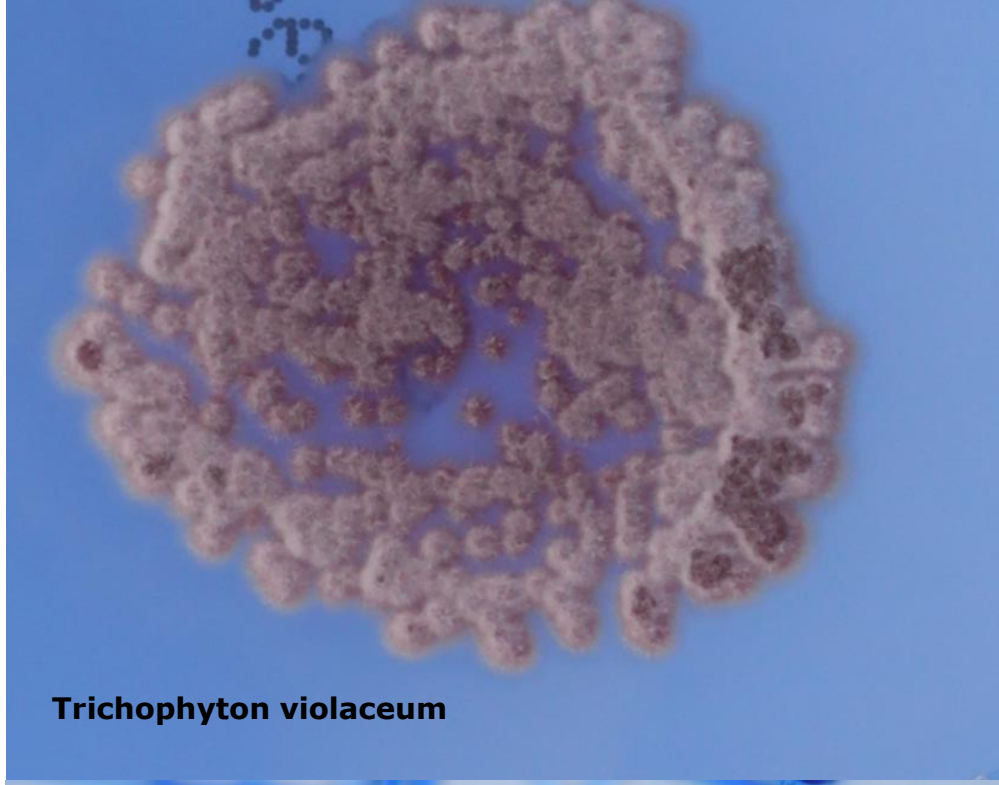
- EQA samples are provided as an aliquot of plasmas or sera

Cultural detection methods

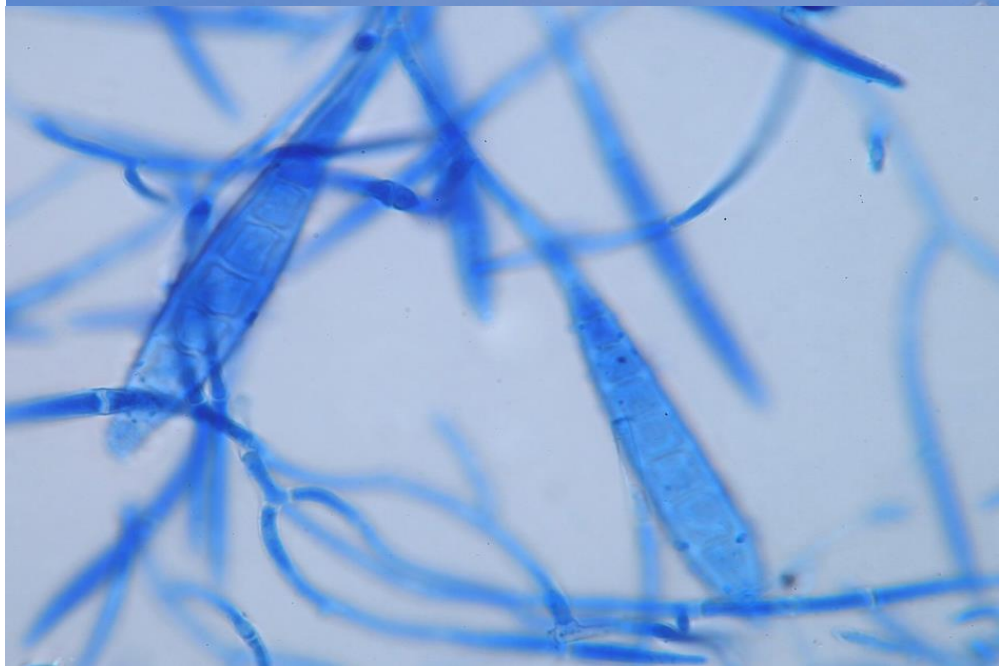
- Shipping of smears/eswabs

Virtual EQAs

- Online proficiency testing / interpretation of high reslution microscopic images in a given clinical context



Trichophyton violaceum



Providing EQA samples – antigen & antibody

- Identification & recruitment of donors (e.g. Malaria/Treponema pall.)
- Alternatively: Aliquoting of quarantine plasmas
- Characterization of the aliquots by screening test procedures
- Expansion of the existing plasma biobank (>200 plasmas)
- Characterization / cross-validation with further tests

Multiparametric EQA sample for NAT

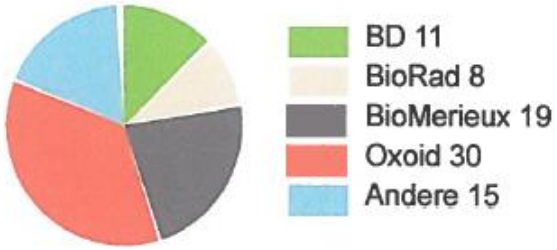
Analyt / Probe	1	2	3	4
Bordetella pertussis	neg pos 15	neg 10 pos 5	neg 15 pos	neg pos 15
Chlamydia pneumoniae	neg 12 pos	neg 1 pos 11	neg 11 pos 1	neg 12 pos
Mycoplasma pneumoniae	neg pos 15	neg 14 pos 1	neg pos 15	neg 15 pos
Coxiella burnetii	neg pos	neg pos	neg pos	neg pos
Francisella tularensis	neg pos	neg pos	neg pos	neg pos
Legionella pneumophila	neg pos 12	neg 12 pos	neg 11 pos 1	neg 11 pos 1
MRSA	neg 26 pos	neg 26 pos	neg pos 27	neg pos 27
Chlamydia trachomatis	neg 21 pos	neg pos 22	neg 20 pos 1	neg 21 pos 1

MRSA Screening

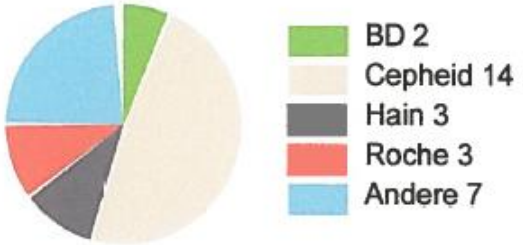
molecular **culture**

Screening-Ergebnisse	pos	neg	fragl	Zusatzresultat	Nachweismethoden
Probe 1	0	112	0	Koagulase-Neg. Staphylokokken(11) S. epidermidis(4) S. aureus(1)	
Probe 2	111	1	0	S. aureus(28)	
Probe 3	112	0	0	S. aureus(28)	
Probe 4	7	104	1	S. epidermidis(11) S. aureus(2) Koagulase-Neg. Staphylokokken(2)	

Verteilung Selektiv-Agar ✓

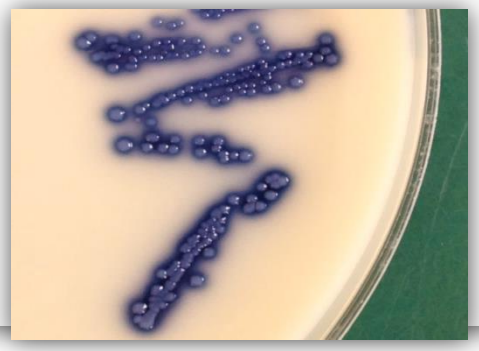


Verteilung MDX Hersteller ✓

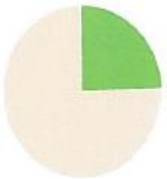


EQA: VRE Screening

Keime	Mol. Genetik
Probe 1	
29 E. faecium	23 - 8 vanA
Probe 2	
1 E. faecium 6 E.faecium, kein VRE 23 kein VRE	30 - 1 vanA
Probe 3	
1 E. faecalis 6 E.faecalis, kein VRE 23 kein VRE	30 - 1 vanA
Probe 4	
29 E. faecium	23 - 8 vanA

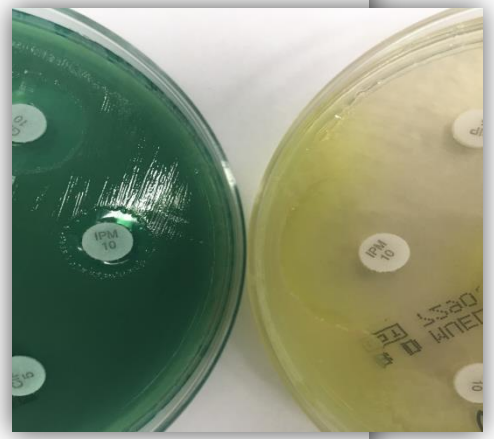


Mol. Gen. Methoden



EQA: Screening MDR gram neg./CRE

Keime	Klassifikation	Carbapenemase-Gene
Probe 1		
22 E. cloacae 11 -	38 negativ	
Probe 2		
38 E. coli 1 -	39 3MRGN	
Probe 3		
38 K. pneumoniae 1 -	39 4MRGN	17 KPC 1 OXA-48
Probe 4		
28 E. coli 11 -	36 negativ	

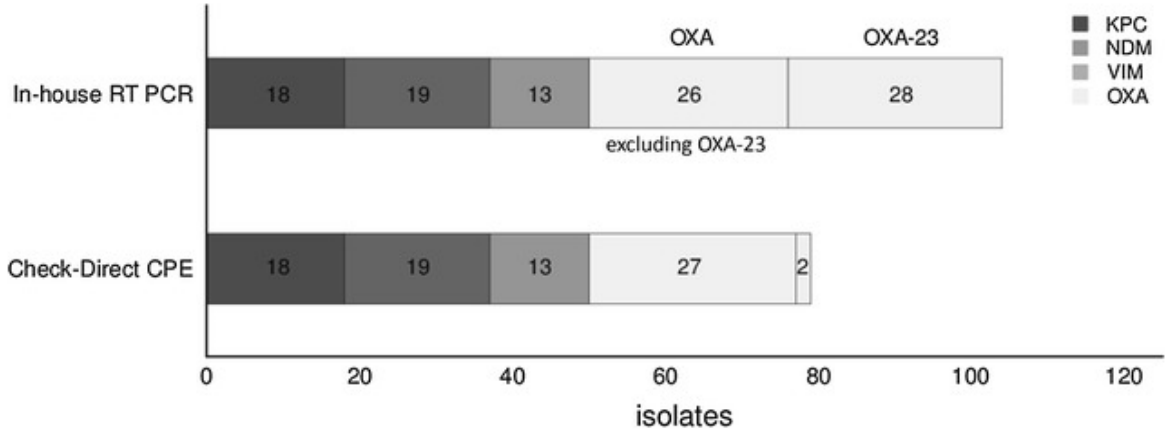


Differences in resistance patterns/genes

PMC full text: Ann Clin Microbiol Antimicrob. 2017; 16: 48.
Published online 2017 Jul 10. doi: 10.1186/s12941-017-0223-z

Ann Clin Microbiol Antimicrob

Fig. 1



Comparison of the in-house PCR assay and the Check-Direct CPE assay for the identification of carbapenem

Examples of the most frequently encountered carbapenemases [67]

Acronym	Name or type	First isolated
KPC	*Klebsiella pneumoniae* carbapenemase	1996
VIM	Verona integron-encoded metallo-β-lactamase	1997
OXA-48	OXA-type carbapenemase	2001
NDM	New Delhi metallo-β-lactamase	2008

Smiljanic et al. Ann Clin Microbiol Antimicrob (2017) 16:48
DOI 10.1186/s12941-017-0223-z

Annals of Clinical Microbiology and Antimicrobials

RESEARCH Open Access

Comparison of in-house and commercial real time-PCR based carbapenemase gene detection methods in *Enterobacteriaceae* and non-fermenting gram-negative bacterial isolates

M. Smiljanic[1], M. Kaase[2,3], P. Ahmad-Nejad[1] and B. Ghebremedhin[1*]

Abstract

Background: Carbapenemase-producing gram-negative bacteria are increasing globally and have been associated with outbreaks in hospital settings. Thus, the accurate detection of these bacteria in infections is mandatory for administering the adequate therapy and infection control measures. This study aimed to establish and evaluate a multiplex real-time PCR assay for the simultaneous detection of carbapenemase gene variants in gram-negative rods and to compare the performance with a commercial RT-PCR assay (Check-Direct CPE).

Methods: 116 carbapenem-resistant *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates were genotyped for carbapenemase genes by PCR and sequencing. The defined isolates were used for the validation of the in-house RT-PCR by use of designed primer pairs and probes.

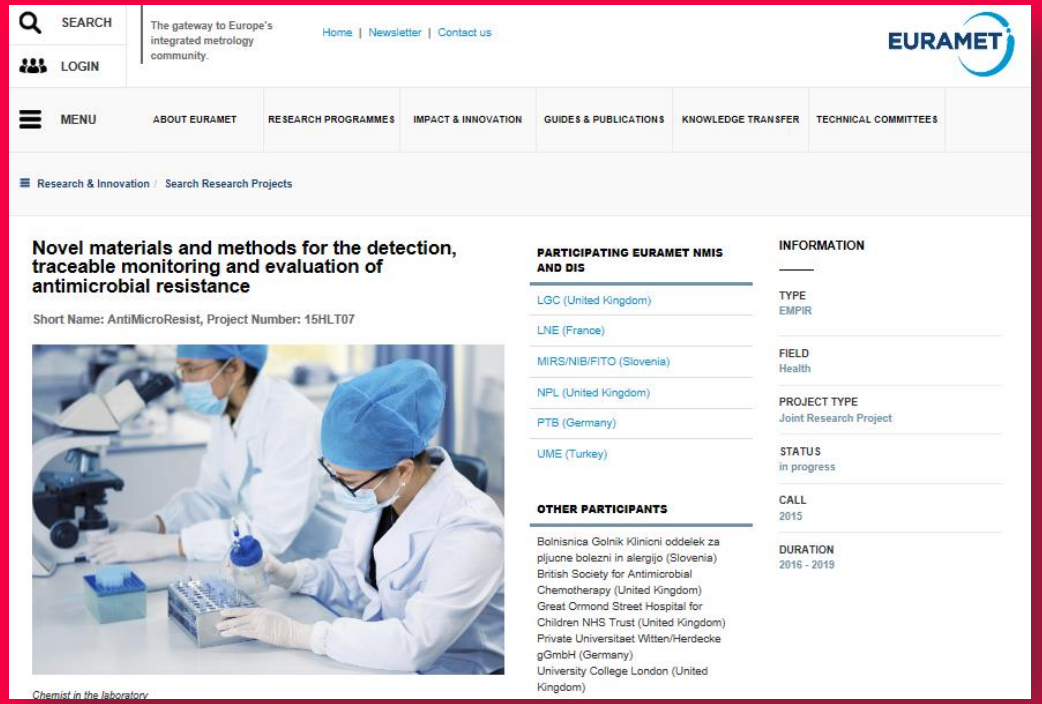
Results: Among the carbapenem-resistant isolates the genes bla_{KPC}, bla_{VIM}, bla_{NDM} or bla_{OXA} were detected. Both RT-PCR assays detected all bla_{KPC}, bla_{VIM} and bla_{NDM} in the isolates. The in-house RT-PCR detected 53 of 67 (79.0%) whereas the commercial assay detected only 29 (43.3%) of the OXA genes. The in-house sufficiently distinguished the most prevalent OXA types (23-like and 48-like) in the melting curve analysis and direct detection of the genes from positive blood culture vials.

Conclusion: The Check-Direct CPE and the in-house RT-PCR assay detected the carbapenem resistance from solid

EU Project AntiMicroResist - AMR

WP1

https://www.euramet.org/research-innovation/search-research-projects/details/?eurametCtcp_project_show%5Bproject%5D=1421



Task 1.3

EMPIR EURAMET
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Development of candidate reference materials for molecular measurements directly from clinical samples associated with AMR assessment

The aim of this task is to develop and assess the performance of candidate reference materials needed to support routine and novel molecular screening of bacterial AMR

Approach to generate a candidate reference material for MRSA detection

Task 1.3: Development of candidate reference materials for molecular measurements directly from clinical samples associated with AMR assessment

Activity	Activity description	Partners
A1.3.1	Following discussion with GOSH, UCG, UCL and UWH, and with input from A1.1.2, NIB and LGC will develop and optimise specific PCR methods for the subsequent quantification of the candidate reference materials. Where e used to quantify the DNA materials used in Task 4.2. A range of at least 3 extraction methods (e.g. CTAB, Qiagen DNeasy an ermine the most suitable upstream method for preparing the DNA for molecular analysis.	NIB, LGC, GOSH, UCG, UCL, UWH
A1.3.2	GOSH will provide at least 10 patient samples that have been previously monitored for infectious diseases. These samples may include, but will not be limited to, DNA extracts from blood. risons in A1.3.5. GOSH will also prepare and quantify at least 10 clinical eria could be prepared and quantified as well.	GOSH, UWH, UCL, UCG
A1.3.3	LGC and UWH will prepare batches of ~200 units of each of the candidate reference materials (at least two materials for current and novel molecular screening) selected in A1.3.2 and will characterise the homogeneity and stability of the material using dPCR or qPCR. Purity will also be evaluated using next generation sequencing. Depending on the findings of A1.1.2 materials are likely to contain either whole bacteria or nucleic acid extracts. Materials will be assesse nded uncertainties (following a method based on that developed in EM his will differ from the application in WP2 as it will not consider SI trace ticipated that the uncertainties will be <50 %. Values will be compared to conventional culture based counting methods performed by GOSH in A1.3.2.	LGC, UWH, NIB, PTB, TUBITAK
A1.3.4	LGC will distribute all of the materials produced in A1 methods used in A1.3.5 and A1.3.6, to the clinical laboratories at GOSH, UWH, UCL, UCG and to at least another fi UWH who organise EQA schemes.	LGC, UWH, UCL, GOSH, UCG
A1.3.5	UWH will coordinate an inter laboratory trial which will include GOSH, UCL, UCG and at least another five clinical laboratories. The materials from A1.3.4 will be assessed using clinical laboratory methods (e.g. PCR). The results will then be used to determine how they perform as a candidate reference material and they will be used to assess laboratory performance. Clinical laborator routine identification test as well as, where possible qPCR, in order to measure the quantity of the candidate reference material. Cli the comparison in order to enable an assessment of the commutability of the candidate reference material. Data will be analysed with the assistance of LGC.	UWH, UCL, GOSH, UCG, LGC
A1.3.6	LGC and NIB will m a pilot comparison of the materials by high throughput qP rm. Agreement between the laboratories will be assessed. ment of AMR. Where appropriate these approaches will also be used in conjunction with Task 4.3.	LGC, NIB
A1.3.7	LGC will write a report describing the performance of the sociated uncertainties, in order to recommend the most appropriate format (i.e. whole bacteria or nucleic acid extracts). LGC w TUBITAK, UCL, GOSH and UCG for comments before finalising the report.	LGC, UWH, NIB, PTB, TUBITAK, UCL, GOSH, UCG

Design assays

Obtain clinical samples/culture isolates

Prepare and characterise EQA materials

Distribute materials

Perform EQA

Also consider POC/high throughput methods

Report findings

Material Plan

Sample No.	Level	Organism
1	High	MRSA
2	Low	MRSA
3	High Low	MSSA and MRSE
4	Low	MRSE

MRSA - Methicillin-resistant Staphylococcus aureus
MSSA - Methicillin-susceptible Staphylococcus aureus
MRSE - Methicillin-resistant Staphylococcus epidermidis

Work package 1

Generation of potential reference material

Testing (different methods/different laboratories)

Pilot EQA → in field testing

Culture methods
Digital PCR
„conventional“ PCR/POCT PCR

(LGC/PTB/IML/…)

Material to be provided for ten laboratories

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Summary

- There is an increasing demand to detect MDR bacteria
- Microbiology changes; many new techniques/methods offer the possibility to analyse patients´samples immediately
- New reference materials and new EQAs should be designed to face the clinical need / to fit for different diagnostic procedures

Thank you for your attention

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