Updates from the National Antimicrobial Committee (NAC)

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Content

- Fosfomycin susceptibility testing
- Trimethoprim-sulfamethoxazole and *Stenotrophomonas maltophilia*
- Survey satisfaction NAC panel 2016

Fosfomycin: introduction

- *Streptomyces fradiae* (1969, Spain)
- Low molecular weight
- Water soluble: good tissue diffusion
- Drug formulation:

- Fosfo-tromethamine/trometamol (soluble salt) **oral use**
- **Fosfo-calcium oral use (less good bioavailability)**
- Fosfo-disodium salt **intravenous use**

- Dosage:

Usual dosage IV: 3x4g/d
High dosage IV: 3x8g/d

Fosfomycin: spectrum of activity

Broad spectrum activity, bactericidal (MBC≈ MIC)

Gram +

- ***Staphylococcus aureus*** (MSSA, MRSA)
- (Streptococci, Enterococci)

Gram -

- ***Escherichia coli***
- *Proteus mirabilis*
- (*K. pneumoniae*, *Enterobacter* spp.)
- *Pseudomonas aeruginosa*

Fosfomycin: resistance mechanisms

- **Chromosomal:** Mutations in uptake system genes
  (transporters: GlpT, UhpT)

- **Plasmidic:** Inactivating enzymes
  - Metallo enzymes: FosA, FosB, FosX
    →**transferable**
  - Kinases: FomA, FomB

- **Reduced affinity** to target (MurA) (natural resistance)
  Staphylococcus saprophyticus, (S. capitis), Morganella morganii, Acinetobacter spp, *Burkholderia cepacia, Stenotrophomonas maltophilia, Anaerobes*

! **No cross resistance** with other classes of antibiotics
! **Resistant mutants less virulent** (fitness cost)
! **High frequency of mutations** during treatment

Fosfomycin AST (1)

CLSI (Enterobacteriaceae):
S ≤ 64 µg/ml; I= 128 µg/ml; R ≥ 256 µg/ml

EUCAST (Enterobacteriaceae / Staphylococci): Only urinary *E. coli*
S ≤ 32 µg/ml; R > 32 µg/ml

No breakpoints (*Pseudomonas aeruginosa*)
EUCAST: potential use of ECOFF (MIC =128 mg/l) for use in combination therapy

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	-	-		-	-	**1.** Colistin MIC determination should be performed with broth microdilution. Quality control must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).
Colistin[1]	2	2		Note[A]	Note[A]	
Daptomycin	-	-		-	-	**2.** Agar dilution is the reference method for fosfomycin. MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturers' instructions for commercial systems. Infections caused by wild type isolates (**ECOFF: MIC 128 mg/L**; corresponding zone diameter 12 mm using the disk potency and reading instructions for *E. coli*) have been **treated with fosfomycin in combination with other agents.**
Fosfomycin iv[2]	-	-		-	-	
Fosfomycin oral[2]	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	**A.** Use an MIC method (broth microdilution only).
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole	-	-		-	-	

Fosfomycin AST (2)

EUCAST

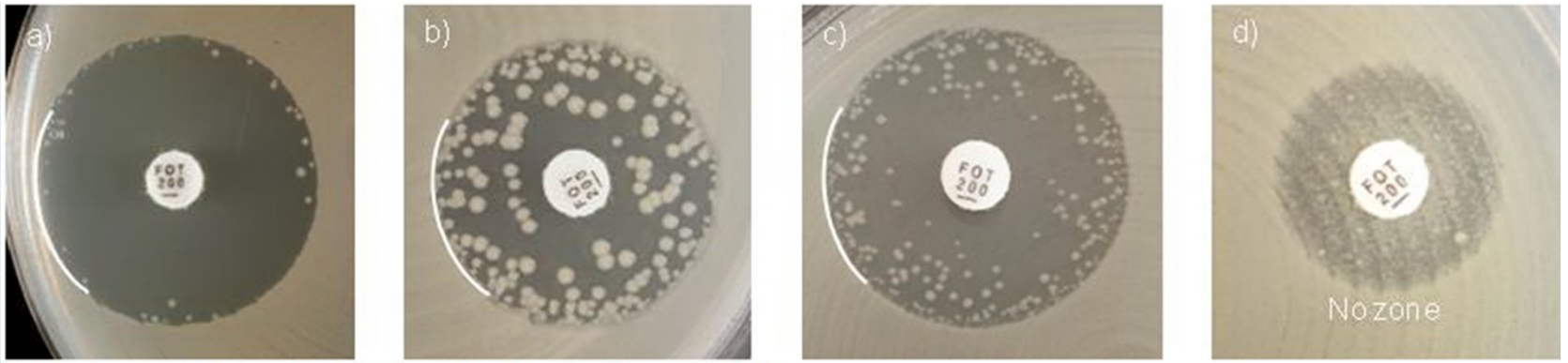
- Reference method: **Agar dilution**

- Alternatives?
- Disk diffusion (only *E. coli, other Enterobacteriaceae use MIC method*)
- Broth microdilution
- Automated AST systems

- ALL techniques:+ **glucose-6-phosphate (G6P) (**25 mg/L in medium)

Fosfomycin AST: disk diffusion

- Disk diffusion



Examples of inhibition zones for *Escherichia coli* with fosfomycin.
a-c) Ignore all colonies and read the outer zone edge.
d) Record as no inhibition zone.

1. Use a MIC method (broth microdilution as alternative for agar dilution?)
2. Fosfomycin 200 µg disks must contain 50 µg glucose-6-phosphate.
3. Zone diameter breakpoints apply to *E. coli* only. For other Enterobacteriaceae, use an MIC method.
4. Ignore isolated colonies within the inhibition zone (see pictures).

Fosfomycin AST: BMD (1)

broth **m**icro**d**ilution

- Broth microdilution

Thermo SCIENTIFIC

2 March 2016

TECHNICAL BULLETIN

Reading Fosfomycin Susceptibility Test Results

A study undertaken by Thermo Fisher Scientific has shown that MICs read in this way are very reproducible. 98.3% of MICs fell within +/- one doubling dilution with 341 MICs performed using frozen reference plates with G-6-P compared to 97.6% with 165 MICs using Sensititre dried plates. Differences were, however, occasionally noted in the MIC range obtained when a strain was tested on

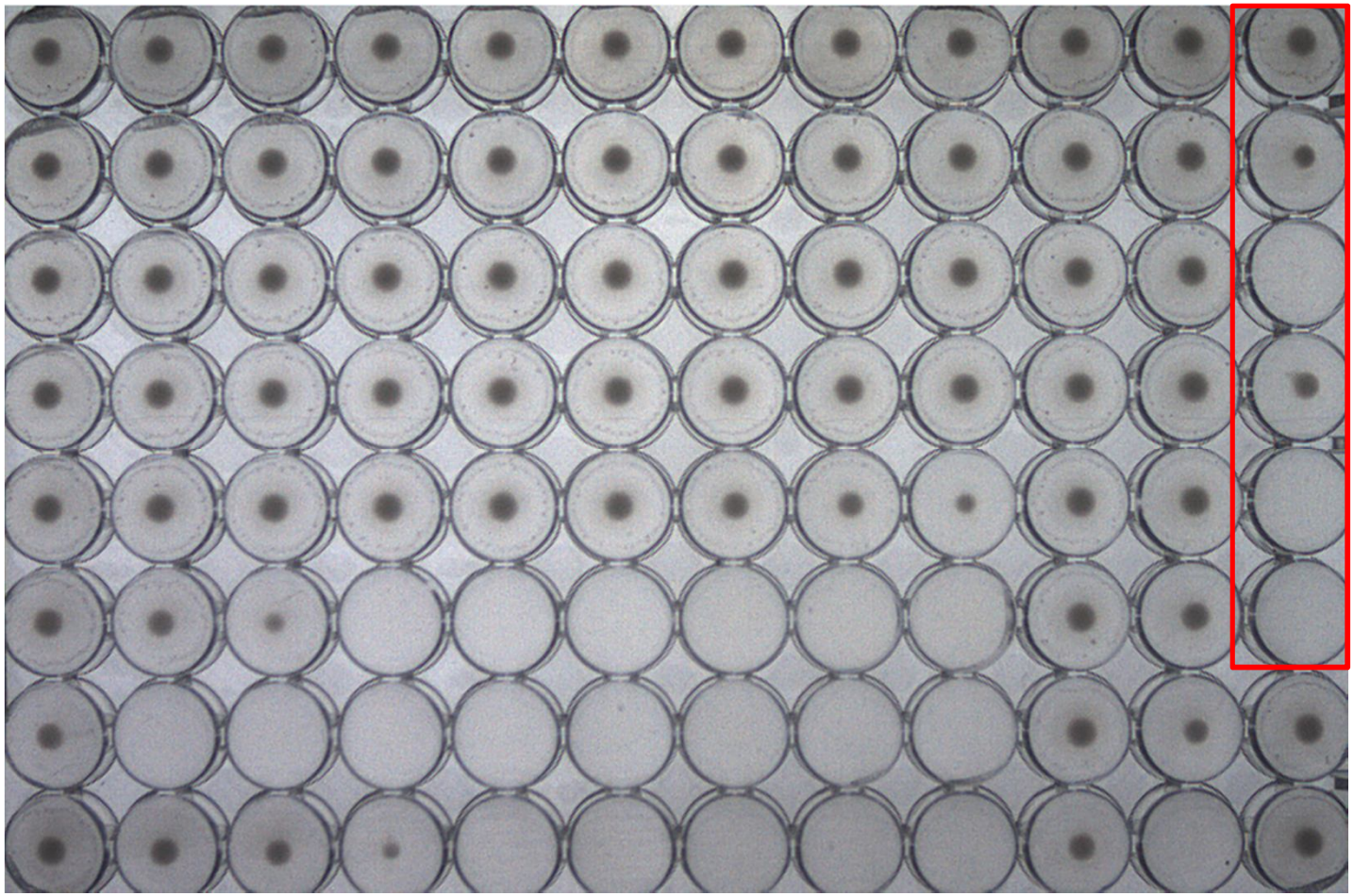
Presence of « outliers » (skip wells): <-> growth of resistant mutants

They nevertheless present difficulties when reading susceptibility tests. Such growth is usually comparable to the positive growth control. The incidence of MICs showing outlier growth is shown in Table 1. Outlier growth is defined as growth in a well bounded by well(s) containing no growth. The MIC is defined as the lowest concentration of antibiotic that inhibits growth. Such growth should be disregarded when reading results with fosfomycin.

Fosfomycin AST: BMD(2)

broth **m**icro**d**ilution

K. pneumoniae



Fosfomycin:
use of automated AST systems

FDA/ISO cleared?
Quality validation studies?

Yes: *E. coli*, low resistance rates (2-5%)
Yes: *S. aureus*, low resistance rates (2-5%)

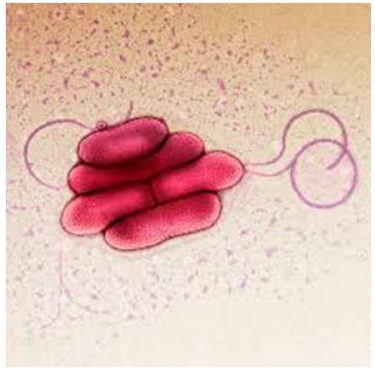
No: *K. pneumoniae*, high resistance rates (20-30%)
No: *Pseudomonas aeruginosa*, no breakpoints

Trimethoprim-sulfamethoxazole and *Stenotrophomonas maltophilia*

Specific reading instructions

BVIKM-SBIMC symposium 29th March 2018
Dr Bénédicte Lissoir
National Antibiotic Committee

Stenotrophomonas maltophilia



- Aerobic non fermentative Gram-negative bacterium
- Ubiquitous environmental microorganism
- Often associated with colonization
- Opportunistic pathogen (immunocompromised patients and patients with cystic fibrosis)

Intrinsic resistance – *S.maltophilia*

Rule no.	Organisms	Ampicillin	Amoxicillin-Clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Ticarcillin-clavulanic acid	Piperacillin	Piperacillin-tazobactam	Cefazolin, Cefalothin Cefalexin, Cefadroxil	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Fosfomycin	Tetracyclines	Tigecycline	Polymyxin B/Colistin
2.1	*Acinetobacter baumannii*, *Acinetobacter pittii*, *Acinetobacter nosocomialis* and *Acinetobacter calcoaceticus* complex	R	R	Note[1]					R	R	R			R	R						R	R	R[2]	Note[2]	
2.2	*Achromobacter xylosoxydans*	R							R	R	R				R											
2.3	*Burkholderia cepacia* complex[3]	R	R	R	R	R	R	R	R	R	R			R	R			R	R	R[4]	R	R			R
2.4	*Elizabethkingia meningoseptica*	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R									R
2.5	*Ochrobactrum anthropi*	R	R	R	R	R	R	R	R	R	R	R	R	R	R											
2.6	*Pseudomonas aeruginosa*	R	R	R					R	R	R				R				R	Note[5]	R		R	R		
2.7	*Stenotrophomonas maltophilia*	R	R	R	R		R	R	R	R	R			R	R	R	R			R[4]	R[6]	R	R[7]			

R = resistant

[1] *Acinetobacter baumannii* may appear to be susceptible to ampicillin-sulbactam due to activity of sulbactam with this species.

[2] *Acinetobacter* is intrinsically resistant to tetracycline and doxycycline but not to minocycline and tigecycline.

[3] *Burkholderia cepacia* complex includes different species. Some strains may appear susceptible to some β-lactams *in vitro* but they are clinically resistant and are shown as R in the table.

[4] *Burkholderia cepacia* and *Stenotrophomonas maltophilia* are intrinsically resistant to all aminoglycosides. Intrinsic resistance is attributed to poor permeability and putative efflux. In addition, most *Stenotrophomonas maltophilia* produce the AAC(6')Iz enzyme.

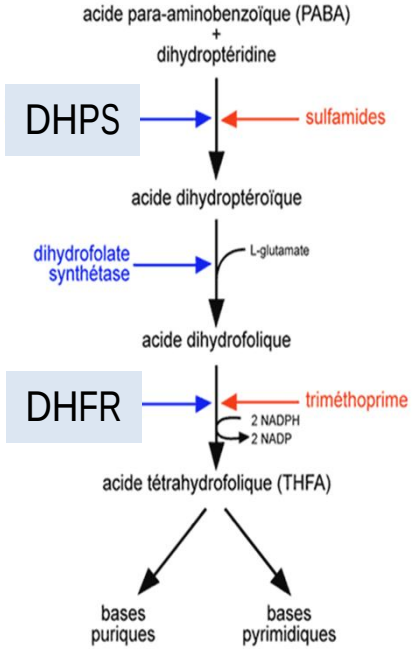
[5] *Pseudomonas aeruginosa* is intrinsically resistant to kanamycin and neomycin due to low level APH(3')-IIb activity.

[6] *Stenotrophomonas maltophilia* typically is susceptible to trimethoprim-sulfamethoxazole, but resistant to trimethoprim alone.

[7] *Stenotrophomonas maltophilia* is intrinsically resistant to tetracycline but not to doxycycline, minocycline and tigecycline.

www.eucast.

Trimethoprim-sulfamethoxazole



- Sulfonamides / trimethoprim (diamino pyridine)
- Bactericidal (Gram + & Gram -)
- Blocks 2 consecutive steps in the bio-synthesis of nucleic acids and proteins of bacteria
- Uses : *Pneumocystis jiroveci* pneumonia, bacterial meningitis (not specific enough!?), chronic bronchitis, Shigellosis, traveler's diarrhea, urinary tract infections, *Stenotrophomonas* treatment, nocardiosis ...

– Wide variety of resistance mechanisms : alteration of permeability, overexpression of efflux pump systems, modification of target enzymes (DHPS <-> sulfonamide; DHFR <-> trimethoprim),...

Trimethoprim - sulfamethoxazole

- Natural resistance to trimethoprim – sulfamethoxazole :
  - *Pseudomonas aeruginosa*
  - *Anaerobic microorganisms*
  - *Campylobacter sp*
  - *Helicobacter pylori*
  - *Mycobacteria*
  - *Mycoplasma, Ureaplasma*
  - *Neisseria sp*
  - *Borrelia sp*
  - *Treponema sp.*

EUCAST - AST recommendations for *S. maltophilia*

A	B	C	D	E	F	G
Stenotrophomonas maltophilia						**EUCAST Clinical Breakpoint Tables v. 8.0, valid from 2018-01-01**

Trimethoprim-sulfamethoxazole is the only agent for which EUCAST breakpoints are currently available. For further information, see guidance document on www.eucast.org.

MIC determination (broth microdilution according to ISO standard 20776-1)
Medium: Mueller-Hinton broth
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1ºC, 18±2h
Reading: For trimethoprim-sulfamethoxazole, the MIC should be read at the lowest concentration that inhibits approximately 80% of growth as compared with the growth control well.
Quality control: *Escherichia coli* ATCC 25922

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges from the back of the plate against a dark background illuminated with reflected light (see below for specific instructions).
Quality control: *Escherichia coli* ATCC 25922

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Trimethoprim-sulfamethoxazole[1,2]	4	4	1.25-23.75	16[A]	16[A]	**1.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration. **2.** Breakpoints are based on high dose therapy, see table of dosages. ~~at least 0.24 g trimethoprim and 1.2 g sulfamethoxazole administered together twice daily~~. **A.** Isolates showing any sign of inhibition zone ≥ 16 mm should be reported susceptible and growth within the inhibition zone should be ignored. The density of growth within the zone may vary from a fine haze to substantial growth (**see pictures below**).

CA-SFM and CLSI AST recommendations for *S. maltophilia*

- Antibiotics other than cotrimoxazole to be tested:

French committee (CA-SFM, 2017 v2.0)

Divers	Concentrations critiques (mg/L) S ≤	R >	Charge du disque (µg)	Diamètres critiques (mm) S ≥	R <	Notes Chiffres arabes pour les commentaires portant sur les concentrations critiques (CMI) Lettres pour les commentaires portant sur les diamètres critiques d'inhibition
Ticarcilline - acide clavulanique	16	64		-	-	Pour évaluer la sensibilité, la concentration d'acide clavulanique est fixée à 2 mg/L.
Ceftazidime	8	16		-	-	
Minocycline	4	8	30	19	15	
Lévofloxacine	2	4	5	17	12	

CLSI 2017, M100-S27

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm) S	I	R	Interpretive Categories and MIC Breakpoints (µg/mL) S	I	R
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS								
O	Ticarcillin-clavulanate	–	–	–	–	≤16/2	32/2–64/2	≥128/2
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
B	Ceftazidime	–	–	–	–	≤8	16	≥32
TETRACYCLINES								
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16
FLUOROQUINOLONES								
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8

- Ticarcillin / Clavulanic acid
- Ceftazidime
- Levofloxacine
- Minocycline (tigecycline ?)

Disc diffusion criteria (CA-SFM,CLSI): only for levofloxacin, minocycline

Stenotrophomonas maltophilia and trimethoprim-sulfamethoxazole

- An isolate showing any sign of inhibition zone ≥ the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones.

Ignore growth and read an inhibition zone if any zone edge can be seen.
= Susceptible if zone diameter ≥ 16 mm

Growth up to the disk and no sign of inhibition zone = Resistant

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Quick reminder

The NAC developed a challenge panel:

including both Gram-negatives and Gram-positives

Covering most important resistance mechanisms

For the implementation of EUCAST breakpoints or EUCAST methods in the clinical laboratories

Panel send out to requesting laboratories as from April 2016

Response rate

Send out: 24/07/17

Deadline: 11/09/17

Reminder: 21/09/17

Final response rate january 18: 44/71 (62%)

7 labs: not yet used the panel; storage for future use

Appreciation & use

Median score : 8 (4 - 10)

Use:

- 17 labs : 1 occasion
- 14 labs : > 1 occasion

Purpose

Validation of existing method & guidelines: 13

Implementation of new guidelines: 5

Implementation of new method & validation m/g: 5

Implementation of new method: 4

Implementation of new m/g & validation m/g: 4

Implementation of new method & new guidelines: 2

Other: 4

Storage

Original panel RT:	1
Original panel refrigerator:	3
Original panel freezer:	13
Aliquots freezer:	25
Original panel & aliquots freezer:	1

Problems growth/contamination (35 labs)

21 labs: none

Problem strains:

- *S. pneumoniae* (strain #50): 9
- *S. pneumoniae* (strain #48): 7
- *S. pneumoniae* (strain #52): 6
- *S. agalactiae* (strain #35): 3
- *S. aureus* (strain #53): 3
- *P. aeruginosa* (str #3) – *K. pneumoniae* (str #15): 1

Problems identification (35 labs)

29 labs: none

Problem strains:

- *E. coli* (str #7) identified as *E. aerogenes* (2 labs)
- *P. aeruginosa* (str #4) identified as *E. aerogenes*
- *S. pneumoniae* (str #48) identified as *S. mitis/pneumoniae*
- *S. aureus* (str #53) identified as *S. capitis/warneri*

Problems antibiogram (35 labs)

28 labs: none

Str 4# (P. aeruginosa): many discordances, needs to be retested

Str#L2 (E. coli): levo I i.o. S

Str#38 (S. agalactiae): clinda R i.o. S (Vitek expert system)

Str #11 E. aerogenes genta R i.o. S & str #38 (S. agalactiae): clinda R i.o. S

Str #11 E. aerogenes (cefur R) & str 24 C. koseri (cefur S) but EUCAST 2015: no breakpoints

Suggestions for future panels

38 labs interested – 33 interested in collaborating

Colistin-resistant Enterobactericeae:	25 labs
Linezolid -resistant S. aureus:	18 labs
Linezolid-resistant Enterococcus:	17 labs
Tigecycline-resistant Acinetobacter:	10 labs
Other combinations germ/AB:	8 labs

Lessons learned

Information about storage

Inoculate certain strains upon reception (e.g. pneumococci)

The future

Then NAC considers a new/updated panel

Members of the bureau of the National Antimicrobial Committee (NAC)

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- Bénédicte Lissoir (GHdC – Charleroi)
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