

Critically Appraised Topic

**Referral strategy for duplicate
isolates: a first evidence-based
approach**

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Repeat isolations: quid?

- When a micro-organism is **repeatedly isolated** from the **same patient** from a **similar body site** within a **short period of time** (< 7 days) , this secondary isolation might be identical to the first identification if **morphologically** consistent and **enzymatically** confirmed.

→ Criteria for repeated isolation

1. Same patient
2. Same sample type
3. Current micro-organism morphologically consistent with previous, enzymatical tests (indol, oxidase, ...) confirmative
4. Time interval < 7 days

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Questions

1. (Dis)agreements referral procedure:
acceptable?
 - ID
 - AST
2. Effect of antibiotic pressure?

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Literature

- **No evidence-based studies** published concerning repeat isolates

- **Guidelines**
 - Garcia et al: *Clinical Microbiology Procedures Handbook*.
 - CLSI M100-S20 (2010)

- guidelines based on **expert opinions**

Clinical Microbiology Procedures Handbook (Garcia et al)

6. Use the following guidelines to report repeated isolation of the same organism.
 - a. Do not perform full identification and susceptibility testing on microorganisms, if the patient has had a positive culture from the same source within the last (x) days with what apparently is the same organisms(s) and full identification and susceptibility testing were done on the previous isolate(s).
☑ **NOTE:** For determination of (x) days, a good general rule is to repeat identifications every 7 days, if the morphology is the same. An exception would be for nonhemolytic staphylococci, all of which should be checked with a coagulase test. Policies on how often to repeat antimicrobial susceptibility testing (AST) vary and should be based on evaluation of local AST results and therapies used to treat disease. General guidelines include 7 days for oxacillin-susceptible staphylococci and most gram-negative rods, 4 days for *P. aeruginosa* and selected other gram-negative rods, and 30 days for vancomycin-resistant enterococci. If extended-spectrum beta-lactamases are present locally, additional susceptibility surveillance may be indicated.
 - b. Ensure that the current organism is morphologically consistent with the previous isolate(s) prior to reporting them as identical. Perform minimal procedures to confirm the identification (oxidase, indole, catalase, etc.), if possible.
 - c. Report the genus and species identification.
 - d. When referring identification to prior identification, indicate in the report that the identification is “presumptive” followed by the following comment after the organism name: “Refer to culture from [date] for complete identification [and susceptibility testing].” Use caution so that referred cultures are not referred to referred cultures.
 - e. If susceptibility testing was performed (e.g., not sure it is the same, previous positive overlooked, etc.), record these results but do not report them, unless they differ from the prior result. Such reporting can distort the data in the antibiogram produced by the laboratory for epidemiological surveys.

Garcia Referral Criteria

- Presumptive identical identification
- *Morphologically consistent*
 - *Confirmation tests: indol, oxidase,...*
- No full ID & AST required if previous isolation was analyzed < 7 days ago
- **General rule:**
Repeat ID & AST when > 7 days
CAVE: non-hemolytic staphylococci:
→ always perform coagulase testing
- *P. aeruginosa*: repeat when > 4 days
- ESBL => additional AST surveillance (?)
- Refer to previous culture
for ID & AB



Guidelines CLSI M100-S20 (2010)

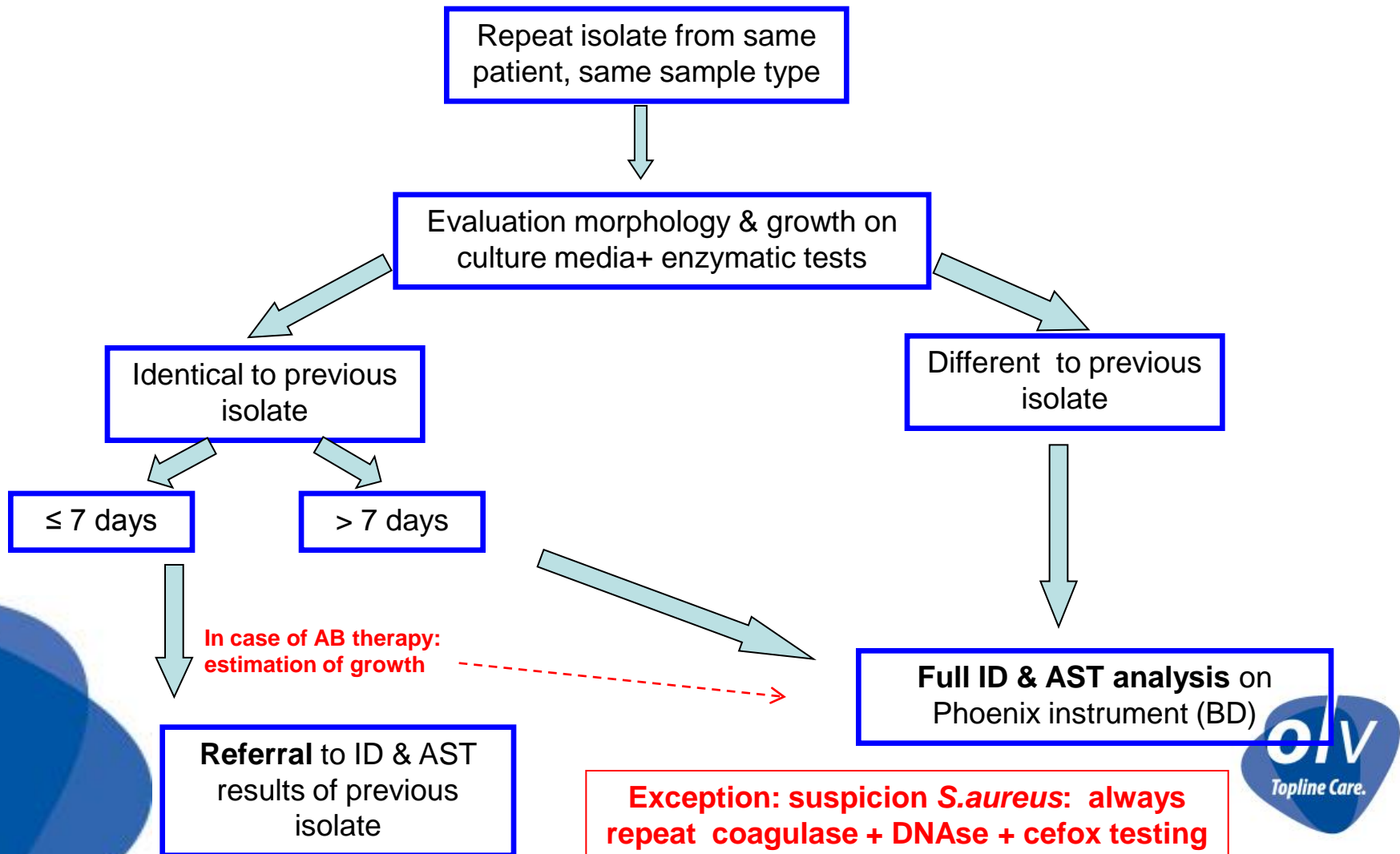
Development of Resistance and Testing of Repeat Isolates

Isolates that are initially susceptible may become intermediate or resistant after initiation of therapy. Therefore, subsequent isolates of the same species from a similar body site should be tested in order to detect resistance that may have developed. This can occur within as little as three to four days and has been noted most frequently in *Enterobacter*, *Citrobacter*, and *Serratia* spp. with third-generation cephalosporins; in *P. aeruginosa* with all antimicrobial agents; and in staphylococci with quinolones. For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.

In certain circumstances, testing of subsequent isolates to detect resistance that may have developed might be warranted earlier than within three to four days. The decision to do so requires knowledge of the specific situation and the severity of the patient's condition (eg, an isolate of *Enterobacter cloacae* from a blood culture on a premature infant). Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with the medical staff.

- After initiation of therapy: CAVE resistance
- Repeat testing dependent on specific situation
- Laboratory guidelines should be determined internally

Workflow Repeat isolates OLVZ Aalst



Prospective study repeat isolates

Workflow validation

Materials & Methods:

- 15/02/2011-15/05/2011
- All gram-negative repeat isolates retested for ID & AST

Repeat ID & AST (Phoenix analysis)

→ **Evaluation referral procedure by comparison:**

→ **ID: agreement primary & secondary isolation**

→ **AST: categorical results (S, I, R)**

- Discordant ID: both isolates retested
- Sample types: urinary + other sample sources
- New EUCAST compliant Phoenix panels



Criteria Interpretation results

Reference : ID & AST results from primary isolate

Identification: (dis)agreement

- FDA : min. 90 % agreement for method acceptance
- OLV Aalst: $\geq 95\%$

Susceptibility results : categorical (dis)agreements

- Minor errors – Major errors (ME) – Very major errors (VME)
- FDA:
 - Major errors $\leq 3\%$
 - Very Major errors $\leq 1.5\%$



Categorical (dis)agreement AST results

Category	Interpretation	Categorical result
Agreement	Identical results	Identical
Very Major Error (VME)	Repeat test S Primary isolate tested R	S instead of R
Major Error (ME)	Repeat test R Primary isolate tested S	R instead of S
Minor Error (MinE)	Repeat test R/S Primary isolate tested I or vice versa	I instead of R or S or vice versa

Measured ME = reporting S instead of R → VME in referral (& vice versa)

1st : S S
repeat: R → **X** → S instead of R is reported (= referral)
 \downarrow \downarrow
Measured as major error Reported as very major error



Which AST to evaluate?

Table 1: Intrinsic resistance (R) in Enterobacteriaceae

Enterobacteriaceae are also intrinsically resistant to penicillin G, glycopeptides, fusidic acid, macrolides (with some exceptions¹), lincosamides, streptogramins, rifampicin, daptomycin and linezolid.

Rule no.	Organisms	Ampicillin	Amoxicillin-clavulanate	Ticarcillin	Piperacillin	Cefazolin	Cefoxitin	Cefamandole	Cefuroxime	Aminoglycosides	Tetracyclines/tigecycline	Polymyxin B/Colistin	Nitrofurantoin
1.1	<i>Citrobacter koseri</i>	R		R	R								
1.2	<i>Citrobacter freundii</i>	R	R			R	R						
1.3	<i>Enterobacter cloacae</i>	R	R			R	R						
1.4	<i>Enterobacter aerogenes</i>	R	R			R	R						
1.5	<i>Escherichia hermannii</i>	R		R	R								
1.6	<i>Hafnia alvei</i>	R	R			R	R						
1.7	<i>Klebsiella</i> spp.	R		R	R								
1.8	<i>Morganella morganii</i>	R	R			R			R		R	R	R
1.9	<i>Proteus mirabilis</i>										R	R	R
1.10	<i>Proteus vulgaris</i>	R				R		R	R		R	R	R
1.11	<i>Proteus penneri</i>	R				R		R	R		R	R	R
1.12	<i>Providencia rettgeri</i>	R	R			R				R ²		R	R
1.13	<i>Providencia stuartii</i>	R	R			R				R ²		R	R
1.14	<i>Serratia marcescens</i>	R	R			R		R	R	Note ³		R	
1.15	<i>Yersinia enterocolitica</i>	R	R	R	R	R	R	R					
1.16	<i>Yersinia pseudotuberculosis</i>											R	

¹ Azithromycin is effective in vivo for the treatment of typhoid fever and erythromycin may be used to treat travellers' diarrhoea.

² All *Providencia* spp. produce a chromosomal AAC(2')-Ia enzyme. *Providencia* spp. should be considered resistant to all aminoglycosides except amikacin and streptomycin. Some isolates express the enzyme poorly and can appear susceptible to netilmicin *in vitro*, but should be reported as resistant as mutation can result in overproduction of this enzyme.

³ All *Serratia marcescens* produce a chromosomal AAC(6')-Ic enzyme that may affect moderate the activity of all aminoglycosides except streptomycin and gentamicin.

Antibiotics included for urinary isolates

KIEM	AM PICIL LIN	AM OXC LAV	CEF URO X	CEF TRI AX	CEF TAZ	CEF EPI M	PIP TAZ O	TEM OCI LL	ME RON EM	AMI KAC IN	CIP ROX IN	NIT ROF UR	TMP SXL	FOS FOM	AZT REO NA M	# AB
<i>E. coli</i>																15
<i>Proteus mirabilis</i>																14
<i>Klebsiella sp.</i>																14
<i>E. aerogenes</i>																13
<i>Citrobacter koseri</i>																14
<i>P. aeruginosa</i>																10

Antibiotics included for isolates from other sample sources

	AM PICI LLIN	AMO XYC LAV	CEF URO XIM	CEF TRIA X	CEF TAZI DIM	CEF EPI ME	PIP TAZ O	MER OPE NEM	AMI KAC IN	CIP ROX IN	AZT REO NAM	TMP/ SXL	Aantal antibiotica vergeleken per kiem
<i>E. coli</i>													11
<i>P.mirabilis</i>													11
<i>P. vulgaris</i>													10
<i>Klebsiella sp.</i>													10
<i>Enterobacter sp.</i>													9
<i>M. morgani</i>													8
<i>H. alvei</i>													9
<i>S. marcescens</i>													8
<i>P. stuartii</i>													9
<i>P. aeruginosa</i>													7
<i>Acinetobacter sp.</i>													7
<i>S. maltophilia</i>													1

Results ID

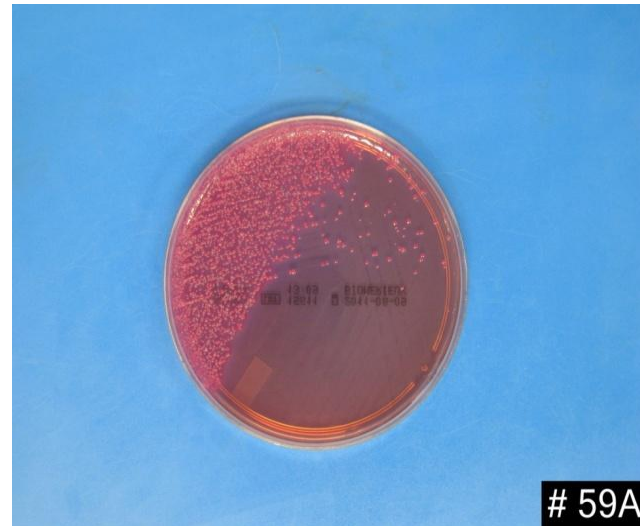
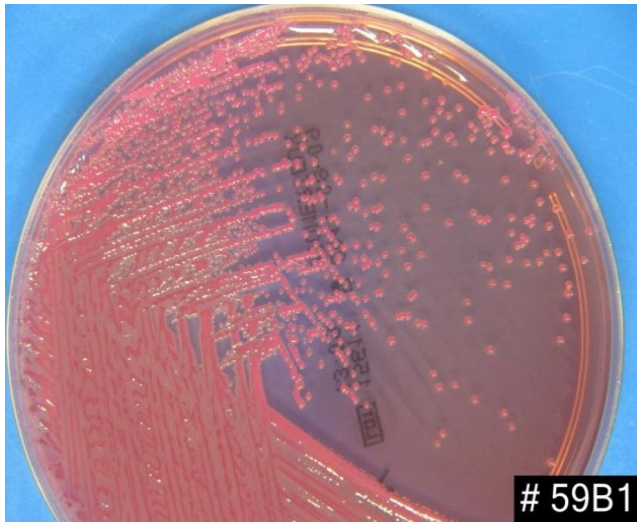
Micro-organisms	Urinary	Other
<i>E. coli</i>	20	24
<i>Proteus species</i>	8	3
<i>Klebsiella species</i>	8	3
<i>Enterobacter species</i>	2	9
<i>Citrobacter species</i>	1	0
<i>M. morganii</i>	0	2
<i>H. alvei</i>	0	1
<i>S. marcescens</i>	0	1
<i>P. retgerii</i>	0	1
<i>P. aeruginosa</i>	4	9
<i>S. maltophilia</i>	0	3
<i>Acinetobacter species</i>	0	1
TOTAL	43	57

Results ID: Agreements

- 3 ID conflicts (3%) → Mismatch ID were (both) retested
- **Agreement : 97%**
- Disagreements:

Date	ID	Therapy	Cause ?
31/03	<i>K. oxytoca</i>	None	Excess time frame (9 days)
09/04	<i>C. farmeri</i>	None	
23/03	<i>S. maltophilia</i>	None	Oxidase test? Morphology?
28/03	<i>P. aeruginosa</i>	None	
09/05	<i>E. coli</i>	Pip/Tazo	Morphology? Inevitable?
12/05	<i>E. cloacae</i>	Pip/Tazo	

Disagreements identification (1)



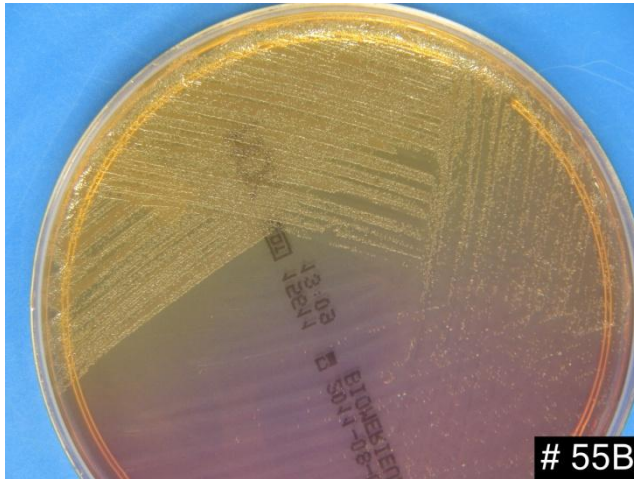
1st isolation:

K. oxytoca

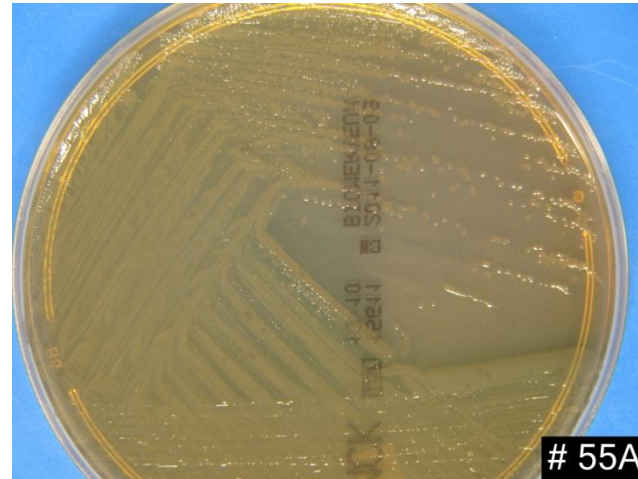
→ Excess referral time frame

Repeat: ***E. coli***

Disagreements identification (2)



1st isolation: *S. maltophilia*



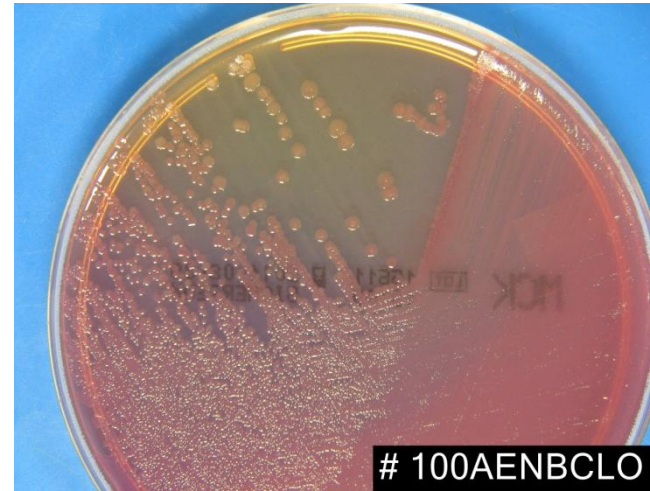
Repeat: *P. aeruginosa*

- Oxidase testing? Not performed
- Morphology? *P. aeruginosa* : <18h incubation: “too young to evaluate” (Garcia)
- **Always perform oxidase testing** on lactose-negative gram-negative colonies!

Disagreements identification (3)



1st isolation: *E. coli*



Repeat: *E. cloacae*

→ Morphology?

→ Inevitable?

Results AST evaluation

- Secondary isolate : repeat test ID & AST
→ Comparison results with previous AST results
- Evaluation of referral procedure ~ categorical agreement

1	Monster	Afgenom	Object	Material	Organis	Brdl.	AM PI	AMO XCLAV	CEF URO	CEF TRIAX	CEF TA	CEF EPI	PIP TAZ	TEM OC	MER OPEN	AMI KA	CIP RO	FUR AD	TMP SXL	FOS FOM	AGREE	AB
2	3853862	20/04/11	1048993	Urine ESO	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S		
3	3857544	25/04/11	1048993	Urine ESO	Kle.pneu	80	R	S	S	S	S	S	S	S	S	S	S	S	S	S	OK	Ø
4	3861260	28/04/11	1128456	Urine SP8	Kle.pneu	m100	R	R	R	R	R	R	R	S	S	S	S	R	S	S		
5	3864514	02/05/11	1128456	Urine VER	Kle.pneu	m100	R	R	R	R	R	R	R	S	S	S	S	S	S	S	1 VME	Ø
6	3837387	06/04/11	1130531	Urine MID	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S		
7	3839225	07/04/11	1130531	Urine MID	Kle.pneu	40	R	S	S	S	S	S	S	S	S	S	S	S	S	S	OK	Ø
8	3850889	18/04/11	1160339	Urine VER	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S		
9	3851195	19/04/11	1160339	Urine VER	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S	OK	Ø
10	3798430	03/03/11	1205546	Urine VER	Kle.pneu	m100	R	R	R	R	R	R	R	S	S	S	S	S	S	S		
11	3801915	07/03/11	1205546	Urine VER	Kle.pneu	m100	R	R	R	R	R	R	R	S	S	S	S	S	S	S	OK	Ø
12	3840515	08/04/11	1624149	Urine MID	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	R	S	S		
13	3840784	08/04/11	1624149	Urine MID	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	R	S	S	OK	GI
14	3889289	23/05/11	2053584	Urine ZAK	Kle.pneu	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S		
15	3890627	24/05/11	2053584	Urine ZAK	Kle.pneu	50	R	S	S	S	S	S	S	S	S	S	S	S	S	S	OK	Ø
16	3781446	17/02/11	2062641	Urine VER	Kle.oxyt	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S		
17	3785133	21/02/11	2062641	Urine VER	Kle.oxyt	m100	R	S	S	S	S	S	S	S	S	S	S	S	S	S	OK	Ø

Global study results AST evaluation

- n = 96
- 4 isolates were excluded from AST evaluation
 - => 3 ID mismatch + 1 discordant sample source

[illegible]

Disagreement per antibiotic

	AMPICIL LIN	AMOX CLAV	CEFURO XIME	CEFTRIA XONE	CEFTA ZIDIME	CEFE PIME	PIP TAZO	TEMOCI LLIN	AZTREO NAM	MEROPE NEM	AMIKACI NE	CIPROFL OXACIN	TMP SXL	FOSFO MYCINE	NITROFU RANTO IN	TOT AL
AGREEMENT	50/53	59/64	74/77	79/80	93/94	92/94	90/94	38/39	92 /9 4	94/94	92/94	90/94	45/45	43/43	33/35	1064/ 10 94 97.27
MINOR ERROR	2	4	0	1	0	1	2	0	2	0	1	2	0	0	0	15 1.37
MAJOR ERROR	1	1	3	0	1	1	1	0	0	0	1	2	0	0	1	12 1.09
VERY MAJOR ERROR	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	3 0.27
TOTAAL	53	64	77	80	94	94	94	39	94	94	94	94	45	43	35	1 094

Disagreement per antibiotic


	AMPICIL LIN	AMOX CLAV	CEFURO XIME	CEFTRIA XONE	CEFTA ZIDIME	CEFE PIME	PIP TAZO	TEMOCI LLIN	AZTREO NAM	MEROPE NEM	AMIKACI NE	CIPROFL OXACIN	TMP SXL	FOSFO MYCINE	NITROFU RANTO IN	TOT AL
AGREEMENT	50/53	59/64	74/77	79/80	93/94	92/94	90/94	38/39	92 /9 4	94/94	92/94	90/94	45/45	43/43	33/35	1064/ 10 94 97.
MINOR ERROR	2	4	0	1	0	1	2	0	2	0	1	2	0	0	0	15 1.37
MAJOR ERROR	1	1	3	0	1	1	1	0	0	0	1	2	0	0	1	12 1.09
VERY MAJOR ERROR	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	3 0.27
TOTAAL	53	64	77	80	94	94	94	39	94	94	94	94	45	43	35	1 094

- β -lactam AB: amoxicillin, AMC, PTZ (55,5% of all disagreements)
- Fluoroquinolones: Ciprofloxacin (15% of all disagreements)

Influence of antibiotic therapy

	None	Antibiotic therapy	No info	TOTAL
n =	47	42	7	96
AB	569	426	99	1094
Minor Err	0.52%	1.87%	4.04%	1.37%
Major Err	0.52%	1.41%	3.03%	1.09%
Very Major	0,17%	0,23%	1,01%	0.27%

Results AST in referral procedure



3861260	28/04/11	1128456	Urine SPS	Kle.pneu m100	R	R	R	R	R	R	R	S	S	S	S	R	S	S		
4 01		(M)		moni.																
3864514	02/05/11	1128456	Urine VER	Kle.pneu m100	R	R	R	R	R	R	R	S	S	S	S	S	S	S	1 VME	Ø
5 01		(M)		moni.																

Very major error on Phoenix analysis = Major Error when evaluating referral procedure:

- When S instead of (previous) R is measured: R is reported instead of S
→ R instead of S is reported for the second isolate = **Major Error for referral**
- ***Measured very major errors are major errors and vice versa in referral procedure***

Referral procedure error rate:

- **Minor Errors: 1.37%**
- **Major Errors: 0.27 %**
- **Very Major Errors: 1.09%**

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Conclusion

Results Evaluation

- ID referral : 97% agreement $\geq 95\%$ ACCEPTED
- AST referral :
 - **Minor Errors: 1.37%** ACCEPTED
 - **Major Errors: 0.27 %** $\leq 3\%$ ACCEPTED
 - **Very Major Errors: 1.09%** $\leq 1.5\%$ ACCEPTED
- Referral procedure applicable when **all criteria** are met
- Antibiotic therapy has **influence** on AST categorical agreement, yet acceptable
- Microbiologist evaluates need for full ID & AST : clinically indicated & antibiotic usage, resistance suspected,...

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To do's

1. Presentation study results at BD Benelux Users Day meeting 08/12/2011 (Lindner hotel , Antwerpen)
2. Revision procedure when Maldi-TOF implemented.
3. Multicenter evaluation of referral procedure: Bilulu-project



"That's all Folks!"