

Comments on Proficiency Testing Survey B9 Microbiology 2016-1

Specimen A: Urinary tract infection

Requirement: Potentially pathogenic bacteria (genus + species) + resistance test

Enterococcus faecium isolated in this urinary tract infection demonstrated vancomycin resistance (VRE). The molecular analysis confirmed the presence of the *vanA* gene. The MIC for vancomycin was > 256 mg/L and 8 mg/L for teicoplanin. In addition, a high level of resistance against gentamicin was determined, but not against streptomycin. High resistance of enterococci against gentamicin should at least be tested with systemic infections.

Going forward, please do not list any antibiotics tested for diagnostic reasons (for example clindamycin). According to EUCAST, nitrofurantoin and fosfomycin are only recommended for *enterococcus faecalis* in cases with common urinary tract infections. For *Enterococcus*, EUCAST does not specifically list resistance tests for doxycycline, minocycline, and tetracycline, whereas CLSI does. We have accepted the data for these antibiotics, but will no longer evaluate them next time; therefore the full number of points will not be achieved for an insufficient quantity of tested antibiotics. We have assessed indication of ceftazidim as "nonsensical" because cephalosporines are a priori ineffective against enterococci.

Please note that as of 2016, EUCAST has also defined the enterococci inhibition zones for ciprofloxacin and levofloxacin; unfortunately this strain was also resistant to guinolones.

These VRE are problematic from a hospital hygiene perspective. If glycopeptides (vancomycin or teicoplanin) have to be used empirically against *Staphylococcus aureus* in a hospital due to high MRSA frequency, the selection pressure for VRE is especially high. If therapy is necessary in the case of a VRE with additional resistances, it is imperative to contact an infectious disease specialist. Unfortunately, daptomycin and linezolid are being administered more frequently. Tigecycline was likewise sensitive with our strain.

Identification	Number
Enterococcus faecium	55
Enterococcus gallinarum	5
Enterococcus species	1
Gram-positive cocci	1

Specimen B: Urinary tract infection

Requirement: Potentially pathogenic bacteria (genus + species) + resistance test

Escherichia coli contained in this specimen were easily identified by all participants.

This *E. coli* has a molecular biology-verified class-D carbapenemase of the OXA-48 type and an extended-spectrum beta-lactamase (ESBL) of the CTX-M type.

It should be noted that in the case of ESBL, an adjustment of the cephalosporines from "sensitive" to "resistant" is no longer provided according to EUCAST (according to CLSI as well). The cephalosporines are reported as they are read. The same also applies for carbapenems with an existing carbapenemase. We accepted all results for the carbapenems. The marker for determining OXA-48 is temocillin, which then appears "resistant" (< 11 mm according to EUCAST). It is crucial that the resistance mechanism is communicated so that the patient is isolated and, if necessary, the specialist can ignore the EUCAST rule in the event of critical infections.

Going forward, please also list a carbapenem on the result sheet when carbapenemase is suspected and likewise a cephalosporin when ESBL is suspected.

We accepted all values for norfloxacin. Nalidixic acid (a precursor of quinolones, which served as point mutation markers) was resistant with our strain. According to CLSI, the quinolones will still be adjusted if nalidixic acid is resistant. EUCAST no longer applies this rule due to cases in which norfloxacin was resistant and nalidixic acid was sensitive.

EUCAST rule 12.9 http://www.eucast.org/expert rules and intrinsic resistance (p. 151, table 12) states that tobramycin must be noted resistant if tobramycin is intermediary, gentamicin is resistant and amikacin is sensitive. This was the case with our strain. This time, resistant and intermediary for tobramycin gave the full number of points.

Determining ESBL or carbapenemase is essential because it indicates hospital hygiene measures and serves to monitor infections.

IdentificationNumberEscherichia coli62

Specimen C: Prosthesis-associated infection

Requirement: Potentially pathogenic bacteria (genus + species)

Propionibacterium acnes was easily identified by most participants in this specimen from a prosthesis-associated infection. This is a gram-positive, coryneform rod, which is better anaerobically cultivated than aerobically. In addition to identification by means of MALDITOF, it could also be easily identified through the positive catalase reaction, CAMP factor, nitrite, and indole. During glucose fermentation, the metabolic fatty acid propionic acid is formed.

P. acnes is a part of the skin flora but also a known pathogen in prosthetic valve "endocarditis." In over 90%, the blood culture isolates of *P. acnes* are contaminations, but with foreign body material every isolate of P. acnes should be more closely assessed.

Identification	Number
Propionibacterium acnes	59
Propionibacterium species	1
Corynebacterium species	1
Gram-positive cocci	1

Specimen D: Ulcer

Requirement: Potentially pathogenic bacteria (genus + species)

Pseudomonas stutzeri, a gram-negative non-fermenting rod, was easily identifiable for most participants. Vitek, API 20 NE, and MALDI-TOF gave no problems with identification. Our strain was oxidase-positive, TSI group 4, C390 resistant and showed positive growth at 42°C. Flat, crumbly colonies appeared on sheep blood agar after 24 hours of incubation with CO².

P. stutzeri is a ubiquitous bacteria.

Identification	Number
Pseudomonas stutzeri	59
Pseudomonas oryzihabitans	1
Pseudomonas species	1
Gram-negative rods	1

Specimen E: Pneumonia

Requirement: Potentially pathogenic bacteria (genus + species) and resistance test

Haemophilus influenzae is a facultative anaerobe bacteria. It needs X and V factors, which are contained in boiled blood agar, in order to grown well. It grows on sheep blood agar with the help of *Staphylococcus aureus* (which produces V factor) in the satellite phenomenon. Our strain was easily identifiable by API NH or MALDI-TOF.

H. influenzae is a typical pathogen in upper respiratory tract infections. There, it can cause inflammatory diseases, such as epiglottitis, bronchitis or pneumonia.

Our strain was beta-lactamase-negative and resistant to ampicillin, amoxicillin-clavulanic acid, cefepime, and ceftriaxone, and was sensitive for ciprofloxacin, meropenem, and tetracycline. This is a resistance mediated by modified PBP and not by beta-lactamase.

It was important to observe the concentration of the antibiotic discs. This was very clear with amoxicillin-clavulanic acid. The analysis showed that all participants who had reported amoxicillin-clavulanic acid as "sensitive" had used the AMC 30 concentration. The participants who reported "resistant" as a result tested either AMC 3 or performed MIC. EUCAST specifies AMC 3—accordingly the isolate is AMC-resistant.

Below is the compilation of results (number of participants with R/I/S). Unfortunately, for administrative reasons it was not possible to integrate the results into the assessment form. (The respective target value is marked in bold)

<u>Antibiotic</u>	<u>R</u>	<u>I</u>	<u>s</u>
Ampicillin	53	0	2
Ceftriaxone	47	0	7
Augmentin	30	0	8
Ciprofloxacin	0	0	43
Cefepime	26	0	2
Meropenem	4	0	26
Tetracycline	2	1	25

Identification	Number
Haemophilus influenzae	59
Gram-negative rods	3

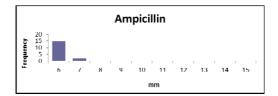
Kind regards,

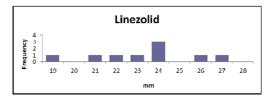
Prof. Dr. R. Zbinden

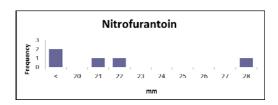
Houder

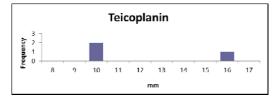
F.S. Hufschmid-Lim

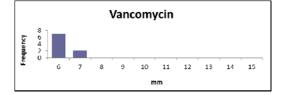
Resistance test of specimen A











Resistance test of specimen B

