Verein für **medizinische Qualitätskontrolle**
Association **pour le contrôle de Qualité medical**
Associazione **per il controllo di qualità medico**

Comment on the Proficiency Testing Survey B9 Microbiology 2014-2

Sample A: Midstream urine
Requirement: Potentially pathogenic bacteria (genus + species) + resistance testing

This was a strain of *Klebsiella pneumoniae* with normal resistance, i.e. resistant to ampicillin, and sensitive to everything else. As mentioned in the discussion 2014-1, EUCAST has changed the clinical limits for amoxicillin / clavulanic acid in uncomplicated urinary tract infections: for MIC from 8 to now 32 mg/l; for the disc test (20µg amoxicillin - 10µg clavulanic acid) from 17 mm to now 16 mm. For other infections, the threshold value for MIC remains at 8 but for the disc test the threshold value was increased from 17 to 19 mm. The Swiss Committee for the antibiogram (SAC) proposes to use an intermediate zone (16-18 mm) in order to circumvent the technical problems.
For fosfomycin MIC is required per EUCAST, but the evaluation of inhibition zones is in preparation. Per EUCAST, nitrofurantoin is intended only for *Escherichia coli.*

According to the discussion of sample A sent out on 2014-1, we ignored testing cefoxitin; should not enough antibiotics have been tested as a result, there would be a deduction; please also note in the future.

	Number
Klebsiella pneumonia	64
Gram neg. rods	1

Sample B: Wound swab
Requirement: Potentially pathogenic bacteria (genus + species) + resistance testing

The isolated *Staphylococcus aureus* was easy to identify. Penicillin sensitive, i.e. a sufficiently large diameter of the inhibition zone was present with a blurred, faded edge. Isolates with a zone of penicillin inhibition above the threshold (per EUCAST 1 Unit disc ≥26mm, per CLSI 10 Unit disc ≥29mm) and a blurred inhibition zone edge can be reported as sensitive. If the inhibition zone diameter is above the threshold, but a sharp edge is observed, then the strain should be reported as penicillin—and by extension ampicillin—resistant. EUCAST still recommends beta-lactamase, but it should be tested from the edge of the cefoxitin- or oxacillin disc. Our strain was beta-lactamase negative.

As of 2014, specifications regarding penicillin/ampicillin in coagulase-negative staphylococci are no longer provided by EUCAST. However, the method described above also works for coagulase-negative staphylococci. We will communicate SAC's opinion regarding this matter.

The laboratories that reported millimeters mostly measured correctly, but due to an edge assessed by some participants as sharp, the result was mistakenly reported as "resistant." It appears there are some uncertainties regarding "blurred" and "sharply defined" which is why we included pictures from EUCAST in the comment. With 0.064 mg/l, the MIC of penicillin (Etest) was sensitive. A fuzzy zone could also be observed there.

Report fuzzy zone diameter ≥26mm → sensitive

Report sharp edge with a diameter ≥26mm → resistant!

This time we only required 4 antibiotics. Unfortunately, many participants tested neither oxacillin nor cefoxitin, which we accepted this time; however, in the future, according to our instructions we will interpret absent indirect indication whether there is MRSA as a missing antibiotic and make a corresponding deduction.

Vancomycin should be tested by MIC. This was accordingly taken into account by almost all laboratories. If the disc test is performed, the teicoplanin disc can be tested per the proposal by SAC.

According to EUCAST, nitrofurantoin is recommended as therapy only for UTI.

	Number
Staphylococcus aureus	64
Gram pos. cocci	1

Sample C: i.v. Catheter
Requirement: Potentially pathogenic bacteria (genus only)

This was a strain of *corynebacterium amycolatum*, the most common—non-lipophilic—corynebacteriumin found in clinical material, which may cause catheter and other infections (Eur J Clin Microbiol Infect Dis 1999; 18: 518-21; Infect Dis 2002; Em 8: 97-9) and which occasionally occurs on the skin.

The colonies are gray-white and generally rough. In the Gram stain typical coryne-form rods are seen. *Corynebacterium jeikeium* is lipophilic, i.e. it does not grow as well on blood agar and growth is enhanced by Tween or serum (e.g. on a TSI slant). *Corynebacterium xerosis* produces a yellow pigment and, in contrast to *C. amycolatum*, does not produce propionic acid. In *Corynebacterium minutissimum* O/129 is sensitive, in contrast to *C. amycolatum* (MH agar with sheep blood); but *C. amycolatum* is also sensitive to O/129 on MH with horse blood. *C. amycolatum* is included the database of Api Coryne and Maldi-TOF and can thus be easily identified.

	I
Corynebacterium amycolatum	26
Corynebacterium species	30
Corynebacterium minutissimum	1
Corynebacterium xerosus	1
Corynebacterium jeikeium	2
Corynebacterium Group 3	2
Tsukamurella species	1
Kocuria species	1
No growth	1

Sample D: Blood
Requirement: Potentially pathogenic bacteria (genus + species)

Despite its name, *Clostridium tertium* also grows aerobically and is typically found in the blood of patients with hematologic-oncologic diseases. *Lactobacillus* is a typical misdiagnosis; the strong gas formation by *C. tertium* as well as high mobility allows them to be distinguished from another. With a diagnosis of e.g. AML the microbiologists should consider this.

C. tertium shows acidification of the entire TSI tube (Group 1); the following sugars were fermented: glucose, sucrose, maltose, xylose, mannose, fructose. Esculin and nitrate were positive; catalase, urease, and the cAMP assay were negative. Gas formation was present and mobility was positive (cloudy in the MIO-tube). The fatty acid pattern with acetate and butyric acid with little lactate are a fit for *C. tertium. C. tertium* is not included in the Api Coryn database; therefore, good results were not expected there; *C. tertium* is contained in the databases of anaerobic systems Rapid ID 32A and Api 20. *C. tertium* is in the MALDI-TOF data base.

	Number
Clostridium tertium	53
Clostridium species	2
Bacillus circulans	1
Serratia plymuthica	2
Mannheimia haemolytica	1
Erysipelothrix species	1
HACEK Group 1	1
Lactobacillus species	1
Bacillus cereus	1
Aggregatibacter aphrophilles	1
Gram neg. rods	1

Sample E: Tracheal secrete
Requirement: Potentially pathogenic bacteria (genus + species)

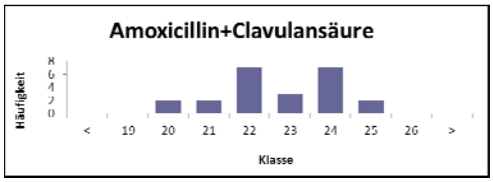
We did not rate this sample.
It was *Enterobacter aerogenes*.
This strain was very difficult to identify with conventional methods. Api20E suggested *Enterobacter sakazakii* (82.1%, t-value 0.79), the results by Vitek2 were *Raoultella ornithinolytica* with a probability of 99%. Maldi-TOF identified this strain very well; the identification was confirmed by sequencing.
(This strain was ESBL negative but AmpC was over-expressed. Carbapenemase was not detectable by phenotype and molecular biology.)

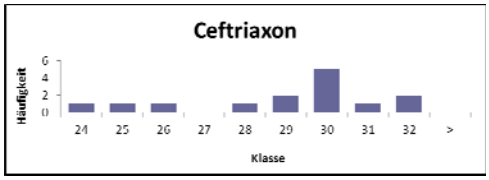
	Number
Enterobacter aerogenes	47
Enterobacter sakazakii	5
Enterobacter species	2
Enterobacteiaceae	1
Raoultella ornithinolytica	5
Klebsiella pneumonaie	1
Klyvera ascorbata	2
Gram neg. rods	1
Cronobacter sakazakii	1

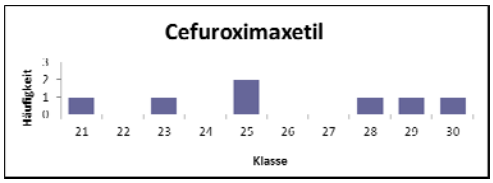
With best regards

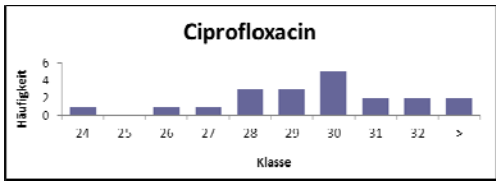
Prof. Dr. R. Zbinden *F.S. Hufschmid-Lim*

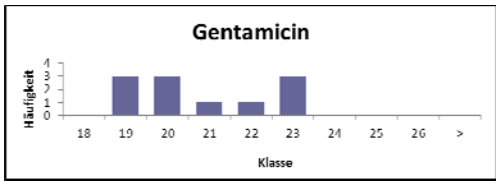
Resistance testing of Sample A

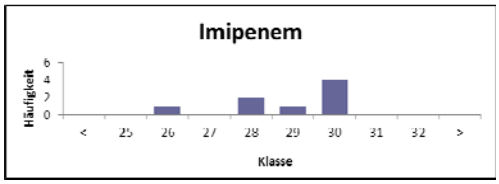


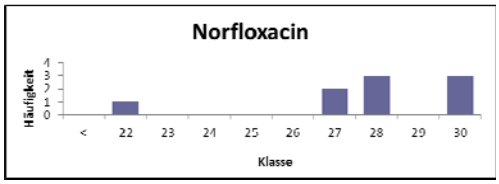




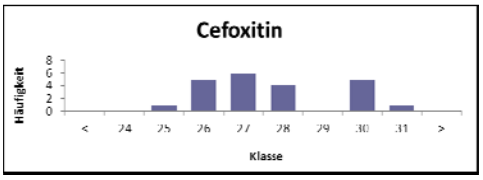


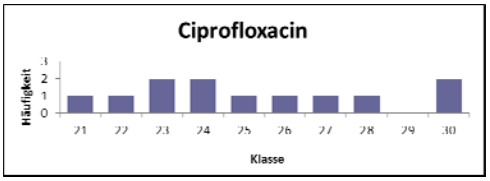


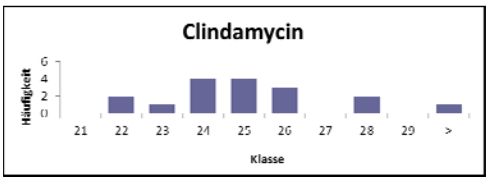


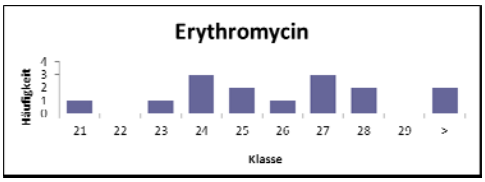


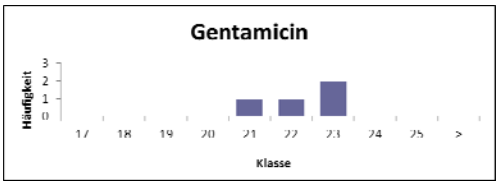
Resistance testing of sample B

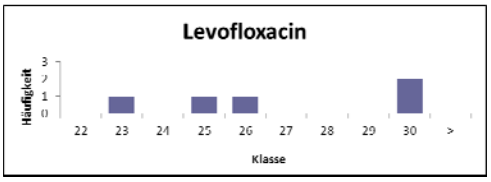


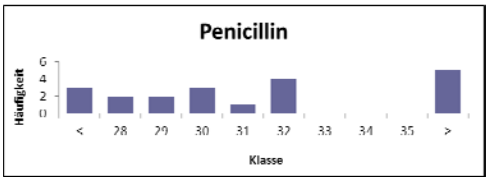


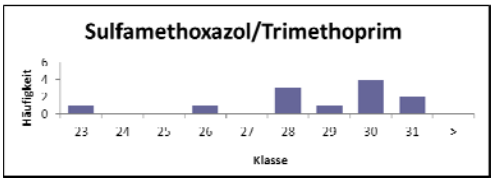












MQ Institut für Klinische Chemie
University Hospital Zurich CH-8091 Zurich
Phone 044 255 34 11 Fax 044 261 12 83
www.mqzh.ch · info@mqzh.ch