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

Comment on the survey sample B9 Microbiology 2015-1

Specimen A: Urinary Tract Infection
Requirement: Potentially pathogenic bacteria (genus + species) + resistance testing

This was a strain of *Escherichia coli,* the most common cause of urinary tract infections that was identified by all participants without difficulty by VITEK 2, API 20E or MALDI-TOF.

This *E. coli* - strain was sensitive to almost all antibiotics. For amoxicillin/clavulanic acid we accepted sensitive and intermediate. It is mentioned in EUCAST that some countries grade wild type isolates of *E. coli* and *P. mirabilis* as intermediate for ampicillin and amoxicillin/clavulanic acid by setting the thresholds for sensitive to a virtually unobtainable 50 mm. Unfortunately, with the measurement accuracy of the inhibition zones it is unavoidable that inaccurate sensitive zones of inhibition are read both for ampicillin and amoxicillin/clavulanic acid. EUCAST proposes different thresholds for amoxicillin/clavulanic acid (16 mm and 19 mm) for uncomplicated urinary tract infections and other 'systemic' infections. The Swiss Committee for the Antibiogram (SAC) proposes to apply an intermediate zone (16-18 mm) in order to circumvent the technical problems. This reduced the risk of very major errors; see publication: Maurer FP, Courvalin P, Böttger EC, Hombach M. Integrating forecast probabilities in antibiograms:
a way to guide antimicrobial prescriptions more reliably? J Clin Microbiol 2014. 52: 3674-3684. The strain primarily exhibited an "intermediate" zone for augmentin in our preliminary experiments. EUCAST has not provided values for doxycycline, tetracycline and minocycline; according to EUCAST, these antibiotics are rated "resistant" without testing.
According to EUCAST, MIC is required for fosfomycin, but the evaluation of inhibition zones is in preparation. We allowed—according to CLSI—sensitive zones of inhibition, because in addition to nitrofurantoin, fosfomycin was also successfully used for ESBL.

	Number
Escherichia coli	63

Sample B: Wound infection
Requirement: Potentially pathogenic bacteria (genus + species) + resistance testing
This isolated *Staphylococcus aureus* was easy to identify. Penicillin was resistant, i.e., a small inhibition zone diameter with a sharply defined edge was present. Isolates with a zone of inhibition against penicillin above the threshold (per EUCAST, 1 Unit discs ≥ 26 mm, per CLSI, 10 Unit discs ≥ 29mm) and an extension of the edge of the inhibition zone can be reported as sensitive. If the inhibition zone diameter exceeds the threshold, but a sharp edge is observed, then the strain should be reported as penicillin-resistant, but also by way of derivation, ampicillin-resistant. EUCAST no longer recommends beta-lactamase. SAC, however, still recommends beta-lactamase testing in unclear cases (penicillin zone of inhibition diameter above the threshold, but without a clear extension). But this should be tested from the edge of the cefoxitin- or oxacillin disc. Our strain was beta-lactamase positive.
It is extremely important to use the right disc concentration for ampicillin and penicillin (ampicillin, 2 Units and penicillin, 1 Unit). Some participants used the AM 10 Unit and P 10 Unit, leading to false sensitive values. For penicillin, there is no intermediate zone, which is why we evaluated this as false.
Since 2014, EUCAST has not been providing data to penicillin/ampicillin in coagulase-negative staphylococci. However, the method described above also works for coagulase-negative staphylococci. We will communicate SAC's opinion to this matter on the Swiss Society for Microbiology website, namely to use the same zones of inhibition for penicillin as are used for *S. aureus*.
Vancomycin should be tested by means of MIC. This has been taken into account by all laboratories. If the disc test is performed, in accordance with the suggestion by SAC, teicoplanin discs can be tested.

	Number
Staphylococcus aureus	62
Not specified	1

Sample C: Pneumonia?

Requirement: Potentially pathogenic bacteria (genus + species)

Our strain was *Rothia mucilaginosa,* previously known as *Stomatococcus mucilaginosus*. It is part of the normal flora of the mouth and upper respiratory tract. But there are cases known in which *R. mucilaginosa* was detected as the causative agent of pneumonia; therefore, a certain pathogenicity can nevertheless be attributed to this strain. Cases of meningitis and bacteremia have been described in particular in children: Chavan RS et al. Significant morbidity and mortality attributable to *Rothia mucilaginosa* infections in children with hematological malignancies or following hematopoietic stem cell transplantation. Pediatric Hematology Oncology 2013. 30: 445-454; Lee AB et al. Bacterial meningitis from *Rothia mucilaginosa* in patients with malignancy of undergoing hematopoietic stem cell transplantation. Pediatr Blood Cancer 2008. 50: 673-676.

The identification did not pose difficulties to most participants. *R. mucilaginosa* is a Gram-positive cocci, which is oxidase-negative, catalase-variable, and appear partially "rodoid." They are facultative anaerobes and grow on most non-selective media as whitish, rubbery colonies that are difficult to remove from agar ("rubber cocci"). Negative growth in 6.5% NaCl and hydrolysis of gelatin and esculin distinguishes them from staphylococci, micrococci and enterococci.

	Number
Rothia mucilaginosa	53
Rothia dentocariosa	6
Staphylococcus coagulase negative	1
Staphylococcus lugdunensis	1
Stomatococcus mucilaginosus	1
Not specified	1

Sample D: Infection following visit to Africa

Requirement: Potentially pathogenic bacteria (genus + species)

This Gram-positive rod was *Corynebacterium diphtheriae. C. diphtheriae* was readily identified by MALDI-TOF and Api Coryne. Catalase and nitrite were positive, cAMP negative; in Api Coryne, α-glucosidase was positive. The negative glycogene production excluded *C. diphtheriae* biotype *gravis*. The positive nitrate formation speaks for *C. diphtheriae* biotype *mitis* (biotype *belfanti* is nitrate *negative).* Our strain was toxin-negative. This strain was isolated from a superficial wound on the little finger in a patient following a visit to Africa. We have isolated *C. diphtheriae* on several occasions from similar patients. Please do not forget that these kinds of strains—including toxin-negative ones—must be reported.

	Number
Corynebacterium diphtheriae	46
Corynebacterium diphtheriae mitis/belfanti	9
Corynebacterium diphtheriae gravis	2
Corynebacterium accolens	1
Corynebacterium minutissimum	1
Corynebacterium species	1
Corynebacterium ulcerans	1
Not specified	2

Sample E: Bacteremia in a newborn

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

Streptococcus mitis colonizes the oral cavity, gastrointestinal tract, and female genital tract. *S. mitis* may also occur in the normal skin flora and appear as a contaminant in blood cultures. At the same time, it is also the most common cause of endocarditis. It is important to accurately evaluate the clinical significance of *S. mitis* in blood cultures. Some cases of *S. mitis*-bacteremia are known in neonates.
Our strain was catalase-negative, optochin-resistant, bile-negative. Vitek proposes *S. mitis/oralis*, and a good identification of *S. mitis* is achieved by MALDI-TOF. Sequencing of the *recA* gene allowed only identification as part of the *S. mitis-group;* a more accurate identification was not possible.
The special characteristic of our strain was its penicillin- and ceftriaxone-resistance. There are no EUCAST breakpoints for piperacillin/tazobactam; it is to be derived from ampicillin. In our case, piperacillin/tazobactam must therefore be reported as "resistant." While this strain was isolated from the blood culture of a Swiss child, further investigation (thanks to Dr. Felix Fleisch) revealed that the mother had previously been on business trip to the Far East. We want to bring this strain to your attention so that you are alerted to such highly resistant viridans streptococci.

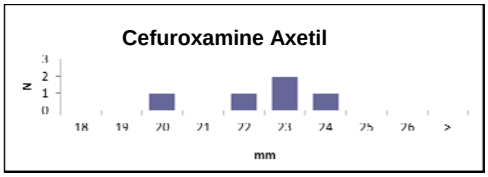
We did not rate this sample.

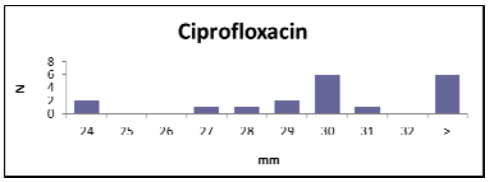
	Number
Streptococcus mitis	20
Streptococcus mitis group	13
Streptococcus mitis/oralis	17
Gemella haemolysans	2
Streptococcus oralis	2
Streptococcus pneumoniae	1
Streptococcus species	1
Streptococcus viridans	1
Aerococcus urinae	1
Escherichia coli	1
Gemella morbillorum	1
Not specified	3

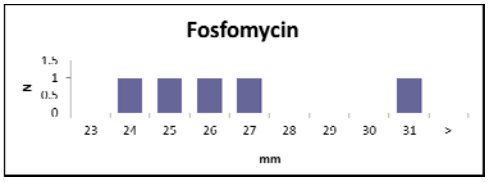
With best regards

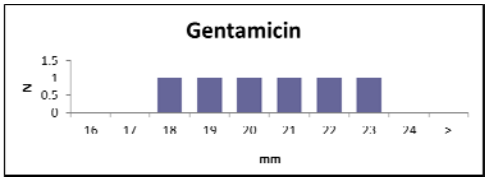
Prof. R. Zbinden MD *F.S. Hufschmid-Lim*

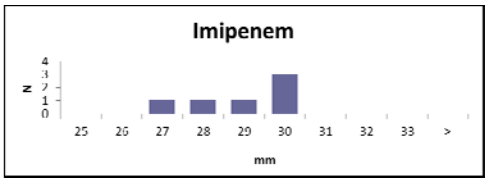
Resistance testing of sample A

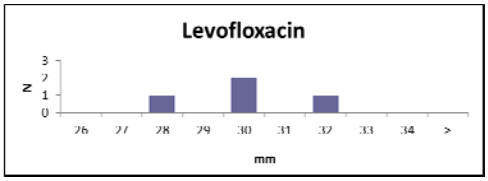


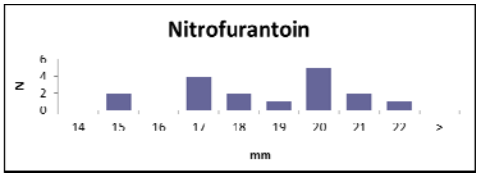


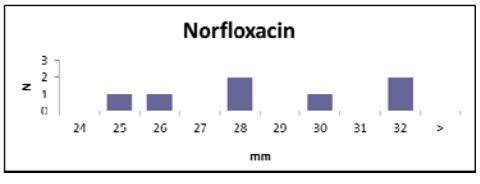


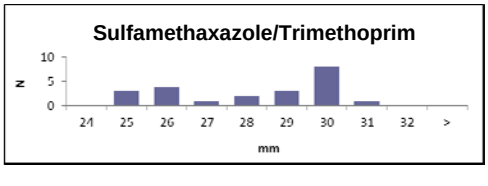












Resistance testing of sample B