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 2014-3

Sample A: Urine
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 successfully identified without difficulty via VITEK 2, API 20E or MALDI-TOF by all participants.

E. coli was fully sensitive. EUCAST has not specified values for doxycycline, tetracycline and minocycline; according to EUCAST, these antibiotics would be set as "resistant" without testing. But we will allow it nevertheless.

Please see the previous discussion with respect to amoxicillin/clavulanic acid in *Enterobacteriaceae*. The Swiss Committee for the antibiogram (SAC) suggests using an intermediate zone (16-18 mm) in order to circumvent the technical problems.

Per EUCAST, MIC is required for fosfomycin but the evaluation of inhibition zones is in preparation.

	Number
Escherichia coli	65

Sample B: Tracheal secrete
Requirement: Potentially pathogenic bacteria (genus + species) + resistance testing

This was a strain of *Pseudomonas aeruginosa* that did not pose difficulties in diagnosing (presence of fluorescein, formation of pyozyanin, positive oxidase TSI Group 4, beta-hemolysis, growth at 42 ºC, and C-390-resistance).

The (natural) resistance against ampicillin, amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole and (*in P. aeruginosa* acquired) resistance to ciprofloxacin, levofloxacin, ertapenem, imipenem were consistently detected. With meropenem, we allowed resistant and intermediately as accurate.

Meropenem resistance can be coupled with either imipenem resistance or exist independently; in the latter case, there is an upregulation of other efflux pumps. Therefore, dissociation of the sensitivities may be present.

When colistin is indicated, it must be tested by MIC per EUCAST. In CLSI, there are no inhibition zones.

Tobramycin was reported by most participants as "resistant." According to EUCAST expert rules this means for aminoglycosides in *P. aeruginosa, Enterobacteriaceae* and *Acinetobacter baumannii* (Leclercq et al. EUCAST expert rules 151; **http://www.eucast.org/expert_rules/**), that with tobramycin resistance and simultaneous resistance to amikacin and gentamicin, amikacin should be reported as "resistant." The production of the acquired AAC (6 ')-I enzyme that modifies amikacin may be phenotypically missed.

For this reason, and because of uncertainty in the measurement of the aminoglycosides, we allowed all results.

	Number
Pseudomonas aeruginosa	64
Gram neg. rods	1

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

This sample is a strain of *Streptococcus pyogenes* (Group A streptococcus). There were no problems with identification.

S. pyogenes forms Gram-positive cocci in chains, is facultative anaerobe, beta-hemolytic, catalase negative and positive for pyrrolidonyl arylamidase (PYR positive).

S. pyogenes (from the Greek πύον pus - pus-inducing streptococci) is a common bacterium that, among others, can cause scarlet fever, and purulent tonsillitis in humans. On the skin, depending on the location and depth of the infectious reaction, impetigo, erysipelas or phlegmons may develop. With poor defense status, local infection may develop into a generalized infection (sepsis). Not to mention the possible formation of toxins (pyrogens and other toxins).

Beta-hemolytic streptococci are always sensitive to penicillin.

	Number
Streptococcus pyogenes	62
Beta-hemolytic streptococci group A	2
Gram-positive cocci	1

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

Our strain was *Turicella otitidis*. It was identified by almost all participants. *T. otitidis* forms long unbranched Gram-positive rods in the Gram-preparation.

It has an oxidative metabolism, is immobile, positive for catalase and CAMP and is contained in the Api Coryne database. *T. otitidis* was also readily identified by MALDI-TOF. In the CTA Bio *T. otitidis* shows acid formation in absence of any sugar.

Generally, *Corynebacterium auris* is difficult to distinguish from *T. otitidis*; it does not show long rods in Gram-preparations, but sticky colonies.

Recently, Professor A. von Graevenitz and Professor G. Funke wrote a review of the past 20 years on *T. otitidis* and *C. auris* (Infection 2014; 42:1-4). It is noted that *T. otitidis* can often be isolated from the ear swab (outer ear) and with otitis media. The genus *Turicella* is derived from Turicum, the Latin name for Zurich.

	Number
Turicella otitidis	58
Corynebacterium auris	1
Corynebacterium jeikeium	1
Corynebacterium propinquum	1
Gram neg. rods	1
No growth	1

Sample E: Urine

Requirement: Potentially pathogenic bacteria (genus + species)

We did not rate this sample.

It was *Corynebacterium pyruviciproducens*. The many different responses showed that *C. pyruviciproducens* is not easy to identify because there is still not enough known about this *Corynebacterium*. In our routine laboratory, diagnosis was not successful either because the lipophilicity (growth on Tween 80-plate) was wrongly assessed as negative. To date, this germ is not included in Bruker's MALDI Biotyper database. Accordingly, identification was not achieved by MALDI-TOF.
The strain was lipophilic (in-house Tween-plate), positive for catalase and CAMP and for TSI Group 1 (clearly grows better with rabbit serum, which also speaks for lipophilicity); nitrite, urease, esculine, glucose, sucrose, maltose and fructose were also positive. The strain was immobile and negative for xylose and mannose. β-Glucuronidase was positive.
The Api Coryne provided excellent identification of *Corynebacterium glucuronolyticum*, which also corresponds with our strain by CTA Bio with the exception of lipophilicity. The interpretation of the lipophilicity is also interpreted differently in recent literature (D. Goldberger, V. Hinic, S. Turan, E. Schultheiss, AL Pacheco, R. Frei and K. Bernard. Extended Characterization of *Corynebacterium pyruviciproducens* based on clinical strains from Canada and Switzerland. J. Clin. Microbiol. 2014; 52: 3180-3); This publication also describes that unambiguous identification is also possible by 16S-r RNA gene sequencing, but there are polymorphisms in the first 200 base pairs.

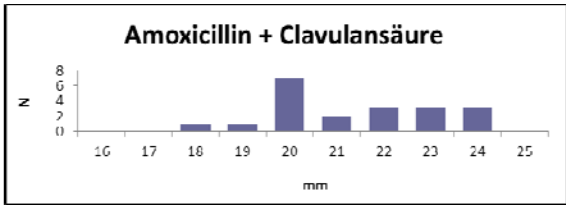
Practically speaking, *C. glucuronolyticum* identified by conventional methods that forms small colonies on sheep blood agar is suggestive of *C. pyruviciproducens*. For clarification of lipophilicity, we incubated 3 sheep blood plates (1x supplemented with rabbit serum, 1x supplemented with Tween 80 and 1 without additives) inoculated with *C. pyruviciproducens* and incubated for 24 hours at 37°C and CO_2. It was found that significantly better growth was seen on the sheep blood plate with rabbit serum than on both other plates. Thus, lipophilicity can be assessed much better with rabbit serum than with Tween 80. We were unable to find the reason for this. It is possible that the concentration of Tween 80 in the Tween plate is too high for *C. pyruviciproducens.*

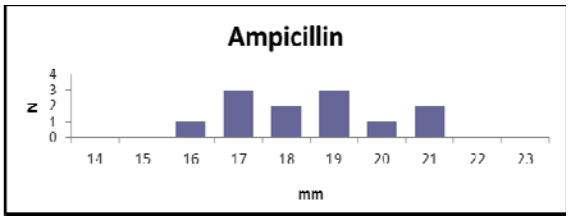
	Number
Actionbaculum schaalii	3
Bacillus species	1
Corynebacterium glucuronolyticum	14
Corynebacterium pyruviciproducens	11
Corynebacterium renal	10
Corynebacterium urealyticum	3
Corynebacterium species	9
Corynebacterium pseudogenitalium	1
Corynebacterium riegelii	1
Escherichia coli	1
Lactobacillus delbrueckii	1
Propionibacterium acnes	1
Granulicatella adiacens	1
Gram neg. rods	5
Gram-positive cocci	1
No growth	1
No report	1

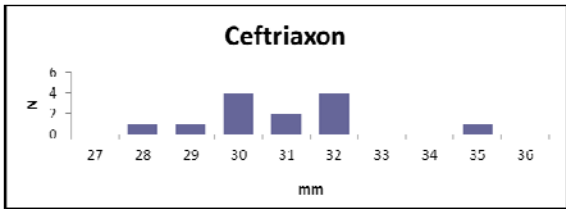
With best regards

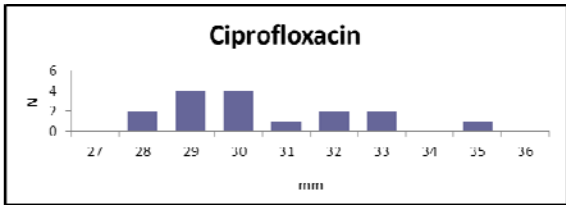
Prof. Dr. R. Zbinden *F.S. Hufschmid-Lim*

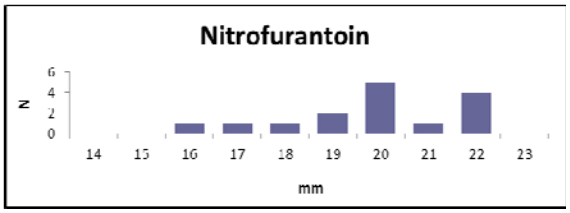
Resistance testing of Sample A

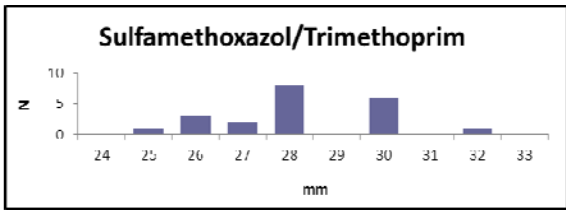




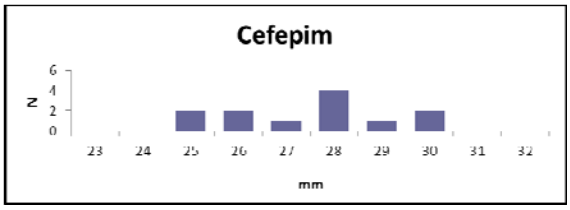


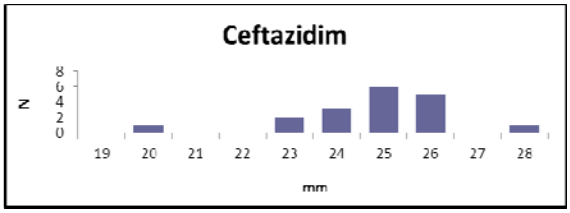


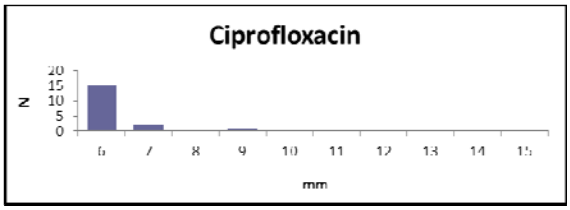


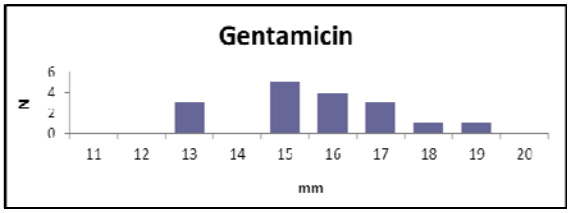


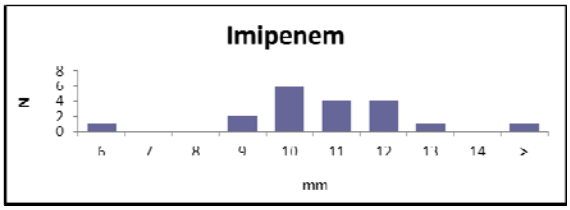
Resistance testing of sample B

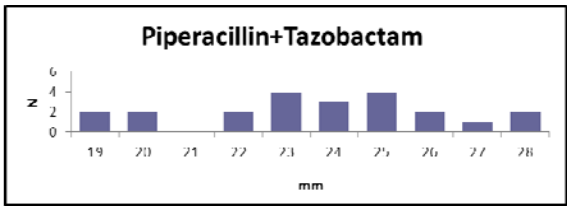












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