



Comment on Survey Specimen B9 Microbiology 2014-1

Specimen A: Midstream urine
Requirement: Potentially pathogenic bacteria (genus + species) / susceptibility testing

This is a strain of *Escherichia coli* with an extended spectrum beta-lactamase (ESBL). Cefepime and cefotaxime were resistant (we accepted all results for ceftazidime), but amoxicillin/clavulanic acid and piperacillin/tazobactam were sensitive; per EUCAST, a change to resistant—also according to CLSI—is not warranted. In 2014, EUCAST changed the clinical limits for amoxicillin/clavulanic acid for uncomplicated urinary tract infections: for MIC from 8 to currently 32 mg/l; for the disc test (20µg amoxicillin - 10µg clavulanic acid) from 17 mm to currently 16 mm. The MIC limit values for other infections remained at 8, but for the disc test the limit value of 17 was increased to 19 mm.

This change in the limits—in particular the different limits for urinary tract infections and other infections—is problematic for many reasons; the Swiss Antibigram Committee will address these and provide a suggestion to the laboratories on how to handle this.

This strain was intermediately sensitive to ertapenem (MIC 1 mg/l). We accepted all the results. Increasingly, we see ESBL, where (similar to AmpC producers, see discussion 2013-4 specimen A) changes of porins in the outer cell membrane and overexpression of efflux pumps may result in reduced sensitivity to ertapenem.

Cefoxitin should not be reported in the microbiology report because cefoxitin is intended only for AmpC screening; this time we did not evaluate cefoxitin. Cefoxitin is on the select quality control list because of staphylococci. In future, we will ignore cefoxitin reports; please ensure that you have specified enough of the other antibiotics. Fosfomycin and nitrofurantoin were sensitive; in accordance with EUCAST, MIC is in principle required for fosfomycin, but the evaluation of inhibitors is in preparation.

We would like to remind you that for *Enterobacteriaceae* you have the possibility of distinguishing between complicated mechanisms (ESBL or hyperproduction of AmpC in combination with membrane changes) and carbapenemases by contacting one of the expert laboratories designated by the Swiss Antibigram Committee. We included the appropriate form at our last discussion, which can also found on the homepage of the Schweizerische Gesellschaft für Mikrobiologie (Swiss Society for Microbiology) (www.swissmicrobiology.ch); please contact the corresponding specialized laboratory in advance to clarify the exact procedure.

Escherichia coli

Number
65

Specimen B: Urinary Tract Infection**Requirement: Potentially pathogenic bacteria (genus + species) / susceptibility testing**

Enterococcus faecium isolated from this urinary tract infection exhibits high-level gentamicin resistance. As also discussed in the last meeting [regarding] 2013-4 specimen B, enterococci always present low-level resistance to aminoglycosides, therefore the presence of so-called high-level resistance in enterococci is of interest. We ask that in future you report only high-level resistance in enterococci; the sole indication of resistance to aminoglycosides is not considered in the evaluation; therefore, in that case, your number of reported antibiotics might not be insufficient.

The same applies to reports regarding cephalosporins and clindamycin; reporting resistance to clindamycin and cephalosporins in enterococci is not wrong, but it is a natural resistance.

For nitrofurantoin, EUCAST lists only one value for *Enterococcus faecalis*, but not for *Enterococcus faecium*. This time we accepted all results, but in the future will evaluate them as incorrect. However, we evaluated the reported results for fosfomycin, tetracycline, and doxycycline as wrong in accordance with our announcement at the meeting 2013-4-Specimen B.

	Number
<i>Enterococcus faecium</i>	63
<i>Enterococcus</i> sp.	1
<i>Enterococcus gallinarum</i>	1

Specimen C: Sepsis**Requirement: Potentially pathogenic bacteria (genus + species)**

The genus *Aerococcus* includes seven different species. They are facultative anaerobic, catalase-negative, Gram-positive cocci, which frequently form tetrads in liquid medium. *Aerococcus urinae* and *Aerococcus sanguinicola* can both cause urinary tract infections (Cattoir et al. 2010. *Aerococcus urinae* and *Aerococcus sanguinicola*, two frequently misidentified uropathogens, Scand J Infect Dis 42: 775-780). Since they are both resistant to ciprofloxacin, they can proliferate on this treatment regimen and sometimes migrate into the blood and cause sepsis (rarely endocarditis); unlike *Aerococcus viridans*, both are sensitive to penicillin. They are frequently misidentified as *A. viridans* (M. Rasmussen 2013. Aerococci and aerococcal infections. J Infect 66: 467-474) since *A. urinae* and *A. sanguinicola* are not included in all commercial databases. However, penicillin-sensitivity indicates this error regarding the identification of *A. viridans*.

Conventional reactions such as the pyrrolidonylarylamidase (PYR), leucine aminopeptidase (LAP), and beta-glucuronidase (BGUR) can differentiate the above species from each other. *A. viridans* and *A. sanguinicola* are PYR positive (*A. urinae* and other aerococci are PYR negative); *A. urinae* and *A. sanguinicola* are LAP positive (*A. viridans* is LAP negative); *A. urinae*, *A. sanguinicola*, and occasionally *A. viridans* are BGUR positive (other aerococci are BGUR negative)

(M. Rasmussen 2013).

Our strain is *A. sanguinicola* (PYR, LAP, BGUR all positive).

MALDI-TOF MS identifies *A. sanguinicola* and *A. urinae* (E. Seenebey et al. 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry is a sensitive and specific method for identification of aerococci. J Clin Microbiol 51: 1303-4).

	Number
<i>Aerococcus sanguinicola</i>	23
<i>Aerococcus</i> sp.	29
<i>Aerococcus viridans</i>	11
<i>Aerococcus urinae</i>	1
Pilze	1

Specimen D: Ascites with intestinal perforation
Requirement: Potentially pathogenic bacteria (genus + species)

Bacteroides fragilis was isolated from this ascites specimen following intestinal perforation. It was isolated from various materials, mostly in the context of gastrointestinal clinic (post-operative wound infections, perforated colon, etc.). *B. fragilis* is the anaerobic, Gram-negative rod bacteria most frequently isolated in the laboratory.

B. fragilis is distinguished by growth on bile-containing agar (resistant to bile), and by positive esculin reaction. Catalase positive and indole reaction is negative. *B. fragilis* is resistant to vancomycin (5 µg, kanamycin (1000 µg) and colistin (10 µg); this diagnostic resistance points to the *B. fragilis* group. Commercial systems allowed an accurate diagnosis. From glucose, *B. fragilis* typically produces acetic acid, little propionic acid, succinate, isobutyric acid, and isovaleric acid. *B. fragilis* is also readily identified to the species level by MALDI-TOF MS.

	Number
<i>Bacteroides fragilis</i>	55
<i>Bacteroides</i> sp.	1
<i>Bacteroides stercoris</i>	2
Gram-negative rods	2
No growth	1
<i>Microbacterium</i> sp.	1
<i>Prevotella</i> sp.	1
<i>Escherichia coli</i>	1
<i>Alistipes putredinis</i>	1

Specimen E: Sinusitis in dog owners
Requirement: Potentially pathogenic bacteria (genus + species)

We have not rated this specimen. We wanted to introduce *Staphylococcus pseudintermedius* with the specimen. For resistance testing with cefoxitin, the EUCAST guidelines of 2014 list *S. pseudintermedius* separately; when screening with cefoxitin, the resistance circle must be ≥ 35 mm for oxacillin-sensitivity to be assumed (representative of penicillinase-resistant penicillins and cephalosporins). *S. pseudintermedius* was first described in 2005 in animals (Devriese et al. 2005. *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int J Evol Microbiol 2005; 55: 1569-73).

S. pseudintermedius is clumping factor-negative, but coagulase-positive, and may also form hemolysins, exfoliatins, enterotoxins, and leukocidins—similar to PVL with *S. aureus*. The methicillin-resistant *S. pseudintermedius* has a similar meaning in dog as MRSA in humans. Humans can also become infected by contact with dogs, which is what we wanted to demonstrate with this strain. (Stegmann et al. 2010. Human infection associated with methicillin-resistant *Staphylococcus pseudintermedius* ST71. J Antimicrob Chemother 65:2047-8).

Differentiation to *Staphylococcus intermedius* cannot be unequivocally made with conventional tests and MALDITOF, but with 16S RNA gene sequencing. Most isolates of dogs are *S. pseudintermedius*.

The importance of human *S. pseudointermedius* strains and their genetic characteristics are largely unknown. In order to investigate these properties in more detail, Professor Vincent Perreten of the Institut für Veterinär-Bakteriologie (Institute of Veterinary Bacteriology) is very keen for you to send him *S. pseudointermedius* strains you may have isolated in your laboratory. Also, strains with questionable identities or unclear phenotype of methicillin resistance can be mailed for further identification.

Please send your *S. pseudointermedius* strains to the following address:

Vincent Perreten, Prof. Dr.
Institut für Veterinär-Bakteriologie
Universität Bern
PO Box
Länggass-Strasse 122
CH-3001 Bern
Phone: +41 31 631 2484
Fax: +41 31 631 2634
vincent.perreten@vetsuisse.unibe.ch

	Number
<i>Staphylococcus intermdius</i>	31
<i>Staphylococcus pseudointermedius</i>	32
Gram positive Kokken	1
<i>Staphylococcus xylosus</i>	1

Best Regards

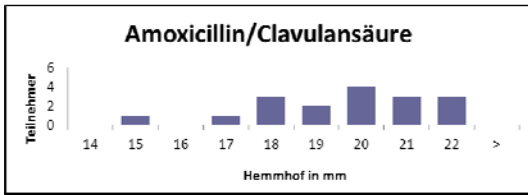


Prof. Dr. R. Zbinden



F.S. Hufschmid-Lim

Susceptibility Testing Sample A



Susceptibility Testing Sample B

