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OPINION
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C. Greko
Dept of animal health and antimicrobial strategies

Gittan Gröndahl
Dept of animal health and antimicrobial strategies
SVA

Regarding reported resistance to penicillin in *Streptococcus equi*

We have been asked for a brief opinion on reported resistance to penicillin in *Streptococcus equi* (either subspecies) and on mutations in the pbp-domains of beta-haemolytic streptococci.

To our knowledge, there are still no reports on confirmed resistance to penicillin in *Streptococcus equi* (regarding standards for confirmation, see below).

We are aware of publications reporting resistance to penicillin in *S. equi*, in particular *S. equi* subsp. *zooepidemicus*, from different sample materials (e.g. Benko et al, 2015, Fonseca et al, 2020, Nocera et al, 2022). These studies have in common that they have used disk-diffusion to test antimicrobial susceptibility (AST). They sweepingly refer to (sometimes very old and thereby invalid) international standards without specifying how they have used quality control strains. None of these authors mention anything about confirmation of their unusual findings.

Common causes of false resistance to penicillin when using disk diffusion are too dense inoculum, contaminated cultures, hydrolysis of penicillin in the disk caused by improper storage and handling routines, and poor or uneven quality of disk from the producer. In addition, erroneous species identification can be a source of error. Therefore, stringent adherence to standards, including regular use of quality control strains and routines for confirmation of unusual or unexpected results, are essential.

A golden rule in routine clinical bacteriology and in research is to confirm unexpected or unusual results. A minimum is to repeat organism identification and antimicrobial susceptibility testing (AST) to test for reproducibility. This is also what current international standards for AST from CLSI and EUCAST recommend. The CLSI standard for AST of animal pathogens, VET01S-5Ed,

recommends as follows for category II, to which findings of non-susceptibility to penicillin in beta-haemolytic streptococci, including *S. equi* (both subspecies), belong:

"Ensure antimicrobial susceptibility test results and organism identification are accurate and reproducible. Consider the following steps:

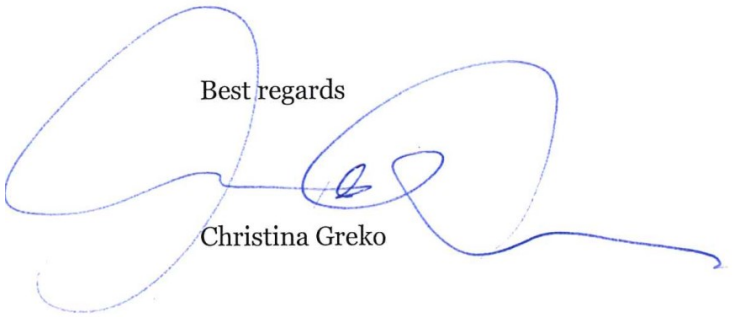
1. *Check for transcription errors, contamination, or defective panel, plate, or card*
2. *Check previous reports on the patient to determine of the isolate was encountered and confirmed*
3. *Repeat organism identification and antimicrobial susceptibility tests with initial method to ensure they reproduce (For Category II, the laboratory may elect to skip step 3 and go to steps 4 and 5. For category III, repeat and/or confirmatory testing may not be needed if resistance is common in the institution.)*
4. *Confirm organism identification with second method performed in-house or at a referral laboratory.*
5. *Confirm antimicrobial susceptibility test results with second method (e.g. in-house or referral laboratory). The second method might be a CLSI reference method (eg, broth microdilution, agar dilution, or disk-diffusion) or a regulatory agency-cleared commercial test."*

A good and responsible practice, should the finding is confirmed, is to contact the national reference laboratory for discussions on possible need for further investigation. In Sweden, SVA is the reference laboratory for antimicrobial resistance in bacteria from animals and food, and we would strongly recommend to let us confirm the result and to perform (or let us perform) genome sequencing to investigate the underlying changes in the bacterial genome. As we are responsible for monitoring of resistance in bacteria from animals, we would also strive to gather more information on the case, on potential spread and on prevalence more generally.

When interpreting results of genome sequencing, it is important to note that multiple mutations in the active domains of pbp2X and other pbps do not necessarily lead to increases in MIC that are of clinical importance. We are aware of one study reporting on multiple mutations in *Streptococcus dysgalactiace* subsp. *equisimilis* leading to MICs above the clinical breakpoint in a few epidemiologically related isolates (Fuursted et al, 2016). But in *S. pyogenes*, the reported increase in MICs in isolates with mutations in pbp2X have not been of a magnitude that it would fall above the break-point for resistance in AST (eg, Beres et al 2022).

Given the importance of penicillin in treatment of infections with beta-haemolytic streptococci in animals and people, it is important that resistance is monitored, and that unusual results are followed-up according to standards. Neither human nor veterinary medicine can afford to lose penicillin as an effective treatment of common infections.

The final development of this opinion was made by Karin Bergström (deputy state veterinarian, DVM, PhD), Märit Pringle (associate professor, DVM, PhD and Christina Greko (associate professor, DVM, PhD, coordinator for this opinion).



Annex: References

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