**Performance of the EUCAST disc diffusion test and supplementary methods to detect enterococci with reduced susceptibility to linezolid or tigecycline -** **The NordicAST 2023 LRE-TRE study**

**Background**

Linezolid, the lead oxazolidinone, and tigecycline are therapeutic alternatives in the treatment of multidrug resistant enterococci and vancomycin resistant enterococci (VRE) in particular (1, 2). Although the prevalence of linezolid (LRE) or tigecycline (TRE) resistant enterococci is still low it is increasing (3).

**Tigecycline**. Resistance is associated with mutations in genes regulating expression of ribosomal protection proteins (*tetM*) or efflux pumps (*tetL*) or genes encoding ribosomal proteins (particularly *rpsJ*, encoding ribosomal protein S10) causing one to four-folds increases in MIC above the current clinical breakpoint for susceptibility (4-8)

**Linezolid.** Several resistance mechanisms may affect oxazolidinone susceptibility in enterococci. These include mutations in the 23S rRNA subunit, changes in L3 or L4 ribosomal protein encoding genes and horizontal acquisition of resistance determinants such as *optrA*, *poxtA* and *poxtA*-*Ef* genes (8-10). The linezolid MIC-level in LRE have been shown to vary considerably challenging breakpoints in current AST. An ATU in the EUCAST disk diffusion method has been suggested (11). Moreover, a recent report from the French reference laboratory suggests prolonged incubation of 2 days to increase reading accuracy of linezolid susceptibility due to hazy growth in agar-based diffusion methods (12).

It is important that laboratories can rapidly detect and report LRE/TRE to support appropriate therapy and infection control measures. Thus, we would like to examine the ability to detect and report LRE/TRE according to EUCAST (http://www.eucast.org/) guidelines in a multicenter study in Nordic laboratories.

**Purpose**

We hereby invite Nordic (Denmark, Finland, Iceland, Norway, and Sweden) laboratories to examine a well characterized, genetically diverse collection of *E. faecium* and *E. faecalis* (n=20) with and without reduced susceptibility to linezolid or tigecycline in a blinded format. The primary aim of this study is to explore the performance of the EUCAST disc diffusion method ([http://www.eucast.org/)](http://www.eucast.org/)%20) and supplementary methods to detect reduced susceptibility to linezolid and tigecycline.

**Study design**

**Timeline.** Invitation to participate in this study will be sent by the end of January 2023. Confirmation of participation (*confirmed participation form*) has to be sent by February 15th, 2023 to the coordinating lab (Norwegian National Advisory Unit on Detection of Antimicrobial Resistance by e-mail to Bjørg Haldorsen ([bjorg.haldorsen@unn.no](file:///C:\\Users\\asu000\\Documents\\Arnfinn\\Kres\\Prosjekt\\Nordic%20CPE%20study\\bjorg.haldorsen@unn.no)), also naming your local study supervisor (contact person) for the study and contact information. A blinded panel of strains (n=20) including well-known quality control strains will be sent to the participating laboratories during week 8-9 (February 20th - March 3rd) along with a result form. Deadline for submitting result will be April 2nd, 2023.

**Bacterial strains.** The strain panel consists of well-characterized reference strains and *Enterococcus* strains (n=20 in total) obtained from human samples since 2012 with a range of MICs against linezolid and tigecycline as well as diverse resistance mechanisms. The strains have been identified by MALDI-TOF and genotyped by whole genome sequencing (WGS) to ensure correct species and resistance determinant identification as well as genetic heterogeneity.

**Methods.**

1. All laboratories must perform bacterial identification at the species level and record methods used.
2. All laboratories must perform antimicrobial susceptibility testing of all strains by the disk diffusion method as recommended by EUCAST using the following panel of antibiotic discs: linezolid (10 µg), tigecycline (15 µg) and gentamicin (30 µg).
3. All laboratories must perform a linezolid and tigecycline MIC-determination using microbroth dilution or a MIC gradient strip test according to the manufacturer’s instructions on all strains.
4. All laboratories must report manufacturer and lot number of antimicrobial susceptibility testing material. The EUCAST laboratory in Växjö will perform an extended quality control of broth, MH agar, MH broth, MIC gradient tests and discs from relevant manufacturers.
5. All laboratories that routinely use a semi-automated AST device for enterococci must perform AST according to the instructions of the manufacturer and report the result for linezolid and tigecycline if included in the AST panel for all strains. If the AST system provides an interpretation (e.g. expert interpretation) regarding resistance mechanisms this should also be reported. Manufacturer, type of AST plates/cassettes/panels must be reported.
6. All laboratories should include the EUCAST recommended enterococcal quality control (QC) strain *Enterococcus faecalis* ATCC 29212 in all methods performed.
7. All results including QC-data must be reported in the enclosed excel sheet.

**The laboratories participating in the study should not perform any genotypic confirmative testing as the strains should be examined in a blinded, non-biased approach. The strains should ideally be analyzed as part of the routine diagnostic services, and not separately by very experienced staff in antimicrobial susceptibility testing.**

**Reporting of results.** Laboratories report their findings in the excel-file provided. Results should be interpreted according to NordicAST Breakpoint Tables v.13.0, 2023 For each of the 20 strains, the laboratory should indicate whether, in a real situation, the finding would have been referred to a reference laboratory or not. Reference analysis results will be provided to each participating laboratory upon receipt of results.

**Organization and publication.** The study originates from and is scientifically approved by the NordicAST committee. We will form a study group (Nordic LRE-TRE study group) consisting of one person from each participating laboratory (the local laboratory supervisor) and a reference group. The reference group consists of *Barbara Holzknecht* (BH; Denmark), *Heikki Ilmavirta* (HI; Finland), *Gunnar Kahlmeter* and *Erika Matuschek* (GK and EM; EDL, Växjö, Sweden), *Kristin Hegstad*, *Bjørg C. Haldorsen* and *Arnfinn Sundsfjord* (KH, BCH and AS; Tromsø Norway), and *Kristjan Orri Helgason* (KOH; Iceland). The reference group is coordinated by KH, BCH and AS. BCH, EM, BH, HI, and KOH are responsible for invitation and recruitment of participating laboratories in Norway, Sweden, Denmark, Finland, and Iceland, respectively. Any publication from this study is obliged to follow the Vancouver protocol for determining authorship. All local LRE-TRE study laboratory supervisors (contact persons) will be included and acknowledged as partners in the Nordic LRE-TRE study group.

**References**

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