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Technical Note

Excellent performance of CHROMagar™ LIN-R to selectively screen for linezolid-resistant enterococci and staphylococci

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ABSTRACT

The increasing number of nosocomial pathogens with resistances against last resort antibiotics like linezolid leads to a pressing need for the reliable detection of these drug-resistant bacteria. National guidelines on infection prevention, e.g., in Germany, have already recommend screening for linezolid-resistant bacteria, although a corresponding screening agar medium has not been provided. In this study we analyzed the performance and reliability of a commercial, chromogenic linezolid screening agar. The medium was capable to predict more than a hundred linezolid-resistant isolates of *E. faecium, E. faecalis, S. aureus, S. epidermidis,* and *S. hominis* with excellent sensitivity and specificity. All isolates were collected at the National Reference Centre between 2010 and 2020.

1. Introduction

Last resort antibiotics such as linezolid, tigecycline, and daptomycin are therapeutic alternatives for the treatment of infections with multidrug-resistant staphylococci and enterococci including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), and vancomycin-resistant enterococci (VRE). Although an increased use of these substances has already led to increasing rates of resistance, corresponding studies that show a clear association between use and resistance progression are limited to single institutions (Bai et al., 2019; Jian et al., 2020; Wessels et al., 2018; Zou and Xia, 2020).

Linezolid is a synthetic oxazolidinone antibiotic that is active against most multidrug-resistant, clinical isolates of staphylococci, and enterococci such as MRSA, MRSE and VRE. Although linezolid was already approved for commercial use in 2000, resistance surveillance schemes and systems did not report increasing linezolid resistance in clinical isolates over time ((ECDC) 2020; Heininger et al., 2020; Markwart et al., 2019; Pfaller et al., 2019; Walter et al., 2015; Walter et al., 2017; Zou and Xia 2020). However, in recent years a trend toward an increased prevalence of linezolid resistance in clinical *E. faecium* and *S. epidermidis* isolates has been recognized at the German National Reference Centre for Staphylococci and Enterococci, while the numbers of linezolid-resistant *S. aureus* remained stable (Klare et al., 2015; Klare et al., 2019; Layer et al., 2019). Therefore, reference laboratories may play an important role in the early recognition of upcoming resistance trends before they might get noticed in surveillance schemes (Klare et al., 2015; Klare et al., 2019; Layer et al., 2019).

In contrast to most protein synthesis inhibitors, linezolid targets the 23S ribosomal DNA of the 50S subunit, thus preventing the formation of a functional 70S-initiation complex. In staphylococci and enterococci, development of oxazolidinone resistance has been associated with mutations in 23S rDNA alleles (Bender et al., 2018; Ruiz-Ripa et al., 2020; Sadowy, 2018). Additionally, variations in ribosomal protein genes may occur (Bender et al., 2018; Rouard et al., 2017). A reservoir of transferable linezolid resistance genes was selected in animal farming and reported for nonclinical isolates of *E. faecium* and *E. faecalis* and coagulase-negative staphylococci, coded by *cfr* (including *cfr*(B)) and/or *optrA* (Cuny et al., 2017; Wang et al., 2015). Corresponding resistance determinants were also reported in enterococcal and staphylococcal isolates from healthy humans and hospitalized patients (Bender et al., 2018; Chen et al., 2019; Cai et al., 2019; Deshpande et al. 2018; Elghaieb et al., 2020; Gawryszewska et al., 2017; Zhou et al., 2019). In 2018, the novel linezolid resistance gene *poxtA* was first described from a clinical MRSA in Italy (Antonelli et al., 2018) and later demonstrated to be wider distributed among enterococci of various origins (Bender et al., 2019; Egan et al., 2020; Elghaieb et al., 2019; Freitas et al., 2020). However, it is not entirely clear whether these transferable resistance determinants can mediate linezolid resistance in various strain backgrounds,

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as e.g., experimental data question the role of *cfr*(B) in linezolid resistance in *E. faecium* or *E. faecalis* (Bender et al., 2016; Liu et al., 2014).

An important step in preventing the dissemination of linezolid-resistant staphylococci and enterococci in the nosocomial setting is the reliable identification and early recognition of colonized hosts. In 2018, the German Commission for Hospital Hygiene and Infection Prevention (KRINKO) published a recommendation for the prevention of infections with "enterococci with special antibiotic resistances" (KRINKO, 2018). The guideline recommended screening for linezolid-resistant enterococci (LRE), when more than a single case is notified within 3 months and an epidemiological link cannot be excluded. However, the technical implementation of an appropriate screening has not been specified. In the last 2 years 2 studies introduced screening agar media for the detection of linezolid-resistant staphylococci and enterococci. The first study, published by Nordmann et al., introduced a selective screening agar called "SuperLinezolid" medium (Nordmann et al., 2019). The authors used a non-selective Mueller-Hinton agar supplemented with aztreonam, colistin and amphothericin B to suppress the normal flora and a linezolid concentration of 1.5 mg/L. The study sample mainly included *S. epidermidis* isolates, for which, performance was very good. In the same year, Werner et al. introduced a selective LRE screening agar, which was based on Enterococcoselagar™ supplemented with 2 mg/L linezolid. Reliable screening to detect all LRE required a prolonged incubation of up to 48 h at 37°C (Werner et al., 2019). The agar revealed a sensitivity and specificity of 96.6% and 94.4%, respectively.

With the present study we assessed the performance of a novel, commercial screening agar called CHROMagar™ LIN-R. The agar allows a species- and genus-specific diagnostics based on a cleavage of chromogenic substrates and a detection of linezolid-resistant staphylococci and enterococci by a supplementation of linezolid. We tested this agar with a well-characterized collection of 48 clinical enterococci (40 *E. faecium* (29 linezolid-resistant [LIN-r]), 8 *E. faecalis* (7 LIN-r), 17 *S. aureus* isolates (14 LIN-r) and 70 coagulase-negative staphylococci (50 *S. epidermidis* (39 LIN-r), 20 *S. hominis* (15 LIN-r)).

2. Materials and methods

2.1. Strain collection

For CHROMagar™ LIN-R validation, we included 40 isolates of *E. faecium* (29 LIN-r) and 8 of *E. faecalis* (7 LIN-r). These strains have already been described and used in a previous assay validation (Werner et al., 2019). In addition, we compiled the following staphylococcal isolates' collection: 17 *S. aureus* (14 LIN-r), 50 *S. epidermidis* (all methicillin-resistant [MRSE]; 39 LIN-r), and 20 *S. hominis* (15 linezolid- and methicillin-resistant [MRSH]; 5 linezolid- and methicillin-susceptible [MSSH]). Isolates were partly described in recent publications (Bender et al., 2015; Layer et al., 2018; Wessels et al., 2018). All isolates had been sent previously from diagnostic laboratories to the National Reference Centre for strain characterization, typing and/or resistance confirmation as part of the routine work of the reference center activities (Supplementary Table S1). The following reference isolates were used: *E. faecalis* ATCC 29212, *E. faecium* ATCC 19434^T, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922.

2.2. Susceptibility testing

Susceptibilities to vancomycin and linezolid were determined by broth microdilution using cation-adjusted Mueller-Hinton broth (Becton-Dickinson, Heidelberg, Germany) according to EUCAST guidelines (EUCAST v8 to v10). Oxacillin resistance was deduced from results of a cefoxitin screen, oxacillin broth dilution MICs according to EUCAST and the detection of the *mecA* gene by PCR. Linezolid resistance is defined as R >4 mg/L for *Enterococcus* spp. and *Staphylococcus* spp. In case of contradictory results, linezolid MIC results were additionally confirmed by Etest® (bioMerieux, Nuertingen, Germany).

2.3. Determination of resistance genotypes

Genetic determinants coding for vancomycin resistance in *E. faecium* (*vanA, vanB*) and oxacillin resistance in *S. aureus* (*mecA*) were identified by the use of multiplex PCR (Bender et al., 2015; Bender et al., 2018). For enterococcal isolates, 23S rDNA mutations were determined by an amplification-restriction-based procedure as described recently (Werner et al. 2004). Additionally, the presence of the transferable LIN-r resistance genes *cfr*(B), *optrA* and *poxtA* was assessed using a multiplex PCR (Bender et al., 2019). For isolates where whole genome sequence data were available, we used LRE Finder to confirm the genetic basis of linezolid resistance in enterococci (Hasman et al., 2019). Staphylococcal isolates were screened for presence of the *cfr* gene by PCR according to (Kehrenberg and Schwarz, 2006). For *S. aureus* strains genes, encoding the central loop of domain V of 23S rRNA, were analyzed for mutations conferring linezolid resistance (Tsiodras et al., 2001). Detailed information can be found in Supplementary Table S1.

2.4. Testing CHROMagar™ LIN-R plates

All isolates were tested on CHROMagar™ LIN-R. CHROMagar LIN-r is a brand of CHROMagar (Paris, France), which is distributed in Germany by MAST Diagnostica (Heidelberg, Germany). In brief, 10 μL of a freshly prepared bacterial suspension of McFarland 0.5 (approximately 1.5×10^6 CFU) were inoculated onto the agar plates. Cell density was in a range that allowed single colony growth after streaking the sample out. Growth, colony morphology and color were determined after 24 hours (overnight) and 48 hours (2d) at 35 to 37°C.

2.5. Statistical analyses

Statistical values were calculated according to https://www.medcalc.org/calc/diagnostic_test.php.

3. Results and discussion

3.1. Validating CHROMagar™ LIN-R for enterococci

All linezolid-susceptible reference isolates performed accordingly on CHROMagar LIN-R (no growth after 48 hours). Out of 48 *E. faecium* and *E. faecalis* isolates, 47 performed as expected on CHROMagar LIN-R: While 35 of 36 linezolid-resistant enterococcal isolates grew on CHROMagar, 12 linezolid-susceptible isolates showed no growth. Importantly, to predict a correct screening result, an incubation of up to 48 hours was required. Growth of linezolid-resistant enterococci was delayed on CHROMagar LIN-R revealing only a slight growth after overnight incubation, meaning that growth was not visible as single colonies and colony color was not fully expressed (Fig. 1). A prolonged incubation was also required with the LRE screening agar introduced recently (Werner et al., 2019). For trained and experienced personal a differentiation based on colony color was possible for *E. faecalis* (turquoise blue) and *E. faecium* (dark blue) (Fig. 1).

A single *E. faecium* isolate UW19492 with an initial linezolid MIC of 8 mg/L (broth microdilution; LIN-r) did not grow on CHROMagar LIN-R. Repeated susceptibility testing by broth microdilution and Etest revealed contradictory MIC results for the respective isolate (broth microdilution: 4 mg/L (LIN-s) and 8 mg/L (LIN-r); Etest: 3 mg/L (LIN-s)). A molecular analysis of this isolate revealed no transferable linezolid resistance determinant and a 23S rDNA alleles' ratio of 4 (wildtype) to 2 (mutant). A wildtype to mutant 23S rDNA alleles' ratio of 4:2 is mostly associated with phenotypic linezolid resistance (Marshall et al., 2002; Werner et al., 2019). Due to these conflicting

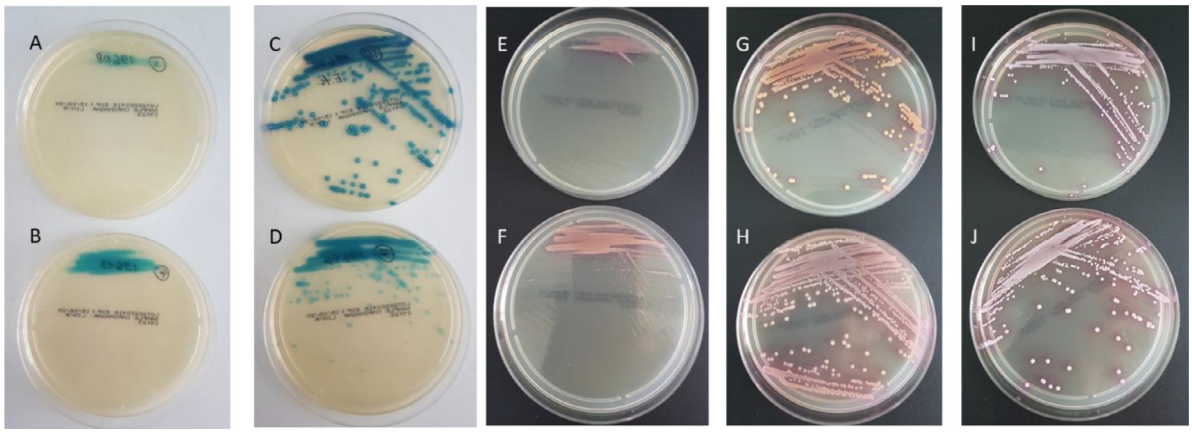

Fig. 1. Growth behavior of linezolid-resistant isolates of *E. faecium*, *E. faecalis*, *S. aureus* and *S. epidermidis*. *E. faecium* with linezolid (LIN) MICs of 8 (A) and 32 mg/L (B) after overnight incubation. *E. faecalis* (C; LIN MIC = 32 mg/L) and *E. faecium* (D; LIN MIC = 8 mg/L) grown for 48 hours. Isolates of *S. aureus* grown for 24 hours (E; LIN MIC = 16 mg/L) and (F, LIN MIC = 8 mg/L) and 48 hours (G, H; LIN MICs = 8 mg/L). Isolates of *S. epidermidis* with LIN MICs of >16 mg/L after 48 hours (I,J).

Table 1
Performance of CHROMagar™ LIN-R to identify linezolid-resistant staphylococci and enterococci.

Bacterium	LIN-r	LIN-s	Grown	Not grown	Specificity (%)	95% CI (%)	Sensitivity(%)	95% CI (%)
Enterococcus spp., *n* = 47*	35	12	35	12	100%	73.5–100	100%	90–100
S. aureus, *n* = 17	13	4	13	4	100%	47.8–100	100%	73.5–100
S. epidermidis, *n* = 50	39	11	38	12	100%	71.5–100	97.4%	86.5–99.94
S. hominis, *n* = 20	15	5	15	5	100%	47.8–100	100%	78.2–100
Sum	101	33	100	34	100%	89.7–100	99.01	94.6–99.97

E. faecium and *E. faecalis* were summarized as *Enterococcus* spp. LIN-r/-s, linezolid-resistant/-susceptible.
* An *E. faecium* isolate which appeared linezolid-resistant (8 mg/L) and linezolid-susceptible (4 mg/L) in repeated MIC tests and with different methods could not be categorized and thus was excluded from this analysis.

phenotypic and genotypic results we were unable to classify this isolate unambiguously as LIN-r or LIN-s and have excluded it from subsequent analyses and calculations (Table 1).

3.2. Validating CHROMagar™ LIN-R for staphylococci

All 4 linezolid-susceptible *S. aureus* isolates did not grow on CHROMagar LIN-R after up to 48 hours incubation at 35 to 37°C. As expected 16 of 17 linezolid-resistant *S. aureus* isolates grew on CHROMagar LIN-R (Table 1). The morphology of *S. aureus* colonies was mainly characterized by yellowish-pink colony color (Fig. 1**).** A single isolate with an initial linezolid MIC of 8 mg/L (LIN-r) showed no colony formation on the screening agar. In repeated MIC determinations the isolate revealed linezolid MIC values of 2 and 4 mg/L (LIN-s). Subsequent molecular analyses revealed a loss of the *cfr* encoding resistance plasmid. This single case demonstrates the excellent predictive power of the tested screening agar by identifying an isolate with an incorrect categorization which has been re-tested and then re-classified as “linezolid-susceptible”.

All eleven linezolid-susceptible *S. epidermidis* and 5 linezolid-susceptible *S. hominis* isolates did not grow on CHROMagar LIN-R after 48 hours incubation at 35 to 37°C. Altogether 42 of 43 linezolid-resistant *S. epidermidis* and *S. hominis* isolates grew on CHROMagar LIN-R. A single *S. epidermidis* isolate showed no colony formation but revealed a broth microdilution MIC of 16 mg/L (LIN-r) and an Etest MIC of 12 mg/L in repeated tests. Thus, this result must be considered “false negative”. Growth of coagulase-negative staphylococci was mainly characterized by whitish-pink colonies (mainly white colonies and a pink surrounding; Fig. 1).

3.3. Comparison to previously published linezolid screening media

In 2019, Nordmann *et al.* introduced a Super Linezolid agar which is based on Mueller-Hinton agar supplemented with 1.5 mg/L linezolid. The medium was additionally supplemented with aztreonam (2 mg/L), colistin (15 mg/L) and amphotericin B (5 mg/L) to suppress microorganisms of the normal intestinal flora. The agar was supposed to be used to detect linezolid-resistant Gram-positive bacteria. However, the collection contained primarily linezolid-resistant *S. epidermidis* (*n* = 13) and only a limited number of linezolid-resistant isolates of other genera and species such as *S. aureus* (*n* = 2), *S. capitis* (*n* = 1), and *E. faecium* (*n* = 1). Due to the low number of isolates other than *S. epidermidis*, a general assumption about performance of this agar with a concentration of linezolid of 1.5 mg/L cannot be made. Recently, our group introduced a LRE screening agar which was based on an enterococcal screening agar (Enterococcoselagar™, Becton-Dickinson, Heidelberg, Germany) supplemented with 2 mg/L linezolid (Werner et al., 2019). The agar was validated with several reference strains and 48 well-characterized enterococcal isolates (the same that were used for this study), which revealed a high sensitivity (98.8%) and specificity (100%). A feasibility study using 400 swab samples of enterococci sent to the Reference Centre for further analyses but of unknown linezolid status also revealed good results. An application study with a clinical partner is currently underway and preliminary results confirm the good specificity and sensitivity of predicting LRE directly from swabs (Weber et al., unpublished results).

There are reports about staphylococcal and enterococcal isolates containing linezolid resistance genes and which exhibited linezolid MICs of 4 mg/L or less (interpreted as linezolid-susceptible) (Li et al., 2015; Wardenburg et al., 2019). Whereas the clinical significance of this observation remains to be determined, it is obvious that such isolates represent a diagnostic challenge in general and in particular for the present CHROMagar™ LIN-R and all other corresponding screening agar media.

4. Conclusion

The present study includes the largest number of linezolid-resistant isolates of *Enterococcus* spp. and *Staphylococcus* spp. which has

been tested so far with a novel, chromogenic linezolid screening agar. CHROMagar LIN-R demonstrated an excellent performance to selectively grow linezolid-resistant isolates of *E. faecium, E. faecalis, S. aureus, S. epidermidis* and *S. hominis* while retaining sufficient specificity to suppress growth of linezolid-susceptible variants of the same species also after incubation of up to 48 hours and at 35 to 37° C. Summarizing all available data from this study, the agar revealed a specificity of 100% and a sensitivity of 99% (Table 1). Even species prediction from plates after 2 times overnight incubation was possible for the experienced and trained expert (Fig. 1). We did not recognize growth differences in relation to the linezolid MIC or a corresponding linezolid resistance mechanism. However, about half of the linezolid-resistant staphylococci already demonstrated single colony growth after overnight incubation, whereas the other half and almost all linezolid-resistant enterococci required longer incubation of up to 2 times overnight. The CHROMagar LIN-r will be commercially available soon. The instruction leaflet (package insert) will consider the results of the internal and external (this study) evaluations. Further analysis must now prove performance under routine clinical conditions with swab samples taken from hospital patients.

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Author contributions

F.L., G.W., conceptualization and project supervision; C.F., R.W., laboratory analyses and raw data collection; B.S., C.C., F.L., R.W., resources and sample pre-assessment; F.L., G.W., R.W., data curation and validation; G.W., writing of the original draft; all authors, review and editing.

Declaration of competing interest

All authors have nothing to declare.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2020.115301.

References

(ECDC), European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2019. In. Stockholm, Sweden: ECDC; 2020.

Antonelli A, D'Andrea MM, Brenciani A, Galeotti CL, Morroni G, Pollini S, et al. Characterization of poxtA, a novel phenicol-oxazolidinone-tetracycline resistance gene from an MRSA of clinical origin. J Antimicrob Chemother 2018;73:1763–9.

Bai B, Hu K, Zeng J, Yao W, Li D, Pu Z. Linezolid consumption facilitates the development of linezolid resistance in enterococcus faecalis in a tertiary-care hospital: a 5-year surveillance study. Microb Drug Resist 2019;25:791–8.

Bender J, Strommenger B, Steglich M, Zimmermann O, Fenner I, Lensing C, et al. Linezolid resistance in clinical isolates of Staphylococcus epidermidis from German hospitals and characterization of two cfr-carrying plasmids. J Antimicrob Chemother 2015;70:1630–8.

Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, et al. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug Resist Updat 2018;40:25–39.

Bender JK, Fleige C, Klare I, Fiedler S, Mischnik A, Mutters NT, et al. Detection of a cfr(B) variant in German enterococcus faecium clinical isolates and the impact on linezolid resistance in enterococcus spp. PLoS One 2016;11: e0167042.

Bender JK, Fleige C, Klare I, Werner G. Development of a multiplex-PCR to simultaneously detect acquired linezolid resistance genes cfr, optrA and poxtA in enterococci of clinical origin. J Microbiol Methods 2019;160:101–3.

Bender JK, Fleige C, Lange D, Klare I, Werner G. Rapid emergence of highly variable and transferable oxazolidinone and phenicol resistance gene optrA in German Enterococcus spp. clinical isolates'. Int J Antimicrob Agents 2018;52:819–27.

Cai J, Schwarz S, Chi D, Wang Z, Zhang R, Wang Y. Faecal carriage of optrA-positive enterococci in asymptomatic healthy humans in Hangzhou, China'. Clin Microbiol Infect 2019;25:630. e1-30.e6.

Chen H, Wang X, Yin Y, Li S, Zhang Y, Wang Q, et al. Molecular characteristics of oxazolidinone resistance in enterococci from a multicenter study in China. BMC Microbiol 2019;19:162.

Cuny C, Arnold P, Hermes J, Eckmanns T, Mehraj J, Schoenfelder S, et al. Occurrence of cfr-mediated multiresistance in staphylococci from veal calves and pigs, from humans at the corresponding farms, and from veterinarians and their family members. Vet Microbiol 2017;200:88–94.

Deshpande LM, Castanheira M, Flamm RK, Mendes RE. Evolving oxazolidinone resistance mechanisms in a worldwide collection of enterococcal clinical isolates: results from the SENTRY antimicrobial surveillance program. J Antimicrob Chemother 2018;73:2314–22.

Egan SA, Shore AC, O'Connell B, Brennan GI, Coleman DC. Linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* from hospitalized patients in Ireland: high prevalence of the MDR genes optrA and poxtA in isolates with diverse genetic backgrounds. J Antimicrob Chemother 2020;75:1704–11.

Elghaieb H, Freitas AR, Abbassi MS, Novais C, Zouari M, Hassen A, et al. Dispersal of linezolid-resistant enterococci carrying poxtA or optrA in retail meat and food-producing animals from Tunisia. J Antimicrob Chemother 2019;74:2865–9.

Elghaieb H, Tedim AP, Abbassi MS, Novais C, Duarte B, Hassen A, et al. From farm to fork: identical clones and Tn6674-like elements in linezolid-resistant Enterococcus faecalis from food-producing animals and retail meat. J Antimicrob Chemother 2020;75:30–5.

Freitas AR, Tedim AP, Duarte B, Elghaieb H, Abbassi MS, Hassen A, et al. Linezolid-resistant (Tn*6246*::*fexB-poxtA*) *Enterococcus faecium* strains colonizing humans and bovines on different continents: similarity without epidemiological link. J Antimicrob Chemother 2020;75:2416–23.

Gawryszewska I, Żabicka D, Hryniewicz W, Sadowy E. Linezolid-resistant enterococci in Polish hospitals: species, clonality and determinants of linezolid resistance. Eur J Clin Microbiol Infect Dis 2017;36:1279–86.

Hasman H, Clausen PTLC, Kaya H, Hansen F, Knudsen JD, Wang M, et al. LRE-Finder, a Web tool for detection of the 23S rRNA mutations and the optrA, cfr, cfr(B) and poxtA genes encoding linezolid resistance in enterococci from whole-genome sequences. J Antimicrob Chemother 2019;74:1473–6.

Heininger A, Zimmermann S, Bootsveld C, Boutin S, Nurjadi D. Low prevalence of combined linezolid- and vancomycin-resistant Enterococcus faecium from hospital admission screening in an endemic region in Germany. J Glob Antimicrob Resist 2020.

Jian Y, Lv H, Liu J, Huang Q, Liu Y, Liu Q, et al. 'Dynamic changes of staphylococcus aureus susceptibility to vancomycin, teicoplanin, and linezolid in a central teaching hospital in Shanghai, China, 2008-2018′. Front Microbiol 2020;11:908.

Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant Staphylococcus isolates. Antimicrob Agents Chemother 2006;50:1156–63.

Klare I, Bender JK, Markwart R, Reuss A, Abu SIn M, Eckmanns T, et al. Properties, frequencies and distribution of vancomycin-resistant enterococci in Germany - Update 2017/2018 [German]'. Epidemiologisches Bulletin 2019;37:365–72.

Klare I, Fleige C, Geringer U, Thurmer A, Bender J, Mutters NT, et al. Increased frequency of linezolid resistance among clinical *Enterococcus faecium* isolates from German hospital patients. J Glob Antimicrob Resist 2015;3:128–31.

KRINKO. Hygienic measures for the prevention of infections with enterococci with special antibiotic resistances - recommendation of the commission for hospital hygiene in Germany (KRINKO). Bundesgesundheitsblatt-Bundesgesundheitsforschung-Bundesgesundheitsschutz 2018;61:1310–61.

Layer F, Strommenger B, Cuny C, Noll I, Klingeberg A, Werner G. Properties, frequencies and distribution of MRSA in Germany - Update 2017/2018 [German]'. Epidemiologisches Bulletin 2019;42:437–42.

Layer F, Vourli S, Karavasilis V, Strommenger B, Dafopoulou K, Tsakris A, et al. Dissemination of linezolid-dependent, linezolid-resistant Staphylococcus epidermidis clinical isolates belonging to CC5 in German hospitals. J Antimicrob Chemother 2018;73:1181–4.

Li S, Zhao L, Zheng B, Shen P, Ji J, Lv J, et al. Identification and characterization of cfr-positive *Staphylococcus aureus* isolates from community-onset infectious patients in a county hospital in China. J Med Microbiol 2015;64:910–5.

Liu Y, Wang Y, Schwarz S, Wang S, Chen L, Wu C, et al. Investigation of a multiresistance gene cfr that fails to mediate resistance to phenicols and oxazolidinones in *Enterococcus faecalis*. J Antimicrob Chemother 2014;69:892–8.

Markwart R, Willrich N, Haller S, Noll I, Koppe U, Werner G, et al. The rise in vancomycin-resistant *Enterococcus faecium* in Germany: data from the German Antimicrobial Resistance Surveillance (ARS). Antimicrob Resist Infect Control 2019;8:147.

Marshall SH, Donskey CJ, Hutton-Thomas R, Salata RA, Rice LB. Gene dosage and linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis*. Antimicrob Agents Chemother 2002;46:3334–6.

Nordmann P, Rodriguez-Villodres A, Poirel L. A selective culture medium for screening linezolid-resistant gram-positive bacteria. Diagn Microbiol Infect Dis 2019;95:1–4.

Pfaller MA, Cormican M, Flamm RK, Mendes RE, Jones RN. Temporal and geographic variation in antimicrobial susceptibility and resistance patterns of enterococci: results from the SENTRY antimicrobial surveillance program, 1997-2016′. Open Forum Infect Dis 2019;6:S54–62.

Rouard C, Aslangul E, Rivière A, Deback C, Butel MJ, Doucet-Populaire F, et al. Mutation in the L3 ribosomal protein could be associated with risk of selection of high-level linezolid-resistant staphylococcus epidermidis Strains. Microb Drug Resist 2017;23:462–7.

Ruiz-Ripa L, Feßler AT, Hanke D, Eichhorn I, Azcona-Gutiérrez JM, Alonso CA, et al. Mechanisms of linezolid resistance among clinical staphylococcus spp. in Spain: spread of methicillin- and linezolid-resistant S. epidermidis ST2′. Microb Drug Resist 2020 May 22. doi: 10.1089/mdr.2020.0122. Online ahead of print.

Sadowy E. Linezolid resistance genes and genetic elements enhancing their dissemination in enterococci and streptococci. Plasmid 2018;99:89–98.

Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, et al. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet 2001;358:207–8.

Walter J, Haller S, Blank HP, Eckmanns T, Sin MAbu, Hermes J. Incidence of invasive meticillin-resistant *Staphylococcus aureus* infections in Germany, 2010 to 2014′. Euro Surveill 2015;20:.

Walter J, Noll I, Feig M, Weiss B, Claus H, Werner G, et al. Decline in the proportion of methicillin resistance among *Staphylococcus aureus* isolates from non-invasive samples and in outpatient settings, and changes in the co-resistance profiles: an analysis of data collected within the Antimicrobial Resistance Surveillance Network, Germany 2010 to 2015′. BMC Infect Dis 2017;17:169.

Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, et al. A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. J Antimicrob Chemother 2015;70:2182–90.

Wardenburg KE, Potter RF, D'Souza AW, Hussain T, Wallace MA, Andleeb S, et al. Phenotypic and genotypic characterization of linezolid-resistant *Enterococcus faecium* from the USA and Pakistan. J Antimicrob Chemother 2019;74:3445–52.

Werner G, Fleige C, Klare I, Weber RE, Bender JK. Validating a screening agar for linezolid-resistant enterococci. BMC Infect Dis 2019;19:1078.

Werner G, Strommenger B, Klare I, Witte W. Molecular detection of linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* by use of 5′ nuclease real-time PCR compared to a modified classical approach. J Clin Microbiol 2004;42:5327–31.

Wessels C, Strommenger B, Klare I, Bender J, Messler S, Mattner F, et al. Emergence and control of linezolid-resistant *Staphylococcus epidermidis* in an ICU of a German hospital. J Antimicrob Chemother 2018;73:1185–93.

Zhou W, Gao S, Xu H, Zhang Z, Chen F, Shen H, et al. Distribution of the optrA gene in Enterococcus isolates at a tertiary care hospital in China. J Glob Antimicrob Resist 2019;17:180–6.

Zou J, Xia Y. Molecular characteristics and risk factors associated with linezolid-resistant *Enterococcus faecalis* infection in Southwest China. J Glob Antimicrob Resist 2020;22:504–10.