J Antimicrob Chemother 2023; **78**: 2185–2191
https://doi.org/10.1093/jac/dkad218 Advance Access publication 20 July 2023

Journal of Antimicrobial Chemotherapy

CHROMAgar™ LIN-R as an efficient screening tool to assess the prevalence of linezolid-resistant enterococci in German hospital patients—a multicentre study approach, 2021–2022

Jennifer K. Bender [1]*, Elsa Baufeld[2], Karsten Becker [2], Heike Claus[3], Anna Dudakova[4], Achim Dörre[5], Nikoletta Fila[6], Carola Fleige[1], Axel Hamprecht[7], Armin Hoffmann[8], Michael Hogardt[9], Achim J. Kaasch[10,11], Axel Kola[12], Nancy Kriebel[1], Franziska Layer-Nicolaou[1], Matthias Marschal[6], Ernst Molitor [13], Nico T. Mutters [14], Jan Liese[6], Claudia Nelkenbrecher[15], Bernd Neumann [15], Holger Rohde [8], Jörg Steinmann[15], Michael Sörensen[16], Philipp Thelen[7], Michael Weig[4], Andreas E. Zautner[10,11] and Guido Werner[1]

[1]Nosocomial Pathogens and Antibiotic Resistances Unit, Department of Infectious Diseases, National Reference Centre for Staphylococci and Enterococci, Robert Koch Institute, Wernigerode, Germany; [2]Friedrich Loeffler Institute of Medical Microbiology, University Medicine Greifswald, Greifswald, Germany; [3]Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany; [4]Institute for Medical Microbiology and Virology, University Medical Center Göttingen, Göttingen, Germany; [5]Department of Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany; [6]Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany; [7]Institute for Medical Microbiology and Virology, Carl von Ossietzky University Oldenburg and Klinikum Oldenburg, Oldenburg, Germany; [8]Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [9]Institute of Medical Microbiology and Infection Control, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt am Main, Germany; [10]Faculty of Medicine, Institute of Medical Microbiology and Hospital Hygiene, Otto-von-Guericke-University, Magdeburg, Germany; [11]Center for Health and Medical Prevention (CHaMP), Otto-von-Guericke University Magdeburg, Magdeburg, Germany; [12]Institute of Hygiene and Environmental Medicine, Charité Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; [13]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany; [14]Institute for Hygiene and Public Health, University Hospital Bonn, Bonn, Germany; [15]Institute of Hospital Hygiene, Medical Microbiology and Infectious Diseases, Nuremberg General Hospital, Paracelsus Medical University, Nuremberg, Germany; [16]Laboratory Prof. Gisela Enders and Colleagues, Stuttgart, Germany

*Corresponding author. E-mail: benderj@rki.de

Received 16 April 2023; accepted 29 June 2023

Background: In recent years, an increasing number of linezolid-resistant enterococci (LRE) was recognized at the German National Reference Centre (NRC) for Enterococci. National guidelines on infection prevention recommend screening for LRE in epidemiologically linked hospital settings without referring to a reliable and rapid diagnostic method. Since 2020, CHROMAgar™ provide a chromogenic linezolid screening agar, LIN-R, suitable to simultaneously screen for linezolid-resistant staphylococci and enterococci.

Objectives: To assess the applicability of CHROMAgar™ LIN-R in clinical settings for detecting LRE directly from patient material and to infer prevalence rates of LRE amongst German hospital patients.

Methods: During the 3-month trial period, clinical samples were plated on CHROMAgar™ LIN-R. Antimicrobial susceptibility testing was performed using VITEK2 or disc diffusion. At the NRC, linezolid resistance was determined by broth microdilution, multiplex-PCR for *cfr/optrA/poxtA* and by a restriction-based assay for 23S rDNA mutations.

Results: The 12 participating study sites used 13 963 CHROMAgar™ LIN-R plates during the study period. Of 442 presumptive LRE, 192 were confirmed by phenotypic methods. Of these, 161 were received by the NRC and 121 (75%) were verified as LRE. Most of LR-*E. faecium* 53/81 (65%) exhibited a 23S rRNA gene mutation as the sole resistance-mediating mechanism, whereas *optrA* constituted the dominant resistance trait in LR-*E. faecalis* [39/40 (98%)]. Prevalence of LRE across sites was estimated as 1% (ranging 0.18%–3.7% between sites).

Conclusions: CHROMAgar™ LIN-R represents a simple and efficient LRE screening tool in hospital settings. A high proportion of false-positive results demands validation of linezolid resistance by a reference method.

Introduction

The synthetic antibiotic linezolid (LIN) can be applied as a last resort treatment option (https://www.who.int/publications/i/item/2021-aware-classification) for infections caused by multidrug-resistant staphylococci and enterococci such as methicillin-resistant *Staphylococcus epidermidis* or *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE). Shortly after the approval of linezolid in the year 2000, phenotypic resistance was reported[1–4] and increased prescription of the substance has shown a clear association with resistance progression.[5,6] Phenotypic resistance is either the result of chromosomal mutations within the drug-binding site in the 23S ribosomal RNA of the 50S subunit and/or of expression of transferable resistance determinants such as the ribosomal protection proteins *poxtA* and *optrA*, or the methyltransferase *cfr* (reviewed by[7,8]). The extent of the contribution of transferable resistance genes, especially of *cfr*, in mediating resistance is still under debate, as some experimental studies failed to demonstrate phenotypic resistance in the presence of *cfr* in enterococci.[9,10] Nevertheless, genotypic identification of a resistance trait as mentioned must not be neglected even in the absence of phenotypic resistance, as these genes might confer resistance under linezolid selective pressure *in vivo*.

Generally, resistance surveillance systems did not report increasing resistance towards linezolid in clinical isolates since the approval of linezolid for clinical use.[11–16] However, data from the German Antimicrobial Resistance Surveillance (ARS) demonstrated a recent increase in linezolid resistance among invasive *E. faecium* isolates from 0.6% in 2019% to 1.2% in 2021 (https://ars.rki.de/). A trend towards an increased prevalence of linezolid-resistant enterococci (LRE) and linezolid-resistant *S. epidermidis* (LRSE) clinical isolates has also been recognized at the German National Reference Centre for Staphylococci and Enterococci (NRC),[17,18] and increasing prevalence has been linked to excessive clinical linezolid use.[19] Nonetheless, a recent study suggests that the prevalence of LRE across Germany and Europe remains at low levels.[15]

To contain LRE in hospital environments and to avoid subsequent transmission and outbreaks with multidrug-resistant enterococci in immunocompromised individuals, appropriate infection prevention and control measures are of particular importance. Hence in 2018, the German Commission for Hospital Hygiene and Infection Prevention (KRINKO) published a national guideline recommending screening for LRE when more than one case is notified within 3 months in such a contained setting and when an epidemiological link cannot be excluded.[20] A rapid diagnostic screening tool was not available at that time. Since 2020, CHROMAgar™ provide a chromogenic screening agar, LIN-R, suitable for selective detection of LRE and linezolid-resistant staphylococci (LRS). Implementation and performance of the screening agar was assessed to some extent, however, not on a large scale.[21–23]

We conducted an observational study at 12 study sites across Germany. We aimed (i) to investigate the applicability of CHROMAgar™ LIN-R under routine practice, (ii) to estimate and compare prevalence rates of LRE at study sites, (iii) to record the proportion of transmissible LIN-r genes and (iv) to correlate genotype with phenotype regarding the presence of LIN resistance genes/mutations and LIN minimal inhibitory concentrations (MICs).

Materials and methods

Study setting

Twelve laboratories serving mainly university hospitals in nine out of 16 German federal states agreed to participate in the CHROMAgar™ LIN-R multicentre study. Study participants were provided with CHROMAgar™ LIN-R agar plates by the German distributor MAST Diagnostica (Reinfeld, Germany) in cooperation with the producer CHROMAgar™ (Paris, France). The study period was set to 3 months, between September 2021 and December 2021; however, in case of delayed agar plate delivery an extension was granted, resulting in a full-length, 3-month screening period. Detected and verified LRE (see next for susceptibility testing and species identification onsite) were sent to the NRC for Staphylococci and Enterococci, Wernigerode, Germany, for further analyses and data consolidation.

Study material

Study participants were asked to extend their routine VRE screening for LRE using CHROMAgar™ LIN-R focussing on rectal swabs. At one partner site, where routine rectal swabbing was not implemented, or on other rare occasions, clinical material such as urine samples were examined.

Antimicrobial susceptibility testing and species identification onsite

Participating laboratories received rectal swabs or other clinical material that were directly streaked on CHROMAgar™ LIN-R. Blue colonies, indicating growth of LRE, were further analysed. MALDI-TOF mass spectrometry (VITEK® MS system, bioMérieux, Marcy-l'Étoile, France or MALDI Biotyper®, Bruker Daltonics GmbH & Co. KG, Bremen, Germany) was used for species identification and either VITEK2 or disc diffusion assays (bioMérieux, Nuertingen, Germany) were carried out for linezolid susceptibility testing. Verified LRE were then sent to the NRC for downstream analysis.

Antimicrobial susceptibility testing at the NRC

Isolates were cultivated on sheep blood agar followed by broth microdilution (BMD) using an in-house and accredited procedure and by applying EUCAST clinical breakpoints for resistance determination (EUCAST v.11; https://www.eucast.org/clinical_breakpoints). For *Enterococcus* spp., linezolid resistance is defined as MIC > 4 mg/L. Linezolid MIC results were additionally assessed using Etest® (bioMérieux, Nuertingen, Germany). As BMD is considered the reference method, we will refer to LRE, determined at the NRC, as isolates demonstrating an MIC > 4 mg/L in BMD.

Determination of putative resistance mechanism(s)

All isolates received by the NRC were subjected to DNA extraction and multiplex-PCR in order to screen for *cfr*, *cfr(B)*, *optrA* and *poxtA* resistance determinants as described recently.[24] Further, G2576T 23S rRNA gene mutations were determined by an amplification-restriction-based procedure as published previously.[25]

Data analysis

Statistical analyses were carried out using R/R Studio (v.4.1.2). For determining LRE prevalence, only the first isolate of a patient was considered, copy strains were excluded. Repetitive screening of negative patients was not accounted for, meaning that a patient with a negative screening result was potentially examined again. Hence, prevalence was defined as the proportion of verified LRE among all non-copy samples investigated on CHROMAgar™ LIN-R. (Table 1 and Figure S1 available as Supplementary data at *JAC* Online). For assessing urine-specific prevalence, only those sites were considered that had analysed ≥10 urine samples (study site 12 excluded, see Table 1). A Mann–Whitney *U*-test (significance level of 0.05) was carried out to compare material-specific prevalence rates. The number of false positives was calculated as the percentage of linezolid-susceptible *Enterococcus* spp. (LSE) of all enterococci detected; here, copy strains were included.

Ethics

The LRE screening was implemented as part of the general screening procedure for VRE or multidrug-resistant bacteria at participating sites and thus was exempt from additional ethical approval.

Results and discussion

The 12 study sites collectively used 13 963 CHROMAgar™ LIN-R agar plates (median 1188; IQR 468–1595) (Table 1). Around 90% of all samples (11 511/12 788; information available from 9/12 study sites) were retrieved from rectal swabs, followed by urine (9.5%; one study site solely collected urine) and other material (<1%; Table 1 and flowchart Figure S1).

Where visual inspection was documented (7/12 study sites), 797 plates (median 53, IQR 48–235) showed growth of any type of microorganism and 48% of these plates revealed contamination by non-targeted organisms such *Saccharomyces* spp. or other fungi (numbers not shown). Growth of *Candida* spp., albeit to a lower extent, has also been observed by a recent study assessing the performance of CHROMAgar™ LIN-R from blood cultures and nasal swab screening samples.[22] Although the technical note of CHROMAgar™ LIN-R states that growth of Gram-negative bacteria and yeast is inhibited (https://www.chromagar.com/wp-content/uploads/2021/11/NT_EXT_119_V1.0-1.pdf), residual contamination is obviously not avoided. However, LRS and LRE should easily be differentiated due to their typical colony appearance of pink and steel blue colour.

In total, 448 enterococci, appearing as blue colonies on CHROMAgar™ LIN-R, were identified within our study period of which 442 results from antimicrobial susceptibility testing (AST) were available (Figure S1). Of those, 192/442 (43%) enterococci were verified as LRE by either VITEK2 or disc diffusion assays on-site (Table 1 and Figure S1). For 250/422 *Enterococcus* spp. linezolid resistance could not be verified, corresponding to 57% false positives (ranging 0%–93% between study sites) (Table 1). The variability of false positives between sites could be the result of inconsistent storage or inappropriate usage of CHROMAgar™ LIN-R, but remained unresolved at the end of the study period. The overall percentage of LSE grown on CHROMAgar™ LIN-R is a strikingly high number compared to 7.5% of LSE, linezolid-susceptible staphylococci, *Lactobacillus* spp. and Gram-negatives as observed by Girlich *et al.*[22] In another recent validation report of CHROMAgar™ LIN-R in routine practice, specificity was estimated at 90% due to growth of non-targeted organisms including LSE and linezolid-susceptible staphylococci.[21] Although we would like to stress that comparing our results from rectal samples with those from nasal swabs or referring to accuracy of a test is imperfect, it is known that heavily inoculated samples may cause growth of linezolid-susceptible bacteria (see CHROMAgar™ LIN-R technical note). Likewise, an incubation period of 36–48 h, which is indispensable to achieve sufficient growth and colouring of LRE, provides enough time for non-targeted organisms to thrive. The number of false positives is a trade-off for sensitivity at the expense of specificity, but reasonable as CHROMAgar™ LIN-R is intended to be used as rapid screening tool. In any case, the manufacturer strongly recommends confirmation of species identification and verification of linezolid non-susceptibility, which was also performed for isolates identified in our study.

Considering only LRE with a secondary AST result and by dismissing all copy strains, we estimated the prevalence as 1% across all study sites (ranging 0.18%–3.7% between sites) (Table 1). Prevalence of linezolid-resistant *E. faecium* was 1.2% for blood culture isolates as inferred from the German ARS system for 2021 (https://ars.rki.de). It must be noted that the ARS system collected resistance rates for two *Enterococcus* species only (*E. faecium* and *E. faecalis*) and from invasive isolates rather than screening samples, thus preventing a direct comparison of the two outcomes.

In our study, information about screening material was available for seven out of 12 participants with >90% of rectal swab origin (see previously). Three sites investigated urine samples, but only two collected >10 urine samples during our study period. Material-specific prevalence was lower for urine samples (0.5%) than for rectal swabs (0.9%) (Table 1); however, this difference was not statistically significant ($P = 0.09$).

When using CHROMAgar™ LIN-R to assess the prevalence of LRE and LRS, Dembicka and colleagues detected one single LRS among 159 patients tested, which corresponds to an overall prevalence of 0.63%.[21] Although this was not the focus of our study, seven participating sites recorded the growth of LRS, yielding a prevalence of 0.34% (data not shown). We again would like to note that we defined prevalence as the proportion of LRS or LRE among all non-copy samples investigated, as we were unable to exclude repetitive negative screening samples from our calculations. This could potentially introduce a bias towards a lower prevalence and is a limitation of this study. However, the risk of bias may be low due the high number of negative samples.

The NRC received 161 of 192 enterococcal isolates verified as LRE at the study sites that were further analysed with respect to AST and putative underlying resistance mechanisms. At the NRC, linezolid resistance was verified for 121/161 (75%) isolates using BMD and for 111/161 (69%) isolates using Etest®. It is worth noting that the predominant share of the linezolid-susceptible isolates (susceptible at the NRC, but resistant at study sites), demonstrated an MIC of 4 mg/L in BMD (34/40, 85%) and Etest® (27/50, 54%), a value just

Table 1. Total numbers and calculated LRE prevalence based on data collected at 12 study sites participating in the German CHROMAgar™ LIN-R multicentre study, 2021–2022

	Study site 1	Study site 2	Study site 3	Study site 4	Study site 5	Study site 6	Study site 7	Study site 8	Study site 9	Study site 10	Study site 11	Study site 12	Total
Material screened—total	1387	470	2220	460	2263	1178	1295	1198	182	247	778	2285	13 963
Material total—w/o copy strains	1387	469	2220	460	2263	1178	1295	1198	181	231	778	2251	13 911
Material only rectal swabs—w/o copy strains	1387	n.a.	2220	n.a.	2263	1178	1295	n.d.	109	n.a.	778	2247	11 477
Material only urine—w/o copy strains	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.	n.d.	1198	15	n.a.	n.d.	2	1215
LRE total	15	7	13	17	4	8	9	5	3	23	5	83	192
LRE total—w/o copy strains	15	6	13	17	4	8	9	5	2	7	5	49	140
LRE only rectal swabs—w/o copy strains	15	n.a.	13	n.a.	4	8	9	n.d.	1	n.a.	5	48	103
LRE only urine—w/o copy strains	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.	n.d.	5	0	n.a.	n.d.	1	6
Prevalence LRE—total (%)	1.08	1.28	0.59	3.7	0.18	0.68	0.69	0.42	1.1	3.03	0.64	2.18	1.0
Prevalence LRE—only rectal swabs (%)	1.08	n.a.	0.59	n.a.	0.18	0.68	0.69	n.d.	0.92	n.a.	0.64	2.14	0.9
Prevalence LRE—only urine (%)	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.	n.d.	0.42	0	n.a.	n.d.	n.c.	0.5
LSE total	0	3	0	89	10	106	11	0	2	0	6	23	250
False positives (%)	0	30	0	84	71	93	55	0	40	0	55	22	57

n.a., data not available; n.c., data not considered due to low sample size; n.d., not determined; w/o, without.

Table 2. Distribution of acquired resistance genes *cfr*, *optrA*, *poxtA* and of 23S rDNA G2576T mutations in phenotypically linezolid-resistant and linezolid-susceptible *E. faecium* and *E. faecalis* isolates of the German CHROMAgar™ LIN-R multicentre study, 2021–2022

E. faecium (*N* = 108)	*cfr*	*optrA*	*poxtA*	23S rDNA G2576T	*n*	Percentage (%)
Susceptible[a] (*n* = 27)						
	–	–	–	+	6	22.0
	–	–	–	–	6	22.0
	–	–	+	–	14	52.0
	–	+	–	–	1	4.0
Total					27	100
resistant[a] (*n* = 81)						
	–	–	–	+	53	65.4
	–	–	–	–	1	1.2
	–	–	+	–	11	13.6
	–	+	–	–	14	17.3
	–	+	+	–	2	2.5
Total					81	100
E. faecalis (*N* = 53)	*cfr*	*optrA*	*poxtA*	23S rDNA G2576T	*n*	Percentage (%)
Susceptible[a] (*n* = 13)						
	–	–	–	–	3	23.1
	–	–	+	–	2	15.4
	–	+	–	–	7	53.8
	+	+	+	–	1	7.7
Total					13	100
resistant[a] (*n* = 40)						
	–	–	–	+	1	2.0
	–	+	–	–	38	95.0
	–	+	+	–	1	2.0
Total					40	100

[a]According to BMD results determined at the NRC.

below the clinical breakpoint according to EUCAST (>4 mg/L). For some bacteriostatic antimicrobial agents, such as linezolid, EUCAST provides specific reading instructions to address the phenomenon of 'trailing growth' (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2022_manuals/Reading_guide_BMD_v_4.0_2022.pdf). Since trailing growth often occurs around an MIC of 4 mg/L, some difficulty arises when aiming to differentiate between resistant and susceptible enterococci during visual inspection. The resulting diagnostic dilemma is yet to be solved.

In our study, 21/27 (78%) linezolid-susceptible *E. faecium* and 10/13 (77%) linezolid-susceptible *E. faecalis* either harboured a G2576T 23S rRNA gene mutation or any of the three resistance genes *cfr*, *poxtA* or *optrA* (Table 2). Of the 40 LSE, only 6 (2.4%) demonstrated an MIC of ≤2 mg/L by BMD but, in some instances, harboured resistance determinants or mutations (3× no mutation/no gene, 2× G2576T mutation/no gene, 1× no mutation/*poxtA*). One linezolid-susceptible *E. faecalis* (BMD and Etest® MIC = 4 mg/L) harboured the *cfr* gene and turned out positive for *optrA* and *poxtA*. Unexpectedly, of the 40 LSE (*n* = 27 *E. faecium* and *n* = 13 *E. faecalis*), only nine (23%) neither showed a G2576T conversion nor one of the known acquired resistance loci (Table 2). It is well known that some of these mobile resistance determinants do not necessarily mediate phenotypical resistance under standard laboratory conditions.[9,10] Also, a LIN MIC creep was demonstrated for MRSA isolates over a period of 11-years in bloodstream isolates from Taiwan, which could be the result of increased gene expression or stepwise accumulation of allelic mutations.[26] Thus, the presence of silent resistance genes or chromosomal alterations must not be neglected and those isolates could be considered 'potentially resistant', meaning they might develop phenotypic resistance under LIN selective pressure more rapidly.

Since BMD is still the gold standard for AST in enterococci, only those isolates with a linezolid MIC > 4 mg/L by BMD (*n* = 121) were considered for the following analyses. Most of those LRE [94/121 (78%)] were vancomycin-susceptible, 26/121 (21%) were vancomycin- and linezolid-resistant and one isolate additionally exhibited tigecycline resistance (*vanB*-positive *E. faecium*) (not shown). MALDI-TOF identification was verified by 23S-specific PCR assigning 40/121 (33%) to *E. faecalis* and 81/121 (67%) to *E. faecium* (Table 2). Vancomycin resistance (*n* = 27) was solely detected for *E. faecium* isolates. This is an interesting and important finding, as most LRE isolates (94/121)

would have been missed with a VRE-centred routine screening procedure. However, these vancomycin-susceptible LRE may constitute an important reservoir for transferable linezolid resistance determinants.

With respect to the prevalence of *cfr*, *optrA*, *poxtA* and the G2576T 23S rRNA gene mutation in our LRE isolates, we detected an uneven distribution among both species (Table 2). While the dominant resistance trait in *E. faecium* was represented by the 23S rRNA gene mutation (53/81, 65%), almost all phenotypically resistant *E. faecalis* possessed the gene encoding the ribosomal protection protein OptrA (38/40, 95% & one *optrA*/*poxtA*-positive *E. faecalis*) (Table 2). Nonetheless, acquired resistance determinants *optrA* and *poxtA* were also present in linezolid-resistant *E. faecium* isolates albeit to a lesser extent (17.3% *optrA*, 13.6% *poxtA* and 2.5% *optrA* and *poxtA*). Interestingly, we could not detect a combination of the 23S rRNA gene mutation and *optrA* or *poxtA* in linezolid-resistant *Enterococcus* spp. (Table 2). Our observations are in line with other studies analysing the basis of linezolid resistance in enterococci and demonstrating the imbalance of resistance traits between *E. faecium* and *E. faecalis*, the latter being in favour of transferable resistance determinants.[27–29] The reason for this phenomenon is currently unknown and should be addressed in future investigations.

In summary, our multicentre study approach assessing the applicability of CHROMAgar™ LIN-R confirmed the ease and beneficial usage in routine screening practice. Confirmatory susceptibility testing is necessary to compensate for reduced specificity when using patient material. We identified an overall prevalence of 1% across all study sites and species-specific resistance traits for *E. faecium* and *E. faecalis*. As highlighted, we observed a markedly high number of genotypically resistant, but phenotypically susceptible *Enterococcus* spp. that might constitute latent risks and important reservoirs for mobile genetic resistance determinants.

Acknowledgements

We would like to thank Alberto Lerner (CHROMAgar™) and Kristin Schröder (MAST Diagnostica GmbH) for providing CHROMAgar™ LIN-R plates. Especially we would like to acknowledge the excellent support by Christine Günther, Sabine Jürgenfeldt, Lisa Marr, Francis Meier, Birgit Pasemann, Petra Vilbrandt and Annette Weller.

Funding

CHROMAgar™ LIN-R agar plates were provided free of charge by MAST Diagnostica GmbH (Reinfeld, Germany) in collaboration with CHROMAgar™ (Paris, France). Neither the manufacturer nor the distributor had any influence on the study design, data collection, evaluation and interpretation of the study results. The work of the NRC is funded by a grant from the Federal Ministry of Health, Germany (current period, 2018–2022).

Transparency declarations

All authors declare no conflict of interest.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online.

References

1 Auckland C, Teare L, Cooke F *et al*. Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. *J Antimicrob Chemother* 2002; **50**: 743–6. https://doi.org/10.1093/jac/dkf246

2 Bassetti M, Farrel PA, Callan DA *et al*. Emergence of linezolid-resistant *Enterococcus faecium* during treatment of enterococcal infections. *Int J Antimicrob Agents* 2003; **21**: 593–4. https://doi.org/10.1016/S0924-8579(03)00090-6

3 Meka VG, Gold HS. Antimicrobial resistance to linezolid. *Clin Infect Dis* 2004; **39**: 1010–5. https://doi.org/10.1086/423841

4 Seedat J, Zick G, Klare I *et al*. Rapid emergence of resistance to linezolid during linezolid therapy of an *Enterococcus faecium* infection. *Antimicrob Agents Chemother* 2006; **50**: 4217–9. https://doi.org/10.1128/AAC.00518-06

5 Jian Y, Lv H, Liu J *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. https://doi.org/10.3389/fmicb.2020.00908

6 Wessels C, Strommenger B, Klare I *et al*. Emergence and control of linezolid-resistant *Staphylococcus epidermidis* in an ICU of a German hospital. *J Antimicrob Chemother* 2018; **73**: 1185–93. https://doi.org/10.1093/jac/dky010

7 Bender JK, Cattoir V, Hegstad K *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. https://doi.org/10.1016/j.drup.2018.10.002

8 Schwarz S, Zhang W, Du XD *et al*. Mobile oxazolidinone resistance genes in gram-positive and gram-negative bacteria. *Clin Microbiol Rev* 2021; **34**: e0018820. https://doi.org/10.1128/CMR.00188-20

9 Bender JK, Fleige C, Klare I *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. https://doi.org/10.1371/journal.pone.0167042

10 Liu Y, Wang Y, Schwarz S *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. https://doi.org/10.1093/jac/dkt459

11 Chen Y, Ji J, Ying C *et al*. Blood bacterial resistant investigation collaborative system (BRICS) report: a national surveillance in China from 2014 to 2019. *Antimicrob Resist Infect Control* 2022; **11**: 17. https://doi.org/10.1186/s13756-022-01055-5

12 Coombs GW, Daley DA, Yee NWT *et al*. Australian Group on Antimicrobial Resistance (AGAR) Australian Enterococcal Sepsis Outcome Programme (AESOP) annual report 2020. *Commun Dis Intell (2018)* 2022; **46**: 1–12.

13 Diekema DJ, Hsueh PR, Mendes RE *et al*. The microbiology of bloodstream infection: 20-year trends from the SENTRY antimicrobial surveillance program. *Antimicrob Agents Chemother* 2019; **63**: 1–10. https://doi.org/10.1128/AAC.00355-19

14 Heininger A, Zimmermann S, Bootsveld C *et al*. 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; **22**: 646–50. https://doi.org/10.1016/j.jgar.2020.05.003

15 Markwart R, Willrich N, Eckmanns T *et al*. Low proportion of linezolid and daptomycin resistance among bloodborne vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* infections in Europe. *Front Microbiol* 2021; **12**: 664199. https://doi.org/10.3389/fmicb.2021.664199

16 Pfaller MA, Cormican M, Flamm RK *et al*. 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. https://doi.org/10.1093/ofid/ofy344

17 Weber RE, Bender JK, Werner G *et al*. Eigenschaften, Häufigkeit und Verbreitung von vancomycin-resistenten Enterokokken in Deutschland —update 2019/2020. *Epid Bull* 2021; **27**: 11.

18 Layer F, Vourli S, Karavasilis V *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. https://doi.org/10.1093/jac/dkx524

19 Olearo F, Both A, Belmar Campos C *et al*. Emergence of linezolid-resistance in vancomycin-resistant *Enterococcus faecium* ST117 associated with increased linezolid-consumption. *Int J Med Microbiol* 2021; **311**: 151477. https://doi.org/10.1016/j.ijmm.2021.151477

20 KRINKO. Hygienemaßnahmen zur Prävention der Infektion durch Enterokokken mit speziellen Antibiotikaresistenzen. *Bundesgesundheitsblatt* 2018; **61**: 51.

21 Dembicka KM, Powell J, O'Connell NH *et al*. Prevalence of linezolid-resistant organisms among patients admitted to a tertiary hospital for critical care or dialysis. *Ir J Med Sci* 2022; **191**: 1745–50. https://doi.org/10.1007/s11845-021-02773-2

22 Girlich D, Mihaila L, Cattoir V *et al*. Evaluation of CHROMagar LIN-R for the screening of linezolid resistant staphylococci from positive blood cultures and nasal swab screening samples. *Antibiotics (Basel)* 2022; **11**: 1–8.

23 Layer F, Weber RE, Fleige C *et al*. Excellent performance of CHROMagar(TM) LIN-R to selectively screen for linezolid-resistant enterococci and staphylococci. *Diagn Microbiol Infect Dis* 2021; **99**: 115301. https://doi.org/10.1016/j.diagmicrobio.2020.115301

24 Bender JK, Fleige C, Klare I *et al*. 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. https://doi.org/10.1016/j.mimet.2019.03.025

25 Werner G, Strommenger B, Klare I *et al*. 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. https://doi.org/10.1128/JCM.42.11.5327-5331.2004

26 Hsieh YC, Lin YC, Huang YC. Vancomycin, teicoplanin, daptomycin, and linezolid MIC creep in methicillin-resistant *Staphylococcus aureus* is associated with clonality. *Medicine (Baltimore)* 2016; **95**: e5060. https://doi.org/10.1097/MD.0000000000005060

27 Egan SA, Shore AC, O'Connell B *et al*. 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. https://doi.org/10.1093/jac/dkaa075

28 Hu Y, Won D, Nguyen LP *et al*. Prevalence and genetic analysis of resistance mechanisms of linezolid-nonsusceptible enterococci in a tertiary care hospital examined via whole-genome sequencing. *Antibiotics (Basel)* 2022; **11**: 1–12.

29 Ruiz-Ripa L, Fessler AT, Hanke D *et al*. Mechanisms of linezolid resistance among enterococci of clinical origin in Spain-detection of *optrA*- and *cfr(D)*-Carrying *E. faecalis*. *Microorganisms* 2020; **8**: 1–17. https://doi.org/10.3390/microorganisms8081155