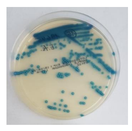

Results of the German multicenter study using CHROMagar™ LIN-R to detect linezolid-resistant enterococci

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Background

Linezolid resistance in enterococci (MIC >4 mg/L) is mediated by 23S rDNA mutations and/or acquisition of resistance genes (*cfr, optrA, poxtA*). German hygiene recommendations require a targeted screening for LRE under specific circumstances. CHROMagar LIN-R is a commercial agar for direct screening of linezolid-resistant enterococci (LRE) and staphylococci. We tested application and performance of this agar with 12 German partner sites, mainly university hospitals, during a 3 months period at the end of 2021.

Methods

An expanded risk-based screening for multidrug-resistant bacteria was performed on hospital wards that previously experienced VRE and LRE infections or colonizations. The number of mainly rectal swabs analyzed ranged from <200 to >2000 per center. Blue colonies growing after a 48h incubation period at 37°C (**Figure 1**) were identified and confirmed by standard lab procedures. Supposed LRE isolates were sent to the National Reference Centre (NRC) for phenotypic confirmation.

Results

False-positivity was high for enterococci (57%, range 0-93% between study sites; **Table 1**). LRE were detected with an overall prevalence of 1% (range 0.18-3.7%; **Figure 1, Table 1**). Material-specific prevalence was lower for urine samples (0.5%) than for rectal swabs (0.9%) (**Table 1**); however, this was not statistically significant (*P*=0.63).

A total of 161 LRE were received by the NRC for further analysis. Altogether 121 isolates (75%) were confirmed as LRE, of which 40 were *E. faecalis* (33%) and 81 were *E. faecium* (67%) (**Table 2**). The majority of LRE was vancomycin-susceptible (78%). Only *E. faecium* isolates were linezolid- and vancomycin-resistant (n= 26). The most frequent linezolid resistance mechanisms in *E. faecium* was due to 23S rDNA mutations (65%) followed by the presence of *optrA* (13%), whereas almost all *E. faecalis* possessed *optrA* (92%) (**Table 2**).

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**).

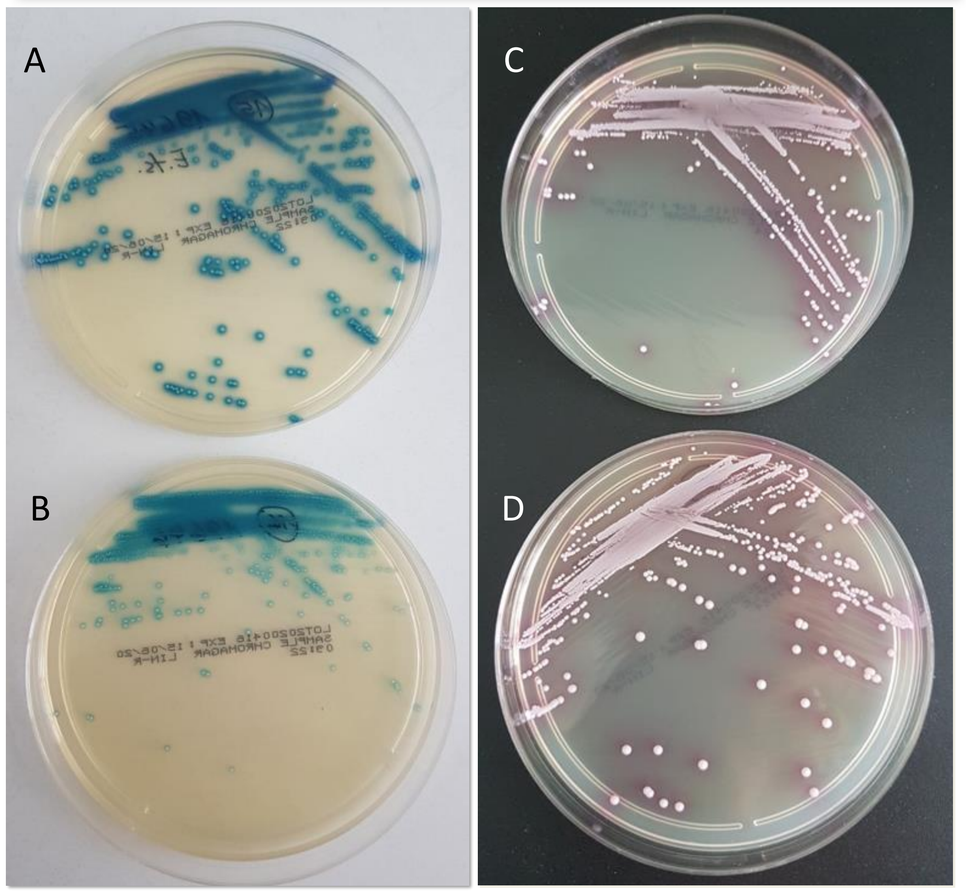

Figure 1. Growth behaviour of linezolid-resistant isolates of *E. faecalis (A), E. faecium (B),* and *S. epidermidis (C,D) after 48 h.* *Enterococcal isolates appear with blue-turquoise color and require 48h incubation at 37°C. S. epidermidis mostly also require 48h incubation time and appear with rose to pink color.*

Table 1. Total numbers and calculated LRE prevalence based on data collected at 12 study sites participating in the German CHROMagar™ LIN-R multicenter 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	1,387	470	2,220	460	2,263	1,178	1,295	1,198	182	247	778	2,285	13,963
Material total – w/o copy strains	1,387	469	2,220	460	2,263	1,178	1,295	1,198	181	231	778	2,251	13,911
Material only rectal swabs – w/o copy strains	1,387	n.a.	2,220	n.a.	2,263	1,178	1,295	n.d.	109	n.a.	778	2,247	11,477
Material only urine – w/o copy strains	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.	n.d.	1,198	15	n.a.	n.d.	2	1,215
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

Abbreviations: w/o, without; LRE = linezolid-resistant enterococci; LSE = linezolid-susceptible enterococci; n.a. = data not available; n.d. = not determined; n.c., data not considered due to low sample size

Table 2. Distribution of acquired resistance genes *cfr*, *optrA*, *poxtA* and of 23S rDNA G2576T mutations in phenotypically linezolid-resistant and -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	%
Resistant* (n=81)						
	-	-	-	+	53	65.4
	-	-	-	-	1	1.2
	-	-	+	-	11	13.6
	-	+	-	-	14	17.3
	-	+	+	-	2	2.5
total					81	100
Susceptible* (n=27)						
	-	-	-	+	6	22
	-	-	-	-	6	22
	-	-	+	-	14	52
	-	+	-	-	1	4
total					27	100
E. faecalis (N=53)	*cfr*	*optrA*	*poxtA*	23S rDNA G2576T	n	%
Resistant* (n=40)						
	-	-	-	+	1	2
	-	+	-	-	38	95
	-	+	+	-	1	2
total					40	100
Susceptible* (n=13)						
	-	-	-	-	3	23.1
	-	-	+	-	2	15.4
	-	+	-	-	7	53.8
	+	+	+	-	1	7.7
total					13	100

*all isolates presented in this table grew on CHROMagar LIN-r plates. The classification into "resistant" and "susceptible" as given here results from results of MIC tests based on broth microdilution determined at the National Reference Centre for Enterococci.

CONCLUSIONS

LRE could be identified using CHROMagar LIN-R with good specificity. Our study design did not allow derivation of sensitivity parameters. Due to a high rate of false-positives, colonies require confirmation by phenotypic and genotypic methods. Prevalence rates of LRE vary between study sites. Linezolid resistance in *E. faecalis* is mediated by a mobile *optrA* gene, which also becomes prevalent among *E. faecium*. Co-existence of linezolid and vancomycin resistance was only detected in *E. faecium*.

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